AB001. The path to genomic medicine

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Abstract: There are numerous obstacles to genomic medicine. These include the large number of rare and novel genomic variants per individual. The American College of Medical Genetics and Genomics (ACMG) has recommended that all pathogenic variants in 56 gene-disease pairs that are identified incidentally in a genomic test be offered to the patient (Green *et al*., 2013, PMID: 23788249). We considered an expanded list of 112 actionable gene-disease pairs, ones where medical intervention is possible to prevent or detect disease early. We estimate the rate of these incidental findings (IFs) in European and African Ancestry groups. However, we found high discordance between classifications of expert reviewers. We have reported both inconsistency across labs in variant classification and a bias towards overcalling pathogenicity (Amendola *et al*., 2015, PMID: 25637381). Thus, there is a need to standardize classification of genomic variants in medical sequencing. To date genomics laboratories have used non-standard classification systems. The ACMG published guidelines for variant classification for Mendelian disorders designed to increase consistency among labs (Richards *et al*., 2015, PMID: 25741868). The Clinical Sequencing Exploratory Research (CSER) Consortium evaluated the use of these rules by nine of the CLIA laboratories supporting CSER projects, considering 99 germline variants. The results were examined to evaluate intra-laboratory differences between variant classifications using the labs own criteria *vs.* adopting ACMG criteria and inter-laboratory differences using either the lab's own system or the ACMG guidelines. Agreement among labs did not differ whether using the laboratory specific *vs.* ACMG criteria (P=0.9); i.e., the ACMG criteria did not yield more consistent variant classification in this exercise. We further analyzed sources of disagreement in the use of the ACMG criteria and identified causes of variance in classifications. In addition to providing useful analyses of how variant classifications approaches vary among laboratories, these data should allow clarification and refinement of the ACMG criteria that may increase consistency in variant classification.

Keywords: Genomic medicine; genomic variants; variant classifications

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AB002. The rare and undiagnosed diseases diagnostic service

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Abstract: The Rare and Undiagnosed Diseases Diagnostic Service (RUDDS) is a Clinical Genomic Diagnostic Pipeline operating within the clinical service at Genetic Services of Western Australia (GSWA). GSWA has provided a state-wide service for clinical genetic care for more than 25 years and it serves a population of 2.5 million people. It includes paediatric, adult, prenatal and familial cancer services in metropolitan and regional WA. Within this framework, and in partnership with the Office of Population Health Genomics, Diagnostic Genomics at PathWest and others, it is delivering a clinically integrated pipeline. This service is aligned to the WA Rare Diseases

Strategic Framework 2015-2018 to address the unmet need of the diagnostic odyssey of those living with rare and undiagnosed diseases. It is: (I) delivered in a patient-centric manner that is resonant with the patient journey; (II) offers multiple options including non-genetic testing; monogenic and genomic (targeted and whole exome) analysis, and matchmaking; (III) is synchronous with precision phenotyping methods, including 3D facial analysis, and phenotype-enabled decision support; (IV) captures new knowledge, including multiple expert review; (V) has multiple points for entry, exit and re-entry to allow people access to information they can use, when they want to receive it; (VI) draws on the clarity gained from the extremity of rare diseases to provide insights for more common diseases; (VII) is integrated with current translational genomic research activities; and (VIII) is designed for flexibility for integrative generation of, and integration with, further clinical research including for diagnostics, community engagement, policy and models of care.

Keywords: Genomics; genetic care; diagnosis; disease

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AB003. The path towards translational medicine for common reproductive diseases

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Abstract: Genetic factors contribute to risk for many common traits and diseases affecting reproduction and fertility. We have used genome-wide association (GWA) studies to understand the genetic architecture and discover genomic regions associated with risk for endometriosis, dizygotic twinning, age at menarche and age at menopause. The next steps are to determine how DNA sequence variation alters regulation and/or function of specific genes and pathways to increase disease risk. Multiple approaches are required to interpret the genetic association results, identify the specific genes likely to be responsible, and obtain the necessary genomic evidence connecting the genetic results to the target genes. Strategies include fine mapping, functional annotation, genomics, and target gene identification through gene expression, epigenetics, and cell-based studies to define direct interactions between causal single nucleotide polymorphisms (SNPs) and target genes. GWA and replication studies have identified seven genomic regions with strong evidence for association with endometriosis risk. The target tissue for functional effects is not known, but current theories suggest changes in the endometrium. We are conducting studies of gene expression and epigenetic regulation in samples of endometrium in carriers of the risk alleles. Development of applications to use GWA data for risk prediction and studies of comorbidity also provide valuable insights into the genetic architecture of endometriosis and overlap in risk with other conditions such as ovarian cancer. Multidisciplinary studies combining genetics, genomics, functional biology, and clinical research will be essential better understand disease biology and translate the new knowledge into better outcomes for patients.

Keywords: Genome-wide association (GWA); genetic architecture; reproduction; fertility; endometriosis

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AB004. Genetic testing for individuals with developmental disabilities and congenital anomalies: choosing between chromosomes, DNA microarrays, and next generation sequencing platforms

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Abstract: Rapid advances in molecular techniques provide multiple testing options available to the clinician. Currently, with the combination of clinical expertise and state-of-the-art molecular genetic testing a specific diagnosis can be identified in approximately 50% of patients evaluated in genetics clinic. Considerations for choosing the best test in a given diagnostic situation will depend on the availability of the testing platform, whether parental samples are required as part of the evaluation, cost effectiveness and the greatest diagnostic yield. In particular, traditional G-banded chromosome studies have a diagnostic yield of 3-5%; they are still the test of choice for a suspected chromosomal disorder such as Trisomy 21, but have the disadvantage of requiring tissue culture facilities. In contrast, DNA microarrays and next generation sequencing platforms do not require tissue culture and have an increased diagnostic yield of 12-15% and 25-30%, respectively. However, the identification of variants of unknown significance (VUS) is common to both DNA microarrays and next generation sequencing. Using these genetic testing platforms may require extensive interrogation of databases and analysis of parental samples to determine the significance of a specific finding. DNA microarrays are the current recommended first-line test for individuals with multiple anomalies, developmental and intellectual disabilities, and autism by the American College of Medical Genetics. The results of 10 years of experience using BAC and oligo DNA microarray panels at Group Health Cooperative (GHC) conservatively identified a genomic imbalance in 16.2% (36/222) of the patients tested, with a similar number of patients (14.9%) with an identified VUS. During a limited clinical study of 20 patients in Viet Nam with congenital anomalies and developmental and intellectual disabilities, 22% were found to have an identified pathogenic genomic abnormality and 22% had a VUS. While the sensitivity associated with new molecular techniques has resulted in an increased diagnostic yield and an expanded spectrum of clinical phenotypes, it has been complicated by the high rate of VUS identification. These issues will be addressed as more patients of varied ethnic backgrounds are studied and international collaborations integrate clinical findings and molecular results across global populations. Medical genetics services are essential to ensure that patients receive the most appropriate genetic testing, result interpretation, and follow-up testing for VUSs, as indicated, to allow appropriate medical care, surveillance and recurrence risk counseling for both the patient and extended family.

Keywords: DNA microarray; next generation sequencing

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AB005. Genomics on site of detection of malaria

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Abstract: Nanopore sequencer, MinION, has enabled sequencing analysis without pre-installation of expensive conventional sequencers or pre-requisite of specific skills in biological experiments. Even electric supply is not always necessary, by connecting MinION to a laptop PC. These features of MinION have opened the opportunity to enable precise genotyping of pathogens on site. In this study, we conducted genotyping of presumed drug resistance-causing SNVs in malaria parasites, *Plasmodium falciparum*. We subjected ten PCR amplicon-mixes covering these SNVs to the MinION sequencing. In spite that the

sequence alignments generated by a Smith-Waterman-based program, SSEACH showed that the average sequence identity was 75%, we found that the mutations at a particular position could be called by the accuracy of 90%, when all the reads covering the corresponding positions were collectively evaluated. We provide the first simple experimental and analytical MinION sequencing procedure, which can be easily followed on site to effectively genotype pathogens of other tropical diseases.

Keywords: MinION; sequencing analysis; sequencers; tropical diseases

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AB006. Personalized and precision medicine: are we there yet?

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Abstract: Personalized medicine is determined by an individual's unique clinical, genomic and environmental information. The molecular understanding of diseases allowed development of preventive healthcare strategies and medical treatments at the pre-symptomatic or earliest stage of the disease. To achieve this promise, DNA-based risk assessment, molecular profiling, targeted therapies and dose selection of therapeutic agents were developed to facilitate customization of patient care. Commercially available genomic tests routinely are applied across a wide range of disease states in predictive or prognostic applications. Many clinicians were concerned about the lack of progress in the clinical application of genomic medicine. The development of genomic diagnostic tools such as array comparative genomic hybridization, exome and whole genome sequencing had a vital role to play in the delineation of new Mendelian loci for previously unrecognized syndromes or identification of additional genes or loci contributing to known disease entities. While the costs of these tests had decreased, the interpretation of information of uncertain significance may require increased 'genomic counseling' consultations to allay anxiety. Personalized medicine at present has limited roles in complex disorders or used as a tool lifestyle change decisions as public health or primary care professionals who may not be sufficiently 'genomic-trained'. Genomic health risk assessments and statistical probabilities are difficult for clients to understand and personalized medicine must be integrated into the existing health systems. In addition, clinical workflow with significant changes required in regulatory and reimbursement policies as well as legislative protection related to patient's confidentiality will be required. The difficulties with use of genome-wide association studies in clinical practice, with its limited phenotype-genotype impact must be addressed. Personalized medicine is expected to revolutionize traditional clinical practice; in reality, it will evolve to deliver on the promise of a safer and effective healthcare for the individual patient.

Keywords: Personalized medicine; genomics; genetic counseling; public health

Cite this abstract as: Thong MK. Personalized and precision medicine: are we there yet? Ann Transl Med 2015;3(S2):AB006. doi: 10.3978/j.issn.2305-5839.2015.AB006

AB007. Genomic medicine: impact of rare disease research on medicine and health care

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Abstract: Most of rare diseases are genetic and the number of identified pathogenic genes is rapidly increasing since the introduction of next-generation sequencers. To date more than 4,500 Mendelian rare disorders with known molecular basis are listed in the OMIM database. In the past, it was believed by the majority of medical community that research on rare diseases scarcely contributes to medical science and

health care in general. Sometimes rare disease research was considered to be a kind of hobby similar to collecting rare stamps or a charity activity to rescue those deserted patients. In the past decade, genome scientists searched for the genetic basis of 'common diseases' based on the 'common variant hypothesis'. It turned out, however, most of the identified common variants identified by genome-wide association studies had relatively small effect on disease susceptibility. Now the genome scientists are turning to 'rare variant hypothesis', where each of rare variants has a strong effect on disease development. Recently rare disease research led to the discovery of important medical findings and the development of novel drugs not only for rare diseases but also for common diseases. For example, the elucidation of pathogenic genes for osteopetrosis, a rare condition in which bone mass is excessively increased, contributed the development of novel drug for osteoporosis, a common fragile bone disorder among aged people. Studies on rare familial renal glucosuria due to SGLT2 mutations gave the idea of a novel therapeutic strategy for diabetes mellitus by dumping excess sugar into urine. It is reasonable to assume that pathogenic genes responsible for Mendelian disorders are crucial for maintaining health in human beings. We need to reiterate the importance of analyzing characteristic clinical features associated with rare genetic variants, which may not be readily elucidated by basic research using cells or animals.

Keywords: Genome; rare disease; Mendelian disorders; next-generation sequencing

Cite this abstract as: Matsubara Y. Genomic medicine: impact of rare disease research on medicine and health care. Ann Transl Med 2015;3(S2):AB007. doi: 10.3978/j.issn.2305-5839.2015.AB007

AB008. SEPARATION and identification glycoprotein in human Fragile X syndrome serum

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Background: Fragile X syndrome (FXS), the most common cause of inherited mental retardation. The absence of FMRP results in FXS leading changes other proteins.
Objective: To detect changes of glycoproteins in human FXS serum.
Methods: Affinity chromatography with lectin concanavalin A (ConA) used to receive glycoproteins. These collected glycoproteins were then separated using 2-D electrophoresis. The protein spots were further excised, trypsin-digested, and analyzed by nanoLC couple with ESI MS/MS and identified by MASCOT v1.8 software.
Results and conclusions: Five glycoproteins showed the different expression levels in the FXS serum. Haptoglobin and IgJ were increased, Ceruloplasmin, Transferrin, Ig kappa were decreased. We found much useful information that related to FXS when these changed glycoproteins were analysed in detail.

Keywords: Fragile X syndrome (FXS); FMRP; FMR1; proteomics

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AB009. Targeting the stratification of neuroblastoma: clinical and biological challenges

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Background: Neuroblastoma (NB) is a solid tumor derived from the sympathoadrenal lineage of the neural crest, and is one of the lowest survival rates in pediatric Vietnamese patients. This is a complex types of cancer because clinical features and genetic diversity heterogeneous. The NB treatment is mostly determined by clinical and biological assessment to classify the patients with low- and high-risk under the guideline of SOPIEN/COG protocols.
Objective: We validate the clinical presentations and biological markers that might assist the clinical decision-making process to improve the survival rates in NB at the Children Hospital II, Hochiminh city.
Methods: RNA and genomic DNA were extracted from 65 cases of NB tumor tissues from the Children Hospital II. We assessed the study of molecular-based genotyping, FISH, and mRNA expression to identify the biomarkers including TrkA, *MYCN* gene amplification, segmental chromosome aberrations (loss of heterozygosity 1p, 11q, or gain of 17q), and compare with clinical presentations are classified as: age at diagnosis, histology, and INSS stages.
Results and conclusions: We identify amplification of *MYCN* in 12 out of 65 (18.46%) NB patients, and chromosome aberrations in 5 out of 65 (7.69%). Additionally, reduced or induced TrkA was correlated with amplified and non amplified *MYCN* respectively. Together, our study supports the pipeline for risk stratification of Vietnamese NB and supports the therapeutics of the disease.

Keywords: Cancer; neuroblastoma; genotyping; MYCN; TRKA

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AB010. Newborn screening worldwide: when and where is molecular testing a part of the screening protocol

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Background: Newborn bloodspot screening (NBS) describes various tests that can occur during the first few hours or days of a newborn's life and have the potential for preventing severe health problems, including death. NBS has evolved from a simple blood or urine screening test to a more comprehensive and complex screening system capable of detecting over 50 different conditions. Molecular testing using dried blood spot specimens was first demonstrated as a viable 2nd-tier NBS technique for both sickle cell diseases and cystic fibrosis in the 1980s. Since that time, use of molecular tests has slowly expanded in NBS programs until now it is a routine part of many screening programs including as a primary screening method for severe combined immunodeficiency disease (SCID).

Objective: This presentation seeks to: (I) provide an overview of NBS activities worldwide; (II) review the origins of molecular testing as part of NBS; and (III) increase awareness of molecular testing activities in NBS today.

Methods: A series of reports described various NBS activities around the world in 2007. These reports were updated in 2015 and this presentation reviews the NBS system and the ongoing activities reported with an eye towards molecular testing. For 2015 reporting purposes, the world was divided into five regions (North America, Europe, Middle East and North Africa, Latin America, and Asia Pacific), and each region was reviewed by NBS experts active in the region. Experts and co-authors of this report include: Brad Therrell and John Adams (North America), Carmencita Padilla (Asia-Pacific), Gerard Loeber (Europe), Issam Kneisser and Amal Saadallah, Middle East/North Africa, Gustavo Borrajo (Latin America).

Results and conclusions: NBS for one or more conditions now covers about one-third of the world's newborns. In more developed programs, molecular testing protocols are included either as 2nd-tier (cystic fibrosis and sickle cell diseases) or primary screening (SCID). Future expansion of NBS will likely include molecular testing protocols.

Keywords: Newborn screening; worldwide screening; molecular methods; screening review; SCID

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AB011. Preimplantation genetic diagnosis—experience from Hong Kong

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Abstract: Preimplantation genetic diagnosis (PGD) is a technology used to determine whether a genetic or chromosomal disorder is present in embryos during an in-vitro fertilization (IVF) cycle. PGD screens embryos prior to their transfer to the uterus. Initially, PGD was developed to detect early onset life threatening single gene disorders for couples who are aware of their hereditary risks through family history or based on carrier testing. Since then, the use of PGD has expanded to detect late onset disorders such as Huntington disease and hereditary cancer predisposition syndromes. PGD treatment has been available in Hong Kong since 2002. In Hong Kong, couples seeking PGD treatment are required to see two doctors, one of whom must have proper training in clinical genetics and/or genetic

counselling. The purposes of genetic counselling include educating patients about the genetic condition and ensuring that patients are given informed choices on the available options. PGD can provide reassurance of having healthy children for couples at high risks passing the genetic change. Yet there are more ethical concerns when the requests become increasingly complex. Multidisciplinary PGD board meeting has a key role in cases with ethical concerns. We illustrate with case examples the practice of PGD-related genetic counselling in Hong Kong.
Funding: SK Yee Medical Research, Hong Kong.

Keywords: Preimplantation genetic diagnosis (PGD); genetic counselling; in-vitro fertilization (IVF)

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AB012. Applying whole exome sequencing (WES) to solve undiagnosed diseases in children in Hong Kong

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Abstract: Whole exome sequencing (WES) combines next generation sequencing technology with capture methods to sequence only the protein-coding part of the human genome. Its application has been successful in the discoveries of new disease-causing genes in both Mendelian and complex disorders. In the context of clinical diagnosis, the WES approach has an overall diagnostic yield of 15-30% for patients with undiagnosed diseases. In Hong Kong, we have applied WES to paediatric rare diseases, by establishing our in-house research pipeline and by utilizing accredited laboratories overseas. Case examples will be used in the presentation to illustrate these two approaches. Finally, we discuss the challenges of using WES in Hong Kong, and the future direction of applying NGS technology to revolutionize the clinical diagnosis and medical researches.
Funding: SK Yee Medical Foundation and The Society for the Relief of Disabled Children, Hong Kong.

Keywords: Whole exome sequencing (WES); undiagnosed diseases; clinical diagnosis; sequencing technology

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AB013. Long-range modulation of *PAG1* expression by 8q21 allergy risk variants

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Abstract: The gene(s) whose expression is regulated by allergy risk variants is unknown for many loci identified through genome-wide association studies. Addressing this knowledge gap might point to new therapeutic targets for allergic disease. The aim of this study was to identify the target gene(s) and the functional variant(s) underlying the association between rs7009110 on chromosome 8q21 and allergies. Eight genes are located within 1 Mb of rs7009110. Multivariate association analysis of publicly available exon expression levels from lymphoblastoid cell lines (LCLs) identified a significant association between rs7009110 and the expression of a single gene: *PAG1* (P=0.0017), 732 kb away. Analysis of histone modifications and DNase I hypersensitive sites in LCLs identified four putative regulatory elements (PREs) in the region. Chromosome conformation capture confirmed that two PREs interacted with the *PAG1* promoter, one in allele-specific fashion. To determine if these PREs were functional, LCLs were transfected with *PAG1* promoter-driven luciferase reporter constructs. PRE3 acted as a transcriptional enhancer

for *PAG1* exclusively when it carried the rs2370615:C allergy predisposing allele, a variant in complete linkage disequilibrium with rs7009110. As such, rs2370615, which overlaps RelA transcription factor (TF) binding in LCLs and was found to disrupt Foxo3a binding to PRE3, represents the putative functional variant in this locus. Our studies suggest that the risk-associated allele of rs2370615 predisposes to allergic disease by increasing *PAG1* expression which may promote B-cell activation and have a pro-inflammatory effect. Inhibition of *PAG1* expression or function may have therapeutic potential for allergic diseases.

Keywords: Allergic disease; *PAG1* expression; chromosome 8q21; target gene; functional variant

Cite this abstract as: Vicente CT, Edwards SL, Hillman KM, Kaufmann S, Mitchell H, Glubb DM, Lee JS, Bain L, French JD, Ferreira MA. Long-range modulation of *PAG1* expression by 8q21 allergy risk variants. Ann Transl Med 2015;3(S2):AB013. doi: 10.3978/j.issn.2305-5839.2015.AB013

AB014. Expanding newborn screening and the initiation of regional follow-up in the Philippines

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Background: Newborn bloodspot screening (NBS) is a well-recognized public health prevention program aimed at identifying newborns that are affected by certain genetic/metabolic/infectious conditions. Early identification of these conditions is particularly crucial since timely intervention can lead to significant reduction in morbidity, mortality, and associated disabilities in affected newborns. Newborn screening systems have existed in many developed countries for over 40 years, but they are still in development in low-income settings. While developing programs are focusing on 1 or 2 prominent congenital conditions, developed programs now screen for more than 50 different conditions. Newborn screening in the Philippines began as a small pilot program in Manila in 1996 and became a nationwide program institutionalized in a 2004 law that requires that NBS to be offered to all newborns, supported by national health insurance. While the screening mandate includes 6 conditions, recent expansion has allowed for inclusion of over 20 conditions at an additional charge. To facilitate clinical follow-up, a network of regional follow-up centers has been implemented using a team approach. A recently implemented genetic counseling program is providing much needed trained personnel to assist with counseling activities across the country

Objective: This presentation will briefly review the implementation of newborn screening in the Philippines and address the considerations involved in expanding to over 20 screened conditions in a resource poor setting. Additionally, it will review the implementation of genetic counseling training and development of a regional clinical follow-up team approach in order to speed and improve needed access to clinical care.

Methods: Expansion of newborn screening to include metabolic conditions detectable with tandem mass spectrometry has included extensive training with support from developed programs in the U.S. and Australia. Likewise, expanded screening for thalassemias and other hemoglobinopathies and miscellaneous other conditions has included similar activities. To augment the clinical and other medical services needed as part of follow-up, a genetic counseling training program has been initiated the regional follow-up teams.

Results and conclusions: NBS in the Philippines now includes screening for over 20 conditions including multiple metabolic and hemoglobin conditions detectable though multiplex laboratory procedures. Centralized, regional follow-up teams including a physician, nurse, counselor and administrative staff have been initiated in all public health regions in the country. National insurance is considering the addition of expanded screening conditions into its ongoing payments for basic newborn screening.

Keywords: Expanded newborn screening; regional follow-up; Philippine newborn screening; genetic counselling

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AB015. Very early for pompe disease contribute to better outcomes: 7-year cohort study in Taiwan

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Background: Pompe disease is a lysosomal storage disorder characterized by the deficiency of acid α-glucosidase (GAA). Whether outcomes differ between very early (few days of age) and early (few weeks of age) ERT is unknown. In our series, 789,797 newborns were screened for Pompe disease. After 2010, we combined nationwide screening system with rapid diagnostic strategies. Treatment could be started at about 10 days of age. We analyzed the outcomes of our patients and compared these data to other IOPD cohort studies.

Methods: In this nationwide program, 789,797 newborns were screened for Pompe disease between January 1, 2008 and January 31, 2015. We diagnosed IOPD in 17 of these newborns, and all were treated and followed in our hospital. We analyzed the outcomes and compared the data to other IOPD cohort studies.

Results: After 2010, the mean age at first ERT was 10 days. Our patients have better biological, physical and developmental presentations and lower anti-rh GAA antibodies after 2 years of treatment, even compared to one group that began ERT just 10 days later than our cohort. No patient had a hearing disorder or abnormal vision. The mean age for independent walking was 11.6±1.3 months, the same age as normal children.

Conclusions: ERT for IOPD patients should be initiated before irreversible damage occurs. Our results indicate that early identification of IOPD patients allows for the very early initiation of ERT. Starting ERT even a few days earlier can lead to better patient outcomes.

Keywords: Pompe disease; ERT; IOPD; newborns

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AB016. Developing diagnostic strategy of multiple congenital anomalies in Indonesia

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Background: Pediatricians quite often must deal with multiple congenital anomalies (MCA). Without a correct diagnosis, many available forms of therapy will be under-or-overused and counseling about prognosis and recurrence risk maybe unrealistic. The basis for diagnosis of MCA involves a combination of defining the physical manifestations and diagnostic genetic testing. Chromosome analysis is a standard practice to unravel the etiology of MCA. Conventional cytogenetic method has limitation in detecting abe1rrations less than 5 Mb in size. Microarray technology could overcome this obstacle. The aim of this study is to develop diagnosis strategy of MCA cases.

Methods: Seventy two MCA cases were recruited from July 2013 until June 2014. Fifty one subjects were diagnosed phenotypically using OMIM and POSSUM databases. Subsequently, chromosome analysis were performed as a first step of diagnosis strategy. Nine cases among those subjects found to have chromosome aberrations, whereas twelve cases showed normal karyotypes. Eight subjects from the normal karyotype group have a good quality of DNA and proceed to microarray examination. Microarray examination were done at Department of Medical Genetics, UMC Utrecht, Netherlands, using Infinium CytoSNP-850K DNA analysis bead chip kit from Illumina. Chips were scanned using Hi-scan scanner from Illumina. Data were extracted using genome studio software. Data were analyzed using Nexus software.

Results: Nine out of twenty cases were found to have chromosome aberrations. Those aberrations are:46,XY,add(13)(q34); 46,XY,6 Mar, 17 dmin; 46,XX,r(4)(p16q35); 46,XY,22ps+; 46,XY,add(5)(p15); 47,XX+G; 46,XX/45XX Rob (13,15/q10.2,q10), 45XX Rob (13,14)(q10,q10); 46,XX, ring 13; 45,XY,der(2)del(2)(q37.3)t(2;15)(q37.2;q11.2). Five out of eight subjects which tested by microarray showed normal array. Two subjects showed well known deletion syndrome, which are Wolf-Hirschhorn

syndrome and Williams-Beuren syndrome. One case has normal array with two large regions Lost of Heterozygosity. **Conclusions:** The first step of MCA's diagnostic strategy is to do phenotype screening using the established databases. Subsequently patients grouped as "known" or "unknown" MCA. Second step is to perform chromosome analysis, either to confirm diagnosis or to find chromosome aberration. Unestablished diagnosis should be examined further using microarray.

Keywords: Multiple congenital anomalies (MCA); conventional chromosome analysis; microarray chromosome analysis

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AB017. The extended newborn screening by tandem mass in Taiwan—results from two national newborn screening centers: Taipei Institute of Pathology & Chinese Foundation of Health

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Abstract: Since 2001, tandem mass spectrometry was introduced for newborn screening by two Taiwan national newborn screening centers: Taipei Institute of Pathology and Chinese Foundation of Health, in Taiwan, respectively. A total of 24 metabolic disorders, including 6 amino acidopathies, 8 organic acid disorders and 9 fatty acid disorders, etc., were screened and the newborns with positive screening result were referred to Taipei Veterans General Hospital for confirmatory diagnosis. Until Dec 2014, a total of 1,391,583 newborns have been screened by these two newborn screening centers. The overall incidence of inborn errors of metabolism identified by tandem mass was approximately 1/6,000. The most common inborn errors were defects of phenylalanine metabolism (phenylketonuria: 1/60,000; 6-pyruvoyltertrahydropterin synthase (PTPS): 1/110,000). Maple syrup urine disease was the second most common amino acidopathy (1/100,000) and, significantly, most MSUD patients (75%) belonged to the Austronesian aboriginal tribes of southern Taiwan. The most frequently detected organic acid disorder was 3-methylcrotonyl-CoA carboxylase deficiency (1/35,000). Glutaric aciduria type 1 and methylmalonic academia were the second most common organic acid disorders (1/100,000 for each disease). The incidence of fatty acid disorder is relatively less than most western countries. Out of them, carnitine transport defect was the most common fatty acid disease (1/100,000). In this report we will present this large population study of tandem mass newborn screening in Chinese population.

Keywords: Tandem mass spectrometry; newborn screening; metabolic disorders; inborn errors

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AB018. Revisited later-onset cardiac type Fabry disease—cardiac damages progressed in silence—experiences from an extremely high prevalent area, Taiwan

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Abstract: All of the current newborn screening studies of

Fabry disease revealed that the incidences of later-onset Fabry disease in their studied populations were much higher than the previous expectancy. It reveals that later-onset Fabry disease could be an important hidden health issue in some populations or even a lot of populations. However, the natural course of later-onset Fabry disease is still largely unknown. A total of 792,247 newborns have been screened for Fabry disease by our team in Taiwan. Through this screening and pedigree study, more than 900 individuals were found to have a later-onset type mutation, IVS4+919G > A. The left ventricular hypertrophy (LVH) onset rate was analyzed through our patient database and gadolinium-enhanced cardiac magnetic resonance imaging (GE-CMRI) was performed in 73 IVS4 adults. LVH onset rate were 77% in male and 35% in female adults who were older than 40 years old. GE-CMRI revealed that 43% of males and 14.3% of females hadn't LVH but had already developed significant myocardial delayed enhancement (MDE). Ten of patients who hadn't LVH with MDE underwent endomyocardial biopsy and all revealed typical Fabry myocardial pathological findings with significant globotriaosylceramide accumulation in their cardiomyocytes. Our findings indicate that the current common consensus when to start ERT for cardiac Fabry patients might be inappropriately focusing on the existence of hypertrophic cardiomyopathy and its related symptoms/signs. An earlier intervention before significant cardiac manifestations have occurred in these later-onset Fabry patients might be necessary for a better outcome of enzyme replacement therapy.

Keywords: Newborn screening; Fabry disease

Cite this abstract as: Niu DM, Hsu TR, Yang CF, Chu TH, Chiang CC, Ho HC. Revisited later-onset cardiac type Fabry disease—cardiac damages progressed in silence—experiences from an extremely high prevalent area, Taiwan. Ann Transl Med 2015;3(S2):AB018. doi: 10.3978/j.issn.2305-5839.2015.AB018

AB019. Osteogenesis imperfecta 2015: new genes, new treatments—an Asia pacific perspective

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Abstract: For 40 years the pathogenesis of the group of brittle bone disorders collectively named osteogenesis imperfect (OI) has been ascribed to mutations in type I collagen. Recent discoveries in matrix biology have transformed our perspectives on the role of mutations in the α_1- and α_2-chains of type I collagen (COLIA1, COLIA2), their post-translational modifications, trafficking and matrix interactions. Furthermore progress in gene discovery has identified 22 genes including the 2 COLI genes, in which mutations result in at least one OI phenotype. The International Bone Dysplasia Committee has grouped the syndromes arising from mutations in these genes into five OI phenotypes. All 3 modes of inheritance, Autosomal Dominant (4 genes) and Recessive (16 genes), X-linked (2 genes) have been discovered. The gene products of the recessive genes have a variety of functions. Mutations in LEPRE1, CRTAP and PIPB regulate prolyl-3-hydroxylation. A recent study in *Crtap*$^{-/-}$ mice showed upregulation of TGF-β target genes and reduced binding of type 1 collagen to the proteoglycan decorin. A similar pattern of TGFB dysregulation was observed in the tissues of heterozygous *Col1a*$_2$ $^{tm1.1\ Mcbr}$ mice. Mutations in FKBP10, SERPINF1 (HSP10), SERPINH1 affect polypeptide trafficking but have other matrix functions. Mutations in PLOD2 and FKBP10 both have extra-skeletal effects on matrices resulting in joint contractures. Mineralisation and osteoclast function are affected by mutations in LRP5, SP7, TMEM38B, WNT1, IFITM5 and CREB3L1 (OASIS), SPARC as do hemizygous mutations in the X-linked gene PLS3. A role for the unfolded protein response (UPR) is observed in the pathogenesis of OI resulting from mutation in CREB3L1. There is some evidence that the frequency of the varying types of OI may vary in and between populations in Asia and the Pacific. OI with Congenital Joint contractures for example is of high frequency in Samoa and Tonga and may well be common in a source community in Asia. Similarly my colleagues have observed a number of families with OI type 5 in the Philippines. This heterogeneity is becoming

relevant to management as there is evidence of resistance to bisphosphonate therapy in patients with homozygous mutations in SERPINF1 also known as OI type VI. Non-COL1 related OI is the most prevalent form of OI in some parts of Africa so that it would not be unusual if non-COLI related OI was more prevalent in some communities in the Asia Pacific region. Targeted exome Multiple Parallel sequencing panels are being developed and may be needed in the future to resolve the question of exact diagnosis to facilitate patient care.

Keywords: Osteogenesis imperfecta (OI); phenotypes; gene mutations

Cite this abstract as: Sillence D. Osteogenesis imperfecta 2015: new genes, new treatments—an Asia pacific perspective. Ann Transl Med 2015;3(S2):AB019. doi: 10.3978/j.issn.2305-5839.2015.AB019

AB020. What is advance in molecular diagnosis for 46,XY and 46,XX testicular disorder of sex development?

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Background: The disorders of sex development (DSD) are defined by congenital conditions in which development of chromosomal, gonadal, or anatomical sex is atypical. It is estimated that genital anomalies occur in 1 in 4,500 births but 1:125 boys has hypospadias. There are three broad groups: 46,XX DSD, 46,XY DSD and sex chromosome aneuploidy DSD. Recently, exome sequencing followed by analysis with a list of all known human DSD-associated genes was used to investigate the underlying genetic etiology of 46,XY DSD patients who had not previously received a genetic diagnosis (E. C. Delot *et al*. ASHG meeting 2014). The authors identified a likely genetic diagnosis in more than a third of cases, including 22.5% with a pathogenic finding and an additional 12.5% with likely pathogenic findings. In addition, 15% had variants of uncertain clinical significance that may be reclassified as literature evolves.

Objective: To identify mutations in causative/candidate/susceptibility genes in patients with 46,XY DSD and 46,XX testicular DSD including *AR*, *ATF3*, *BMP4*, *BMP7*, *BNC2*, *CTGF*, *CYP1A1*, *CYR61*, *DGKK*, *EGF*, *ESR1*, *ESR2*, *FGF8*, *FGFR2*, *GSTM1*, *GSTT1*, *HOXA4*, *HOXB6*, *HSD3B2*, *HSD17B3*, *MAMLD1*, *MID1*, *NR5A1* (*alias SF1*), *SRD5A2*, and *WT1* genes. And to clarify the role of cryptic rearrangements in the development of 46,XY DSD in Vietnamese patients.

Patients and methods: A total of 61 cases with 46, XY were performed mutation analysis using PCR, next generation sequencing. Eight patients with 46,XX testicular DSD were analysed using whole genome and exome sequencing and 6 cases with 46,XY DSD associated with mental retardation and/or other congenital malformations were diagnosed molecular using CGH. Genomic DNA was extracted from lymphocytes of peripheral blood.

Results and conclusions: Two cases with primary adrenal insufficiency and 46,XY DSD from two unrelated families were identified novel homozygous mutation in *HSD3B2* [c.481G>C (p.A161P)]. One case with simple hypospadias without adrenal insufficiency was identified mutation in *HSD3B2* (p.A10T) gene. Six different causative mutations including 3 novel ones of *AR* gene were identified in 9 patients with androgen insensitivity syndrome [p.L701F (c.2103G>T); p.L705F (c.2113C>T); p. W752S (c. 2256 G>T); p.V747M (c.2239 G>A); p.V867M (c.2599 G>A) and p.Q28X (c.82C>T)]. Three causative mutations of *SRD5A* gene (coding for 5-alpha reductase) (p.S220L; p.R237G and p.R227Q) were identified in three patients from three unrelated families. Six cases with 46,XY DSD associated with mental retardation and/or other congenital malformation were identified cryptic rearrangements; 2 cases with 46,XX testicular DSD were identified duplication in *SOX9*. Advances in identification of molecular genetic causes of DSD will help confirmation of diagnosis, appropriate treatment and genetic counseling.

Keywords: Disorders of sex development (DSD); 46,XX testicular DSD; cryptic rearrangements

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AB021. Epigenetics and disease—lessons from imprinting disorders

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Abstract: Different cells in the body are characterised by different functions and different levels of gene expression despite each sharing the same genetic code. This variation in gene activity from cell to cell is achieved by mechanisms and processes that are collectively termed epigenetics. These epigenetic changes alter gene expression without altering the DNA sequence. One epigenetic mechanism that is readily measured is DNA methylation. It is potentially reversible and heritable over rounds of cell division. Furthermore such epigenetic modification of DNA can be influenced by environment, gene interaction or by stochastic error and there is a higher rate of epimutation than DNA mutation. Variation in DNA methylation is a well-recognised cause of human disease and is likely to play a pivotal role in the cause of complex disorders. The challenge is to identify consistent epigenetic alterations of aetiological significance, given that epigenetic modification of DNA differs between tissues, occurs at different times of development within the same tissue and is sensitive to continual environmental factors. This makes it difficult to determine whether epigenetic mutations are a primary cause or secondary to the disease process. Genomic imprinting is one of the best understood examples of epigenetic regulation of gene expression. The expression patterns of imprinted genes are characterised by expression from only one allele (of the pair) in a consistent parent of origin manner. The pattern is set by targeted methylation within the male or female germ line that resists the post fertilisation waves of demethylation of the zygote. Imprinted genes are thought to play an important role in fetal growth and their carefully regulated expression is important for normal cellular metabolism and human behaviour. Several well-known disorders of imprinting are known including Beckwith Wiedemann syndrome, Transient Neonatal Diabetes, Temple syndrome, Wang Kagami Ogata syndrome, Russell Silver syndrome, Angelman syndrome Prader Willi syndrome and Pseudohypoparathyroidism type 1B. Only a proportion of people with these syndromes have a true epigenetic error, as uniparental disomy (inheritance of both chromosome homologues from one parent with no contribution from the other) and copy number variation are more common underlying causes. Studies to determine the cause of seemingly 'true' epigenetic aberrations, identified in imprinting disorders, may provide helpful insights into the causes of epigenetic mutations in general. For example the work on imprinting disorders has led to the identification of *ZFP57*, as a gene essential for DNA methylation maintenance.

Keywords: Epigenetic mutations; DNA methylation; imprinting disorders

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AB022. Harnessing big data to transform clinical care of cardiovascular diseases

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Abstract: Diseases of the heart and vascular system are the leading causes of mortality worldwide. A number of risk factors have already been identified such as obesity, diabetes and smoking; in the recent years, research has shifted its focus on genetic risk factors. Discoveries on the role of genes partnered with the technological developments have enabled advances in the understanding of human genetics and its influence on disease and treatment. There are initiatives now to combine medical records and genetic and other molecular data into a single "knowledge network" to achieve these aptly known as precision medicine. With next generation sequencing readily available at a more affordable cost, it is expected that genetic information of patients will be increasingly available and can be used to guide clinical decisions. Big data generated and stored necessitates broad and extensive interpretation to be valuable in clinical care. Accumulating evidence on the use of such genetic information in the

cardiovascular clinics will be presented.

Keywords: Cardiovascular; precision medicine; genetic information; big data

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AB023. Problem in the prevention and control of thalassaemia in Asia

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Abstract: Thalassaemia is the most common inherited single gene condition in the world. It is especially highly prevalent in the Asian Pacific region and poses a major public health problem. Carrier frequencies of the 2 major types of thalassaemia, the α and the β-thalassaemia, range from 3-40%, and vary from one country to another and within the same country in different ethnic groups. The most severe form of *α*-thalassaemia is Bart's hydrops fetalis. This is a lethal condition that also poses an increased risk for maternal morbidity. Children with β-thalassaemia major are born healthy but require life-long transfusion with expensive iron chelation therapy, or bone marrow transplant to survive. Control of thalassaemia is further complicated by the prevalence of HbE in this region. Individuals who co-inherit HbE and β-thalassaemia have thalassaemia intermedia with severity range from mild to severe anaemia requiring transfusion. The key to control thalassaemia is to identify the population at risk and to offer genetic counselling and providing options to prevent the disease in the family. Routine full blood count and Hb electrophoresis can reliably identify most if not all β-thalassaemia and HbE carriers, and majority of *α*-thalassaemia carriers. Detection of mutation causing thalassaemia enables accurate identification of carrier at risk and allows early prenatal diagnosis to be carried out. This is especially important in identifying at risk individuals who are both α and β-thalassaemia carriers. However, molecular diagnosis is expensive and effective screening requires prior knowledge of the spectrum of mutations in the population. Planning of an effective screening strategy depends on available resources and prevalence of the disease. In many countries, screening is carried out at pregnancy when option for prevention is possible. While screening and prevention may be effective to reduce number of major type of thalassaemia, more resources for optimal treatment can then be made available to those who are seriously affected by the disease.

Keywords: Thalassaemia carrier; anaemia; screening; mutation; counselling

Cite this abstract as: Law HY. Problem in the prevention and control of thalassaemia in Asia. Ann Transl Med 2015;3(S2):AB023. doi: 10.3978/j.issn.2305-5839.2015.AB023

AB024. Chromosome microarray analysis (CMA) for the diagnosis of children with developmental delay and multiple congenital anomalies in Singapore

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Abstract: Chromosome microarray analysis (CMA) is a sensitive method to identify submicroscopic changes too small to be detected by conventional karyotyping. Due to its high-sensitivity in identifying regions with structural variation and hence the genes involved, it is recommended to be the first-tier genetic test for children with intellectual disabilities, development delay or multiple congenital anomalies, and is routinely available in USA and many countries in Europe. Our lab has started offering this as a clinical test based on the research experience on screening >400 children with developmental delay and multiple congenital anomalies since February 2014. To date, 271 patients have been screened using the Agilent 4×180K CGH + SNP array. Copy number variants (CNVs) ranging in size

from 10 kb to 154 Mb were found in 109 patients (40%). Pathogenic and likely pathogenic CNVs were found in 55 (20%). These included 45 with deletions, 8 with duplications and 2 patients with both deletion and duplication. Recurrent microdeletion and microduplication syndromes including the Angelman/Prader-Willi syndrome [5], 1 p36 microdeletion [3], Williams syndrome [2], 22q11.2 distal deletion syndrome [2], 16p13.3 microdeletion syndrome [2], Cat Eye syndrome, Cri du Chat syndrome, Miller Decker syndrome, 3q29 microdeletion, 15q24 microdeletion, and 1q43q44 syndrome were among the variants detected in our patients. CNVs of uncertain clinical significance were detected in 54 (20%) individuals: 32 were duplications, 18 were deletions and one with both deletion and duplication. However, due to the high cost of the test, parental testing was not performed and hence, significance of these variants could not be established conclusively. In conclusion, CMA is a powerful tool in identifying pathogenic chromosomal copy number alternations. However, due to the high cost of the test, parental testing for the cases where variants of uncertain significant are found is often not possible. CMA is useful in identifying pathogenic structural rearrangement. More data are necessary to be collected to enhance interpretation of the results.

Keywords: Chromosome microarray; developmental delay; multiple congenital anomalies; microdeletion; microduplication

Cite this abstract as: Law HY, Brett M, Tan EC, Yong MH, Lai A. Chromosome microarray analysis (CMA) for the diagnosis of children with developmental delay and multiple congenital anomalies in Singapore. Ann Transl Med 2015;3(S2):AB024. doi: 10.3978/j.issn.2305-5839.2015.AB024

AB025. Genome technology applications for perinatal diagnosis and fetal medicine in China

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Abstract: China has nearly one million new born errors with inherited diseases or chromosomal abnormalities in its 20 million new born populations each year. It is a big challenge to avoid new born error with applying various new technologies in prenatal diagnosis. Rapid progress of genome technology in recent years has made it possible to diagnose subtle genetic abnormalities in a clinical setting on routine basis. These technology advances allowed for detailed genotype-phenotype correlations and the identification of the genetic basis of many congenital anomalies. Besides gene polymorphisms of folic acid detection, mutation analysis of thalassemia, classical chromosome analysis, and fluorescence in situ hybridization, etc. classical genetic testings, many new technologies were introduced to the clinic of prenatal and fetal medicine, more advanced technology such as CGH microarray analysis, exome and whole-genome sequencing (WGS) on pre- and postnatal samples of cell-free DNA has revolutionized the field of prenatal diagnosis. Especially, next generation sequencing (NGS) has rapidly adapted to prenatal diagnosis, non-invasive prenatal test (NIPT) was successfully applied into clinic with about half million of pregnant women received NIPT diagnosis in the analysis of aneuploidies and high risk for trisomy 13, 18 and 21 with successful detection rate of great than 99% so far. Currently Chinese central government already specified more than 100 hospitals to utilizing NIPT for high risk planeload fetal screening. Incorporation of these technologies in perinatal and fetal medicine has demonstrated that increased power of detecting diseases in prenatal diagnosis. It is clear that new genome technologies such as CGH array, whole-genome analysis and NIPT by NGS etc., offered more possibilities to identify various inherited diseases, genetic mutation or metabolism errors in new born population and provide more solutions to disease prevent and treatment in the

field.

Keywords: Next generation sequencing (NGS); non-invasive prenatal test; whole-genome sequencing (WGS); new born; prenatal diagnosis

Cite this abstract as: Yang H. Genome technology applications for perinatal diagnosis and fetal medicine in China. Ann Transl Med 2015;3(S2):AB025. doi: 10.3978/j.issn.2305-5839.2015.AB025

AB026. SCN1A mutational analysis in 20 Vietnamese children with Dravet syndrome

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Background: Dravet syndrome is one of the most catastrophic types of epilepsy in infants. It is found that 70-80% of cases of Dravet syndrome are caused by mutations in SCN1A, the gene encoding alpha-1 subunit of the sodium channel. Mutations of the SCN1A gene have an autosomal dominant inheritance pattern. To date, over 1,000 SCN1A mutations have been reported all over the world, however, no SCN1A mutation studies have been performed in the Vietnamese population, and genetic characteristics of Vietnamese Dravet patients are not yet clear. In this study, we analyzed SCN1A gene in 20 Vietnamese patients with clinical features of Dravet syndrome at Children's Hospital 2, Ho Chi Minh City, Vietnam.

Methods: Direct sequencing and multiple ligation-dependent probe amplification (MLPA) were performed to screen the entire coding regions as well as exon-intron boundaries of the gene.

Results: Fourteen mutations (14/20; 70%) were identified including 13 point mutations detected by PCR-Sequencing and 1 large deletion mutation spanning nearly whole exon 7 detected by MLPA. Five mutations were classified as truncations (2 frameshift and 3 nonsense mutations) and 9 were classified as missense mutations. There were 7 mutations were localized at pore-forming loop (connecting S5-S6); 5 mutations were localized at cytoplasmic loops (connecting 2 nearby homologous domains), 1 mutations were localized at transmembrane segments, and 1 mutation in a intronic region. Nine of these 14 SCN1A mutations were novel and parental DNA analysis for the identified mutations in 11 available cases show that all of the mutations were *de nono*. Besides well-known genotype–phenotype correlations, our study results strongly suggests the existence of modifying factors.

Conclusions: The proportion of SCN1A mutations among Vietnamese Dravet patients in this study appeared to be consistent with other populations (70%). Our study also expands the spectrum of SCN1A mutations and confirms the current understanding of genotype–phenotype correlations.

Keywords: Dravet syndrome; SCN1A; Vietnamese

Cite this abstract as: Do TT, Le TM, Bui CB, Vu DM. SCN1A mutational analysis in 20 Vietnamese children with Dravet syndrome. Ann Transl Med 2015;3(S2):AB026. doi: 10.3978/j.issn.2305-5839.2015.AB026

AB027. Developing capacity for variant data sharing in low and middle income countries: HVP's Global Globin 2020 Challenge

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Abstract: The hemoglobinopathies, collectively, are cause for significant morbidity and mortality. Children are the most severely affected. Despite much of the genetics and biology of hemoglobinopathies being known for a long time, and being used successfully in some countries to systematically reduce burden of disease, many low and medium income countries remain practically untouched by recent developments in human genomics involving the

systematic collection and sharing of variation data to fighting hemoglobinopathies (notably thalassaemias and sickle cell disease, but also G6PD). Commitment to systematic variant data collection is increasing, but this is occurring mostly in high-income countries where much of the diagnosis and testing takes place. There is a risk that countries with the highest burden of these diseases are being left behind in a form of "genomic divide". Capacity to generate quality data on variants, to store this information so that it can be shared internationally, needs to be built in these countries. Tackling hemoglobinopathies is an ideal entry point for these countries to develop the necessary infrastructure and expertise that can expand into other areas of health. This genomic capacity will enable building: (I) the genetic evidence base for better management of delivery of local treatment, care and eventually even cure; (II) a foundation for genomic medicine by working with national, regional and local health care professionals to raise public awareness of the genetic basis of hemoglobinopathies. Global Globin 2020 Challenge has been initiated with two goals: (I) to see growth in the quality and quantity of curated inputs into internationally recognized genetic databases from low- and middle-income countries participating in the project, and to harmonize the sharing of all relevant variant data between countries in accordance with international best practice that integrates all the relevant ethical and regulatory frameworks and policies required to protect patients at the same time that the biotechnical procedures are developed; (II) to ensure that the storage, curation and sharing of the relevant DNA variation information is sustainable in the medium and longer term by expanding and strengthening the international network of professionals, including curators, researchers, clinicians, bioinformaticians, counsellors, patient groups and policymakers. Pursuit of these goals will raise the profile of genomic medicine in low and middle income countries in national, regional and international research organizations. It will also develop the capability of professionals required for diagnosing, treating and counseling carriers in low and middle income countries thus giving them a greater voice and profile among genomic researchers globally so they can actively participate in regional and international partnerships related to genomic research. Initially the GG2020 Challenge will focus on a group of countries that have already formed groups of the relevant professionals including: Belgium, China, Cyprus, Egypt, France, Malaysia, Mexico, Mozambique, Nigeria, South Africa, Venezuela, Vietnam, Portugal, and The Netherlands. Other countries are ready to be included as the project expands. HVP will utilize its relationship with both UNESCO and WHO to ensure that the necessary international standards and procedures are developed in a consultative and harmonized manner.

Keywords: Hemoglobinopathies; genomic medicine; burden of disease

Cite this abstract as: Robinson HM. Developing capacity for variant data sharing in low and middle income countries: HVP's Global Globin 2020 Challenge. Ann Transl Med 2015;3(S2):AB027. doi: 10.3978/j.issn.2305-5839.2015.AB027

AB028. Identifying the functional role of *VEZT* gene for endometriosis risk

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Abstract: Endometriosis is a common, chronic gynaecological disease characterised by pelvic pain and subfertility. It is a complex genetic disease affecting of 6-10% women of reproductive age and up to 50% of women with infertility. Evidence from genomewide association (GWA) imputed and meta-analysis studies in women with and without endometriosis identified association on chromosome 12q22, located close to the putative candidate gene *VEZT*. Fine-mapping this region in our 1,029 Australian cases and 958 controls confirmed strong evidence of association for non-coding variants in the 12q22 GWA region with the best imputed SNP rs4762347 in the 3'UTR of *VEZT* (P=0.036; 95% CI, 1.01-1.38; OR =1.18) and four top imputed SNPs in high linkage disequilibrium (LD) with rs4762347. Bioinformatic analysis for potential functional roles for these SNPs using ENCODE data show rs4762347 is highly conserved and alters a regulatory motif for the transcription factor Nkx3. We conducted an initial

experiment to evaluate gene expression of *VEZT* in 36 samples of endometrial tissue from endometriosis cases and controls using high-throughput gene expression RT-qPCR on a microfluidic dynamic array IFC 48x48 chip (Fluidigm Biomark). The results showed that *VEZT* was expressed in endometrial samples from cases and controls. There was a trend for increased expression levels of *VEZT* mRNAs in endometriosis cases compared to controls as well as during the secretory phase compared to proliferative phase of menstrual cycle, although these differences were not statistically significant. The top imputed SNP rs4762347 was chosen to test for its allelic effects on *VEZT* expression. There was suggestive evidence for differences in *VEZT* mRNA expression observed in allelic groups of rs4762347. *VEZT* mRNA expression decreased for transcripts in carriers of minor C allele (CC and CT) of rs4762347 compared to non carriers (TT) ($P<0.01$), but the differences were no longer significant after corrections for multiple testing, hence further gene expression studies should be conducted in a larger sample size.

Keywords: Endometriosis; chronic gynaecological diseas; *VEZT* gene

Cite this abstract as: Luong HT, Painter JN, Sapkota Y, Nyholt DR, Rogers PA, Montgomery GW. Identifying the functional role of *VEZT* gene for endometriosis risk. Ann Transl Med 2015;3(S2):AB028. doi: 10.3978/j.issn.2305-5839.2015.AB028

AB029. Next generation sequencing analysis in hereditary muscle diseases

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Abstract: Still many cases with probable hereditary myopathy are genetically undiagnosed for two major reasons: (I) muscle genes are often big in size and thus it is difficult to at least routinely sequence such genes even though mutations in those genes have been known to cause muscle diseases; and (II) most cases are sporadic without parental consanguinity, which makes us difficult to do linkage study to identify new causative genes. However, the advent of next-generation sequencers is changing the diagnostic and research scenes in myology just as in many other fields. We have set up target resequencing panels that basically cover all known causative genes for hereditary muscle diseases (as of early 2014). We started providing genetic analysis in September 2014 using this system to the cases whose pathological analysis was done at NCNP. So far we have reached diagnosis in 27% of those cases. To identify new causative genes, we applied whole exome sequencing (WES) analysis. In the last 3 years, we have performed more than 750 exome analyses but we found causative mutations in only 11% of cases. This low rate appears to be at least partly due to the fact that Japanese variation database is not well established, in addition to the fact that currently available WES analysis kits do not fully, albeit mostly, cover all exonic regions. In my talk, I will demonstrate some of our analysis data, including the identification of *ORAI1* as a causative gene for tubular aggregate myopathy, and discuss current status and yet-to-be-solved issues of next-generation sequencing in myology field.

Keywords: Next generation sequencing; hereditary muscle diseases; whole exome sequencing (WES)

Cite this abstract as: Nishino I. Next generation sequencing analysis in hereditary muscle diseases. Ann Transl Med 2015;3(S2):AB029. doi: 10.3978/j.issn.2305-5839.2015.AB029

AB030. The evolving role of genetic counseling

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Abstract: Master level genetic counseling training programs have existed in the United States of America

(USA) since 1969. There are now 31 programs to train genetic counselors in the USA, 4 programs in Canada, and 2 programs in Australia. These programs generally train non-medical health professionals (individuals with a BSc or MSc). There are 25 additional programs worldwide, training a combination of physicians, nurses, and non-medical health professionals. Students in the USA, Canada and Australia are required take a certification examination and licensure privileges dependent on the rules of a specific state or providence. The roles of genetic counselors have evolved with genetic medicine. Initial work of genetic counselors involved predominately direct patient care in the prenatal, pediatric and adult genetic clinics. When a hereditary component to cancer was recognized, there was an increasing need for genetic counselors for the consultation and results interpretation in cancer care. With the completion of the Human Genome Project in 2003 and the decreasing cost of next generation sequencing, there has been a plethora of molecular genetic studies that have been available to the genetics provider. Many genetic counselors have migrated to the laboratory setting to assist providers with the selection and interpretation of laboratory studies. With this great expansion of genomic testing available, there has been a concomitant identification of genomic alterations of uncertain significance that require extensive evaluation to determine clinical significance. Genetic counselors have been essential for this work in both the research and clinical setting. Genetic counselors have been recognized as one of the most needed health care professionals in the USA in the next decade. The role of the genetic counselors in emerging countries is complex with the simultaneous need for direct patient care and the support of laboratory services. The development of programs in emerging countries will need to train students with a broad spectrum of skills to include both direct clinical care and to understanding the current complexities of genomic testing. Anticipating social and emotional needs of multiple populations within a country will be an ongoing challenge. Commitment of trained professionals from established clinical genetic programs can facilitate educational support for programs in emerging countries. With this Asia Pacific Meeting, there was a preconference to support genetic counseling in the region. The results of the questionnaire presented to the 70 attendants of this pre-conference will be presented with projections of future needs for genetic counseling training in this region.

Keywords: Genetic counseling; training programs; next generation sequencing

Cite this abstract as: Leppig K, Sternen D, Thompson J, Laurino M. The evolving role of genetic counseling. Ann Transl Med 2015;3(S2):AB030. doi: 10.3978/j.issn.2305-5839.2015.AB030

AB031. Spectrum of IEMs in Vietnamese patients: data from 10 years of selected screening and diagnosis

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Background and objective: Vietnam is the easternmost country on the Indochina Peninsula in Southeast Asia. With an estimated 90 million inhabitants as of 2013, it is the world's 13th-most-populous country, and the eighth-most-populous Asian country. Congenital anomalies accounted about 22% of causes of deaths in children under-5 [2010]. The first service for IEMs was set up at the Northern referral center of Pediatrics-National Hospital of Pediatrics, Hanoi (NHP) in 2004 officially. The NHP in Hanoi provides services to the population of North Vietnam (~30 million people). The aim of this report is to highlight disease spectrum of tandem mass spectrometry (MS/MS) target disease in Vietnam.

Methods: A total of 2,405 high-risk cases with IEMs were studied at NHP during 10 years [2005-2014]. Dry blood and urine samples were analyzed using MS/MS (amino acid & acylcarnitine analysis) & GC/MS (organic acid anaysis) at Shimane University, Japan from 2005. Organic acids analysis for fresh urine samples was performed at NHP using GC/MS at NHP from 2010. Amino acid analysis for plasma samples were performed using HPLC

at NHP from 2012.

Results: Oganic acidemia (OAs), amino acid disorders (AAs), urea cycle disorders (UCDs) and fatty acid oxidation disorders (FAOD) were identified in 235/2, 405 cases (9.8%). A total of 118/235 patients (50.2%) were OAs with 12 different disorders: BKT (33 cases), PPA (21 cases), 5-oxoprolinuria (19 cases), MMA (14 cases), Glutaricaciduria type II (GA II) (11 cases), 3-methylglutaconic aciduria (4 cases), isovaleric academia (3 cases), multiple carboxylase deficiency (MCD) (2 cases), 3-methylcrotonylCoA carboxylase deficiency (2 cases). A total of 42/235 patients (17.9%) were amino acid disorders including 35 cases with MSUD, 7 cases with PKU and 1 case with tyrosinemia type 1. The 36/235 patients (15.3%) were UCDs including OTC deficiency (13 cases), citrulinemia type 1 (1 case) and argininosuccinic aciduria (1 case). 39/235 patients (16.6%) were FAOD including SCAD (3 cases), MCAD (3 cases), VLCAD (8 cases), LCAD (2 cases), CPT 2 (8 cases), CPT 1 (1 case) and primary carnitine deficiency (14 cases). Mortality rate was reduced from 50% in 2005 to 9% in 2014.

Conclusions: Treatable conditions of IEMs were most common in Vietnamese patient identified using MS/MS. Expending newborn screening using MS/MS should be introduced to reduce mortality in Vietnamese children.

Keywords: Tandem mass spectrometry (MS/MS); inborn errors of metabolism (IEMs); newborn screening; Vietnam

Cite this abstract as: Dung VC, Khánh NN, Mai NC, Hương BT, Thao BP, Ngoc CT, Hoan NT, Hai LT, Dung KT, Fukao T, Yamaguchi S. Spectrum of IEMs in Vietnamese patients: data from 10 years of selected screening and diagnosis. Ann Transl Med 2015;3(S2):AB031. doi: 10.3978/j.issn.2305-5839.2015.AB031

AB032. Mutation spectrum in the dystrophin gene disclosed by multiplex ligation-dependent probe amplification in 181 Vietnamese Duchenne/Becker muscular dystrophy patients

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Abstract: Duchenne/Becker muscular dystrophy (DMD/BMD) the most common X-linked muscular dystrophy is caused by mutation in dystrophin gene. Deletion and duplication in the dystrophin gene account for 60-70% of mutation. Multiplex ligation-dependent probe amplification (MLPA) is the most powerful and convenient method to identify exon deletions or duplications in the dystrophin gene because of its overall gene coverage. The present investigation was designed to detect mutation in the dystrophin gene in 181 unrelated Vietnamese Becker/Duchenne patients using MLPA analysis. Among the 181 cases, deletions and duplications encompassing one or more exons were identified in 105 (58%) or 12 (6.6%) cases, respectively. Deletions were found to cluster in the proximal (14.3%) and central hotspot regions (72.4%); 14% were observed to have gross deletions and 1.2% had deletion out of hotspot regions (exon 61-67). The deletion patterns were categorized into 48 patterns. Deletion of exon 48-50 or 45-50 where the most common pattern was deletion of exon 48-50, which was found in 11 cases (10%). Single-exon deletion was found in 14 cases (13%) by MLPA. Further examination disclosed that one of them was not an exon deletion but a single-nucleotide change (c.2227C > T) leading to a nonsense mutation. Outliers from the reading frame rule were 11 DMD (10.4%). Remarkably, 25 and 14 cases were found treatable by exon 51 and 53 skipping, respectively. From these findings, the largest mutation database of Vietnam dystrophinopathy was established.

Keywords: Duchenne/Becker muscular dystrophy (DMD/BMD); dystrophin; multiplex ligation-dependent probe amplification (MLPA); deletion; duplication

Cite this abstract as: Tran VK, Do NH, Tran TH, Ta MH, Tuan-Pham LA, Chi DV, Khanh NN, Ta VT, Matsuo M. Mutation spectrum in the dystrophin gene disclosed by multiplex ligation-dependent probe amplification in 181 Vietnamese Duchenne/Becker muscular dystrophy patients. Ann Transl Med 2015;3(S2):AB032. doi: 10.3978/j.issn.2305-5839.2015.AB032

AB033. Preimplantation genetic diagnosis of spinal muscular atrophy in Vietnam

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Objective: Spinal muscular atrophy (SMA) is a severe neurodegenerative autosomal recessive disorder. Most of patients are caused by the homozygous absence of exon 7 of the telomeric copy of the *SMN* gene (SMNt) on chromosome 5. Setting up a molecular diagnostic protocol for detecting exon 7 gen *SMNT* homozygous deletion in single cell is basic to preimplantation genetic diagnosis of spinal muscular atrophy.

Methods: This study was carried out on 17 patients and their parents. Firstly, lymphocytes of patients and their parents were isolated from fresh blood by ficoll. Taking a lymphocyte on stereoscopic microscope, lysing the cell, amplifying whole genome, then amplifying exon 7 of *SMNT* gene by using a polymerase chain reaction, followed by HinfI restriction digest enzyme of the PCR enabling the important *SMNT* gene to be distinguished from the centromic *SMN* gene (*SMN*c) which has no clinical phenotype to detect mutation. Electrophoresis PCR products after digesting by restriction enzyme and analysis. Besides, the minisequencing technique has also been used to detect the absence of exon 7 of *SMNT* gene based on the difference of one nucleotide at 214-position in exon 7 (C-*SMNT*, T-*SMN*c). Secondly, the application of the protocol was set up on one lymphocyte to preimplantation genetic diagnosis of spinal muscular atrophy on biopsied blastomeres.

Results: Two different protocols which were PCR-RFLP and minisequencing, were set up on 200 lymphocytes from 17 patients and their parents to screen the homozygous deletion in exon 7 *SMNT* gene with the PCR efficiency in 96%. The results were similar with the gene diagnosed from fresh blood. The methods were also efficient, providing interpretable result in 96.55% (28/29) of the blastomeres tested. Three couples were treated using this method. Three normal embryos were transfer which resulted in one clinical pregnancy.

Conclusions: We have successfully applied the technique of PCR-RFLP and minisequencing for the preimplantation genetic diagnosis of spinal muscular atrophy.

Keywords: Spinal muscular atrophy (SMA); *SMN* gene; preimplantation genetic diagnosis

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AB034. Hemoglobinopathies in China and SEA: rapid targeted deep sequencing for molecular screening and clinical genotyping in subjects with hemoglobinopathies

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Abstract: Hemoglobin disorder is one of the most common birth defects in the world. α and β thalassemia are prevalent in tropical and subtropical regions. The imbalance of α and β hemoglobin is the pathological mechanism and the base of clinical classification of α and β thalassemia. In Southern

China, 17 gross deletions account for 70-80%, and 13 point mutations, for 20-30% of α-thalassemia. Fifty-two point mutations account for 97% for β-thalassemia. Six deletions cause δβ-thalassemia or HPFH. Fetal hemoglobin levels are regulated by multiple modifier genes which influences the severity of thalassemia. NGS technology helps significantly the effectiveness of molecular diagnosis and better understanding of the diseases.

Keywords: Hemoglobin disorders; hemoglobinopathies; molecular screening; genotype

Cite this abstract as: Qi M. Hemoglobinopathies in China and SEA: rapid targeted deep sequencing for molecular screening and clinical genotyping in subjects with hemoglobinopathies. Ann Transl Med 2015;3(S2):AB034. doi: 10.3978/j.issn.2305-5839.2015.AB034

AB035. Thalassemia in Vietnam

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Abstract: Thalassemia is a common inherited hemoglobin disorder in Vietnam. The alpha thalassemia, beta thalassemia, and HbE are popular in Vietnam but its variance depends on ethnics. The research for frequency of some ethnics almost in electrophoresis includes: Kinh (beta thalassemia carrier 1.49%, HbE 1.24%), Muong (beta thalassemia carrier 10.7%, HbE 11.7%), Tay (beta thalassemia carrier 11%, HbE 1%). In the recent years, we have conducted researches on thalassemia gene in the Northern and Southern areas of Vietnam. The two researches on beta thalassemia conducted at National Hospital of Pediatrics were Cd17 (33.8%), Cd41/42 (29.4%) following are HbE (19.1%), Cd 71/72 (7.3%), -28 (5.9%), IVS 2-625 (1.5%), IVS 1-5 (1.5%) , IVS 1-1 (1.5%). In Vietnam, we have a thalassemia centre at the National Institute of Hematology and Blood Transfusion and several outpatient clinics at National Hospital of Pediatrics, Children No. 1 Hospital, Blood Transfusion and Hematology Hospital Ho Chi Minh city, Central Hue Hospital. In provincial hospitals, we have transfusion service but very variance. That the number of patients with thalassemia requires regular blood transfusion has been increasing results in big shortage of blood supply. At Department of Clinical Hematology-NHP, we provide patients with screening for HIV, HCV and HBV in every 6 months. Patients were done antibody screening test. Deferoxamine, deferiprone and deferasirox are currently used but in short supply. We are facing the difficulty that almost hospitals in Vietnam lack the drug which is unique for each type of chelation. We have to apply ferritin level to follow the chelation effective and MRI to measure iron overload in patients' liver and heart. We are only able to provide SCT for the modest number of patients with thalassemia. In almost cases, we used sibling donor in SCT for patients with thalassemia. Regarding prevention service, we offer genetic counseling and prenatal diagnosis at three hospitals. We organized prevention program in Hoa Binh province on national budget. The most important future planning is expanding prevention program in provinces with high prevalence and after that in the all country. Our future plan is to set up more thalassemia centres in provincial and central hospitals where overload with patient's demand. We also launch for appeal for blood and iron chelation to patients with thalassemia. In near future, use of haplo SCT to treatment thalassemia patients will be more.

Keywords: Thalassemia; gene; treatment; planning; prevalence

Cite this abstract as: Nguyen HN. Thalassemia in Vietnam. Ann Transl Med 2015;3(S2):AB035. doi: 10.3978/j.issn.2305-5839.2015.AB035

AB036. Analysis of human mitochondrial genome mutations of Vietnamese patients tentatively diagnosed with encephalomyopathy

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Abstract: Human mitochondrial genome consists of 16,569 bp, and replicates independently from the nuclear genome. Mutations in mitochondrial genome are usually causative factors of various metabolic disorders, especially those of encephalomyopathy. DNA analysis is the most reliable method for detection of mitochondrial genome mutations, and accordingly an excellent diagnostic tool for mitochondrial mutation-related diseases. In this study, 19 different mitochondrial genome mutations including A3243G, A3251G, T3271C and T3291C (MELAS); A8344G, T8356C and G8363A (MERRF); G3460A, G11778A and T14484C (LHON); T8993G/C and T9176G (Leigh); A1555G (deafness) and A4225G, G4298A, T10010C, T14727C, T14728C, T14709C (encephalomyopathy in general) were analyzed using PCR-RFLP in combination with DNA sequencing. In addition, a real-time PCR method using locked nucleic acid (LNA) Taqman probe was set up for heteroplasmy determination. Screening of 283 tentatively diagnosed encephalomyopathy patients revealed 7 cases of A3243G, 1 case of G11778A, 1 case of A1555G, 1 case of A4225G, 1 case G4298A, and 1 case of 6 bp (ACTCCT/CTCCTA) deletion. Using the LNA Taqman probe real-time PCR, the heteroplasmy of some point mutations was determined and the results support a potential relationship between heteroplasmy level and severity of the disease.

Keywords: Mitochondrial genome mutations; encephalomyopathy; PCR-RFLP; real-time PCR; heteroplasmy

Cite this abstract as: Nghia PT, Thai TH, Hue TT, Minh NV, Khanh PB, Hiep TD, Anh TK, Loan NT, Van NT, Anh PV, Hung CV, Anh LN. Analysis of human mitochondrial genome mutations of Vietnamese patients tentatively diagnosed with encephalomyopathy. Ann Transl Med 2015;3(S2):AB036. doi: 10.3978/j.issn.2305-5839.2015.AB036

AB037. Inhibition of extracellular signal-regulated kinase pathways by U0126 enhances osteogenic differentiation of bone marrow-derived multipotent mesenchymal stem cells via cross-talk with p38 pathway

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Background and objective: Bone marrow-derived multipotent mesenchymal stem cells (BM-MSCs) can differentiate into osteoblasts via signal transduction pathways that cause nuclear responses. The extracellular signal-regulated kinase (ERK) and p38 signaling pathways control a variety of important cellular events, especially, cell proliferation, differentiation and apoptosis. The aim of this study was to elucidate the role of ERK and p38 signaling pathways involved in the osteogenic differentiation of BM-MSCs.
Methods: BM-MSCs were treated or non-treated with osteogenic differentiation medium (ODM) and specific inhibitors of ERK and p38. Cell proliferation, alkaline phosphatase (ALP) activity, calcium content, expression levels of osteogenic markers and mineralization were measured to assess osteogenic differentiation of BM-MSCs.
Results: ALP activity, calcium content, expression of ALP, osteopontin and osteocalcin, and calcium deposition were significantly enhanced by blocking the ERK pathway with U0126, but they were strongly down-regulated by inhibiting the p38 pathway with SB203580, indicating that U0126

enhanced osteogenic differentiation of BM-MSCs, whereas SB203580 suppressed osteogenic differentiation of BM-MSCs. Interestingly, Western Blot and immunofluorescent analysis showed that treatment with the p38 inhibitor resulted in an increase in the activated form of ERK, whereas treatment with the ERK inhibitor resulted in an increase in the activated form of p38.

Conclusions: These findings suggest that the ERK pathway is repressors of osteogenesis, whereas p38 pathway is an enhancer of osteogenesis of BM-MSCs via the cross-talk between ERK and p38 signaling pathways in BM-MSCs. Inhibition of ERK signaling pathway by U0126 may be useful for promoting osteogenesis during bone formation.

Keywords: Osteogenic differentiation; extracellular signal-regulated kinase; inhibitors; U0126

Cite this abstract as: Doan TK, Park KS, Kim HK, Park DS, Kim JH, Yoon TR. Inhibition of extracellular signal-regulated kinase pathways by U0126 enhances osteogenic differentiation of bone marrow-derived multipotent mesenchymal stem cells via cross-talk with p38 pathway. Ann Transl Med 2015;3(S2):AB037. doi: 10.3978/j.issn.2305-5839.2015.AB037

AB038. NGS-based diagnostics for genetic disorders—promises and pitfalls

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Abstract: Genetic testing forms an integral part of clinical management of heritable genetic disorders as it provides options for molecular diagnosis, carrier identification, prenatal diagnosis, etc. The impetus for the development of diagnostic assays for genetic testing of a disease is often influenced by how much is known about its molecular basis, its incidence among the local population and the clinical demand for testing. Subsequent laboratory implementation of a research test for clinical diagnostic testing requires demonstration of clinical utility and validity. Next-generation sequencing (NGS) approaches are increasingly being adopted for clinical diagnostics as the costs becomes increasingly affordable and its utility to resolve diagnostic odysseys has brought resolutions to many families. Validation of such NGS assays is complex but follows similar established guidelines as for traditional genetic tests. There is high analytical sensitivity for most of these assays although the true clinical sensitivity may remain unknown for some disorders. Targeted analysis using NGS is now available for many gene panels, e.g., involving myopathies, cilipathies, cardiomyopathies, etc. as well as for single gene disorders such as Wilson disease, retinoblastoma, RYR1-related diseases, etc. As the costs for sequencing whole exomes and genomes progressively drop further, it is anticipated that these technologies will also begin to transit into the clinical realm. This talk discusses the impact and challenges of NGS in clinical testing and the diagnostic dilemmas in test interpretations for these lab tests.

Keywords: Next-generation sequencing (NGS); genetic testing; heritable genetic disorders

Cite this abstract as: Lai PS. NGS-based diagnostics for genetic disorders—promises and pitfalls. Ann Transl Med 2015;3(S2):AB038. doi: 10.3978/j.issn.2305-5839.2015.AB038

AB039. Thailand national plan for prevention and care of birth defects and disabilities

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Abstract: Thailand Task Force on Birth Defects and Disabilities was first organized in 2008. From 2011-2014, Birth Defects Association (Thailand) received funding from ThaiHealth Promotion Foundation and the outcomes are: (I) pilot program on Birth Defects Registry (BDR), both on case record form (CRF) and later developed into BDR Online; in 22 out of 77 provinces; (II) eleven health districts model in 11 provinces focus on holistic approach on prevention and care of 5 chosen disorders-down syndrome

(DS), neural tube defects (NTD), cleft lip/palate (CL/P), limb anomalies (LA) and duchenne muscular dystrophy (DMD); (III) manuals and care map for five chosen birth defects for provincial and community hospitals completed; (IV) memorandum of understanding (MOU) was signed in 2012 between four ministries (Health, Education, Social Welfare and Human Security) and interior two organizations (National Health Security Office and ThaiHealth Promotion Foundation) including Birth Defects Association (Thailand); (V) national network was developed consisting of expert paediatricians and obstetricians from eight medical institutions including Queen Sirikit National Children Center in Bangkok; (VI) Country Action Plan' initiated by Department of Medical Services, Ministry of Public Health; (VII) strategic map, master plan and care map developed; (VIII) systematic approach including working with policymakers and stakeholders at country, provincial and community level. From 2015-2017, Birth Defects Association (Thailand) continues to receive fundings from ThaiHealth Promotion Foundation focusing on three objectives: (I) moving forward towards national policy; (II) strengthening the holistic approach in 26 health districts model including one in Bangkok metropolitan area; (III) raising awareness in prevention of birth defects using folate supplementation in women of child bearing age with emphasis on girls in secondary schools.

Keywords: Thailand national plan; birth defects; disabilities

Cite this abstract as: Wasant P. Thailand national plan for prevention and care of birth defects and disabilities. Ann Transl Med 2015;3(S2):AB039. doi: 10.3978/j.issn.2305-5839.2015.AB039

AB040. Biomarkers for Autism: where are we now and what will the future bring?

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Abstract: Autism spectrum disorders (ASD) is a complex disorder in which both genes and environmental factors play important roles. ASD is characterized by a high concordance rate in monozygotic twins pointing to high heritability for this disorder. Notably, the sibling recurrence risk ratio (λs) is 22 for autism. Despite the high heritability clinical symptoms are markedly variable and multiple genetic factors including Mendelian mutations, copy number variations and common polymorphisms all contribute to the genetic load towards passing the clinical threshold to ASD. Indeed, multiple genetic loci have been with various degrees of certainty associated with this disorder. In addition to genetic factors environmental variables and epigenetic signatures are also important modifiers of susceptibility to ASD. For example, studies show that DNA methylation differences can occur in many loci across the genome of ASD subjects. Among biomarkers that have been examined in ASD are metabolic, oxidative stress, mitochondrial dysfunction, methylation, immune dysregulation, amino acids and neuropeptides, peripheral blood gene expression, digit ratio and dysbiosis (gut inflammation) among others. In my talk today after giving an overview (see above) of biomarkers in ASD, I will discuss my own group's approach that includes both gene markers (serotonin transporter, *OXTR* and *AVPR1a*) as well as an innovative endophenotype biomarker approach to better understanding ASD. In a recent study we found that 2D:4D digit ratio (also a proposed marker for ASD) relationship to cognitive empathy (a core deficit in ASD) is dependent in normal subjects on an *OXTR* SNP linked to ASD in some studies. We have also shown that *OXTR* and *AVPR1a* gene polymorphisms are associated in normal subjects with cognitive and emotional empathy respectively. Notably, common polymorphisms in both genes are also associated with ASD. Similarly, the *DRD4* gene shows a gender-sensitive association with cognitive empathy. In a parallel talk at this meeting I discuss in some detail how gene expression studies in lymphoblastoid cells may be a marker for ASD as well as personality traits. I will also discuss a finding with a colleague identifying a functional rare variant in autism using genome-wide screen for monoallelic expression. Altogether, our studies have revealed genetic and other biomarkers that jointly contribute to the social cognitive deficits that represent core diagnostic features of ASD. The biomarkers are not specific to ASD but nevertheless shed considerable light on the underlying common polymorphisms and multiple etiologies of this highly heritable disorder characterized by intellectual and social dysfunctions. One promising area of future studies of

biomarkers in ASD is prenatal diagnosis using ultrasound.

Keywords: Autism spectrum disorders (ASD); biomarkers; serotonin transporter; *OXTR*; *AVPR1a*

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AB041. Genetic diversity of organic and fatty acid disorders detectable in expanded newborn screening in Asian countries

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Abstract: Recently, the increasing number of children with organic acidemias (OAs) and fatty acid oxidation defects (FAODs) has been detected with development of diagnostic tools, like GC/MS or MS/MS, for inborn metabolic disease (IMD). We have performed collaboration study with some Asian countries, and have noticed the diversity of disease contribution and genetic aspects. Diversity of disease distribution: metabolic screening of children at high risk using GC/MS and NS/MS has been performed in collaboration with several Asian countries. In India, Vietnam, and China, as well as Japan, methylmalonic acidemia (MMA) was most common, followed by urea cycle disorder (UCD), propionic academia (PPA). In Vietnam and India, 3-ketothiolase deficiency (BKTD), maple syrup urine disease (MSUD), and oxoprolinuria are also common, although they are extremely rare in Japan. In China, frequency of MMA seemed to be high, in particular combined MMA and homocystinuria many of which are B12 responsible. Genetic diversity: (I) MMA: in China, 42% of MMA are combined type of MMA and HCY due to CblC defect. 609G>A in *MMACHC* gene is a common mutation, covering 55%; (II) BKTD: in Vietnamese patients, c.662C>G in *T2* gene is common, covering 72%; (III) PPA: in Japanese, Y435C mutation in *PCCB* gene is a common mutation in the mild form of PPA, at prevalence of 1 in 86 alleles in Japanese population; (IV) MCAD deficiency: common mutation 985A>C which covers about 90% of alleles among Caucasian is famous. A mutation study of Japanese cases, revealed a common mutation, c.449delCTGA, covering about 45% of the alleles. It is still unknown whether the mutation is Japanese specific, or East-Asian specific. Newborn mass screening (NBS) is increasingly popular in Asian countries. The diversity of disease distribution and genetic mutations will be made clear with the spread of collaboration studies and expanded NBS using MS/MS.

Keywords: Genetic diversity; disease distribution; organic academia (OA); fatty acid oxidation defect (FAOD); expanded newborn screening

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AB042. Therapies for the bone in mucopolysaccharidoses

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Abstract: Patients with mucopolysaccharidoses (MPS) have accumulation of glycosaminoglycans in multiple tissues which may cause coarse facial features, mental retardation, recurrent ear and nose infections, inguinal and umbilical hernias, hepatosplenomegaly, and skeletal deformities. Clinical features related to bone lesions may include marked short stature, cervical stenosis, pectus carinatum, small lungs, joint rigidity (but laxity for MPS IV), kyphoscoliosis, lumbar gibbus, and genu valgum. Patients with MPS are often wheelchair-bound and physical handicaps increase with age as a result of progressive skeletal dysplasia, abnormal joint mobility, and osteoarthritis, leading to: (I) stenosis of

the upper cervical region; (II) restrictive small lung; (III) hip dysplasia; (IV) restriction of joint movement; and (V) surgical complications. Patients often need multiple orthopedic procedures including cervical decompression and fusion, carpal tunnel release, hip reconstruction and replacement, and femoral or tibial osteotomy through their lifetime. Current measures to intervene in bone disease progression are not perfect and palliative, and improved therapies are urgently required. Enzyme replacement therapy (ERT), hematopoietic stem cell transplantation (HSCT), and gene therapy are available or in development for some types of MPS. Delivery of sufficient enzyme to bone, especially avascular cartilage, to prevent or ameliorate the devastating skeletal dysplasias remains an unmet challenge. The use of an anti-inflammatory drug is also under clinical study. Therapies should start at a very early stage prior to irreversible bone lesion, and damage since the severity of skeletal dysplasia is associated with level of activity during daily life. This review illustrates a current overview of therapies and their impact for bone lesions in MPS including ERT, HSCT, gene therapy, and anti-inflammatory drugs.

Keywords: Mucopolysaccharidoses (MPS); enzyme replacement therapy (ERT); hematopoietic stem cell transplantation (HSCT); gene therapy; anti-inflammatory drug

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AB043. Nanopore sequencing for genotyping Dengue virus

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Abstract: Nanopore sequencer, MinION, has enabled sequencing analysis without pre-installation of expensive conventional sequencers or pre-requisite of specific skills in biological experiments. Even electric supply is not always necessary, by connecting MinION to a laptop PC. These features of MinION have opened the opportunity to enable precise genotyping of pathogens in tropical diseases on site. In this study, we attempted genotyping Dengue viruses regarding their serotypes (types 1-4). We directly used serum samples of Indonesian Dengue patients, from which viral genomes were directly amplified by the reverse-transcription-LAMP method in an isothermal reaction condition. We directly used the amplified templates for MinION sequencing allocating one flow cell per sample. We found, although the overall sequencing quality was low (80% sequence identify to the reference genome and the quality value of QV =5 on average), thereby obtained sequence data could discriminate different serotypes of the viruses, whose genome sequences were diverged with the sequence similarity of 70%, with the overall accuracy of 98%.

Keywords: Genome sequences; MinION; genotyping Dengue viruses; serotypes

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AB044. Update in the management of thalassemia

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Abstract: Treatment of thalassemia depends on the clinical diagnosis. Almost all severe alpha thalassemia, Hb Bart's hydrops fetalis, die intra utero or a few minute after birth. However, pregnant woman with Hb Bart's hydrops fetus may develop toxemia of pregnancy and antepartum and postpartum hemorrhage. Those homozygous beta thalassemias (transfusion dependent thalassemia, TDT)

are severely anemic after 6 months. At birth the baby is asymptomatic because Hb F level is high. As Hb F production waning off, replaced by inefficient beta globin chain production, at the age of 6 to 12 months, the baby begins to be anemic with hepatosplenomegaly. A group of non transfusion dependent thalassemia (NTDT) includes the majority of beta thalassemia/Hb E, Hb H disease and a few cases of homozygous beta thalassemias. The thalassemia minor including both alpha and beta thalassemia carriers and some homozygous state like homozygous alpha+ thalassemia, homozygous Hb E are asymptomatic and does not need any regular treatment. All thalassemia major, TDT, patients need regular blood transfusion, every 3-4 weeks, to maintain the hemoglobin around 10-12 g/dL. For those NTDT blood is given only when necessary. Usually patients with hemoglobin level higher than 6 g/dL require no blood transfusion. In infants and children if the hemoglobin levels can be maintained at 7 g/dL or above, defective physical development and bone changes can be prevented until they reached third or fourth decades of life that osteoporosis is almost always presented in those with low hemoglobin level. Iron overload occurs in moderate and severe case without exception. This will lead to dark skin, liver cirrhosis, cardiac arrhythmia and congestive heart failure. Diabetes mellitus secondary to iron deposition in the pancreas and other endocrine dysfunction does develop if the patients live long enough. Iron chelation with 1-2 gm/day of desferrioxamine intravenously or intramuscularly every day, at least 5 days a week, is recommended for those patients who have hemochromatosis. Oral iron chelator such as deferiprone and deferasirox is recently available. This helps patients to have better compliance with the iron chelator. Stem cell transplantation has been tried with a very good result in class I cases. Because some difficulty in finding appropriated HLA matched donor lately people try to perform haploidentical stem cell transplantation with some good results. Lupstaercept (ACE-536) and Sotatarcept (ACE-011), a recombinant fusion protein containing modified activin receptor type IIB and IgG Fc, is being developed for the treatment of anemia due to ineffective erythropoiesis. Preliminary data showed that the compound could increase hemoglobin levels 1.5 g/dL after two weeks of treatment in NTDT case and decrease blood transfusion in 60% of cases with TDT. Research is in progress to find better agents to enhance Hb F production.

Keywords: Thalassemia; stem cell transplantation; Hb F production

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AB045. Molecular markers for disease severity in beta thalassemia/Hb E disease

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Abstract: Thalassemia is a hereditary disease affecting hemoglobin synthesis, characterized by microcytic hypochromic anemia. Homozygote or compound heterozygote patients usually manifested as thalassemia major which require regular treatment. There are five functional genes arranged in the order 5' ε-Gγ-Aγ-ψβ-δ-β 3' that are activated during development. Expression of the individual genes within the β-globin cluster is controlled by the complex interactions between local regulatory sequence (promoter regions) within each gene and the β-locus control region (β-LCR), located 6-18 kb upstream of the ε-globin gene. Beta thalassemia (β-thal) is a very heterogeneous disorder due to variations in inactivation mechanism of the β-genes. Point mutations and small deletions or insertions in the nucleotide sequences are the main molecular defects responsible for most β-thalassemia. In spite of seemingly identical genotypes, severity of β-thal patients can vary greatly. This heterogeneity in the clinical severity may occur from the nature of β-globin gene mutation, α-thalassemia (α-thal) gene interaction and difference in the amount of Hb F production that is partly associated with a specific β-globin haplotype. Co-inheritance of α-thal may ameliorate the severity of β-thal disease in those cases with mild β-thal genotypes. However, many patients who are β°/β+ thal or β°-thal/Hb E do not have a detectable α-thal haplotype but still have a mild clinical symptom suggests that there are other additional factors responsible for the

mildness of the disease. Inheritance of a β-thal chromosome with the Xmn I+ haplotype at the position -158 of the Gγ-globin gene was found to be associated with increased Hb F production and milder anemia in patients with thalassemia intermedia and Xmn I +/+ haplotype is necessary to produce a significant clinical effect. Homozygosity for the Xmn I + haplotype, +/+, was also found in the mild cases of β-thal/Hb E. However, there is no severity difference among homozygous β-thal patients with Xmn I +/+, –/+ or –/–. The GWAS study of the whole genome with more than 6000,000 SNPs of 1,100 β-thal/Hb E patients with mild and severe diseases revealed SNPs in three independent genes that show significant association with the disease severity. The strongest SNPs associated with the disease severity located in three regions; the β-globin gene cluster on chromosome 11, the HBS1L-MYB intergenic region on chromosome 6q23 and the *BCL11A* gene on chromosome 2p15. Further analysis of Hb F level showed that Hb F level was significantly higher in mild patients than moderate and severe patients (%Hb F; mild =42.6±11.5, moderate =35.7±11.1, severe =32.4±12.1; P<0.001). The association of Hb F level and frequency of Hb F-QTLs was studied in 520 cases. All individual SNPs on Hb F-QTLs are associated with Hb F (P value $<10^{-5}$). Thirteen tagging SNPs were selected from three Hb F-QTLs. Of the common haplotypes CA haplotypes of *BCL11A*; CG haplotypes of HMIP; TTCTGTTAA and TTCTGTTAG haplotypes of β-globin gene cluster showed association with high HbF level. Our data indicated that several genetic loci act in concert to influence Hb F levels and disease severity of β-thal/HbE patients. Understanding the genetic modifier in β-thalassemia is important for the management of β-thalassemia patients from PND to prognosis and decision for difficult treatment such as stem cell transplantation. Moreover, this may lead to future alternative treatment of β-thalassemia patients as well.

Keywords: Thalassemia; functional genes; β-thal/HbE; HbF level

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AB046. X-linked dilated cardiomyopathy: the dystrophinopathy in a Thai family

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Background: Idiopathic dilated cardiomyopathy generally presents with congestive heart failure secondary to an increase in ventricular size and impairment of ventricular function. It is one of the leading causes of cardiovascular morbidity and mortality. Most cases have been considered to be sporadic, but recent studies have demonstrated that up to 20% of cases may be inherited, suggesting a strong genetic component for this group of diseases. Inheritance patterns vary and may be X-linked, autosomal dominant, or autosomal recessive. Dystrophin gene defect is one of the known causes of the X-linked dilated cardiomyopathy (XLDCM).

Case report: We reported a Thai 19-year-old young man, an elder brother of two brothers and three sisters, who was referred from a general hospital with pneumonia and congestive heart failure. The patient was later proved having dilated cardiomyopathy with low ejection fraction (EF =20%). He previously had normal motor power and was noticed that his calves muscle was enlarged. The muscle enzyme was elevated, creatine phosphokinase (CPK) =9,772 IU/L, CK-MB =191 IU/L and troponin T (TnT) =0.25 mcg/L. He eventually passed away in the third day of admission in the cardiac care unit (CCU). His parents are first cousin to each other. Both of his father and grandfather died with heart disease in the fourth decade of their lives. He was, then diagnosed as XLDCM.

Results: The DNA of the patient was tested for dystrophin gene deletions by multiplex PCR and MLPA techniques which gave the negative results. The DNA sequencing was performed at the 5' portion of the gene, including the muscle promoter, exon 1, and the exon 1-intron 1 splice site. A missense mutation in exon 9 at nucleotide 1043 was identified that causes an alanine to be substituted for threonine, a highly conserved amino acid, at position 279

(T279A). This mutation results in destabilizing the protein. The T279A mutation of the dystrophin gene, then was tested in all of his brothers and sisters which four of them have the same mutation and expressed the spectrum of cardiomyopathy phenotype.

Keywords: X-linked dilated cardiomyopathy (XLDCM); dystrophinopathy

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AB047. Acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors by the EGFR T790M mutation in a non-small cell lung cancer patient in Vietnam

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Abstract: Acquired resistance of epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in non-small cell lung cancer patients causes re-progression of the disease. This phenomenon prevails after 10-20 months of treatment in most of patients that were primary responses to EGFR TKIs. There was shown to be related to several genetic alterations in which the most frequent (50%) is the secondary mutation T790M in exon 20 of the *EGFR* gene. This study reports a typical clinical case with TKI acquired resistance: patient with the advanced adenocarcinoma of the lung which had already spread to the bone and the latter lung, harboring a LREA deletion mutation in exon 19 of the *EGFR* gene. The tumor progressed after 15 months of erlotinib treatment. The re-biopsy tumor revealed a secondary T790M mutation in exon 20, conferring resistance to erlotinib. The study demonstrated a critical role of molecular diagnostics for TKI acquired resistance through rebiopsies at the time of disease progression.

Keywords: Non-small cell lung cancer; tyrosine kinase inhibitor acquired resistance (TKI acquired resistance); secondary T790M mutation

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AB048. X-chromosomal SNPs variation in populations of Russia

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Abstract: X-chromosome markers are informative tool for studying a genetic diversity in human populations and have become a useful in DNA identification when certain complex kinship cases need to be unravelled. In this work we present population genetic data on X-chromosome-wide SNPs in North Eurasian populations and report XSNP multiplex system for forensic genetics. A total of 2,867 X-chromosomal SNPs were genotyped in 12 populations using Illumina microarray platform. Twelve populations under study (Komi, Mordva, Russians, Kirghiz, Kazakh, Uzbek, Buryat, Yakut, Evenk, Tuva, Khanty, Ket) represent various language families and geographic regions of North Eurasia (Eastern Europe, Central Asia, Siberia and North Asia). North Eurasian populations are highly genetically differentiated with respect to XSNPs allele frequencies. Average level of genetic differentiation (Gst) for 12 populations is 6.03% and ranged from 1.05% to 30.05% per individual SNP. Principal component analysis of allele frequencies demonstrated geographic pattern of population clustering, as well as longitudinal gradient in genetic diversity. The 66 XSNPs characterized by high expected heterozygosity and linkage equilibrium in populations under study were selected for constructing a panel for

forensic genetic applications. Average heterozygosity of selected SNPs varied from 0.4925 to 0.4958. Overall values of power of discrimination for males and females (PDm and PDf) obtained with these XSNPs set are several magnitude higher than those for standard forensic STR panels. Protocol for multiplex amplification of 66 XSNPs in two separate multiplex PCR reactions and MALDI-TOF mass spectrometry genotyping was developed. North Eurasian populations demonstrate high level of genetic diversity and differentiation for X-chromosome-wide SNPs. Based on obtained population genetic data, highly informative multiplex XSNPs panel for forensic genetics was developed.
Funding: This work was supported by the Federal target program "Research and development on the priority directions of Russian scientific-technological complex development" (Agreement #14.604.21.0019).

Keywords: Genetic diversity; X-chromosome SNPs; population genetics; forensic genetics

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AB049. Aldehyde dehydrogenases: from cancer stem cells to inborn errors of metabolism

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Abstract: The aldehyde dehydrogenase (ALDH) superfamily comprises NADP-dependent enzymes that catalyze the oxidation of aldehydes to their corresponding carboxylic acids. To date, 19 *ALDH* genes have been identified in the human genome. In addition to aldehyde metabolizing capacity, ALDHs have additional catalytic (e.g., esterase and reductase) and non-catalytic activities. The latter include functioning as structural elements in the eye (crystallins) and as binding molecules to endobioticsand xenobiotics. Mutations in human *ALDH* genes are the molecular basis of several diseases, including gamma-hydroxybutyricaciduria, type II hyperprolinemia, Sjögren-Larsson syndrome, pyridoxine-dependent epilepsy as well as osteoporosis and gout. ALDH enzymes also play important roles in embryogenesis and development, neurotransmission, oxidative stress and cancer. One of the most exciting recent discoveries regarding ALDHs is their identification as markers of cancer stem cells and their involvement in cancer cell resistance to chemotherapy and radiotherapy. Thus, therapeutic targeting of ALDHs may represent a novel means of more effectively treating patients with cancer or metabolic disease and improving clinical outcomes.

Keywords: Aldehyde dehydrogenase genes (*ALDH* genes); target therapy; inborn errors of metabolism

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AB050. Building population-specific reference genomes: a case study of Vietnamese reference genome

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Abstract: The human reference genome is an essential tool for studying human genomes. The standard reference genome is constructed from genomes of a few donors. The 1,000 genomes project has revealed a huge amount of genetic differences between diverse populations. It is therefore naturally questioned whether the standard reference genome can work well for all human genome studies or population-specific reference genomes are

needed accordingly. In this paper, we present a pipeline for constructing and evaluating a population-specific reference genome. The pipeline was examined on building the Vietnamese reference genome from 100 Kinh Vietnamese genomes obtained from the 1,000 genomes project. Experiments showed that the resulting Vietnamese reference genome was better than the standard reference genome at analyzing Vietnamese genomic data. It helped improve the quality of short reads mapping and genotype calling for Vietnamese genomes. The pipeline is applicable for building and evaluating other population-specific reference genomes.

Keywords: Reference genome; human genomes; genotype; Vietnamese

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AB051. Regulation of IL-2 production through ERK/NFATc3 signalling pathway by A20 in dendritic cells

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Abstract: Dendritic cells (DCs) are the most potent antigen-presenting cells regulating naive T cell responses *in vivo*. A20 is negative regulator of NF-κB activation and inflammation in DCs, key players in the regulation of innate and adaptive immunity. The present study explored whether the effects of A20 on IL-2 production through ERK/NFATc3 activation in DCs. To this end, the mouse bone marrow cells are isolated and cultured with GM-CSF to attain BMDCs. The expression of co-stimulatory and signalling molecules and cell death were examined by flow cytometry, quantitative PCR and western blotting. Cytokine production was determined by ELISA methods. As a result, LPS stimulated to the increase in expression of MHC class II and CD40 as well as activation of NF-κB, STAT-1 and ERK-NFATc3 pathways, which resulted in elevated productions of IL-2, IL-12, and TNF, the effects were further enhanced in A20-deficient DCs. Targeted inhibition of ERK in A20-deficient DCs abolished the increased production of IL-2, the cytokine induced from the activation of NFATc3 in DCs. In addition, cytokine-induced cell apoptosis was unaffected by A20. In conclusion, A20 restricted cytokine production in BMDCs by inhibiting NF-κB, ERK and NFATc3 pathways.

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Keywords: Dendritic cells (DCs); A20; IL-2; NF-κB; ERK; NFATc3

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AB052. The Human Variome Project (HVP) and the HVP ASEAN Node

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Abstract: The Human Variome Project (HVP) is an international NGO that is working to build capacity in responsible clinical genomics around the world. Founded in 2006, and lead in its early years by Professor Richard Cotton, the Project has grown to become a global movement with over 1,300 individual members from 81 countries and close to 200 data provider members. The project works to ensure that the lack of access to information on genetic variants and their effects on human health is not an impediment to diagnosis and treatment. Together with partner organisations including national and regional human genetics societies, national governments

and intergovernmental organisations such as UNESCO—of which the HVP is an NGO official partner—the project establishes standards, guidelines and best practices for the responsible development and operation of genetic variation data sharing infrastructure, facilitates training and education of the public and the clinical genomics field and assists in embedding data sharing into routine clinical practice. The HVP believes that the free and open sharing of genetic variation is fundamental to providing quality clinical care. To ensure that this data can be collected, curated, interpreted and shared in a responsible manner that is respectful of the diverse ethical, legal and social differences of its member countries, the HVP works with local stakeholders in each country to establish HVP Country Nodes. An HVP Country Node is defined as having three components: (I) a repository, or linked network of databases, that collect and store information on variation in the human genome that has been generated within each country and that enables the sharing of that information both nationally and internationally; (II) a governance structure that ensures that the work of the Node is both sustainable in the long term and is consistent with all relevant national and international ethical, legal and social requirements; and (III) a set of policies and procedures that ensures that the repository is operated and maintained in a responsible and accountable manner that is consistent with both national and HVP Standards. The HVP Malaysian Node (MyHVP), one of 23 HVP Country Nodes currently in existence, was established in 2010 and officially launched by Professor Cotton. The MyHVP database was made available on the internet a year later. The HVP Malaysian Node has taken a key role in the region and has worked to establish the HVP ASEAN Regional Node. Among its objectives is to foster closer collaboration among ASEAN member states on issues relating to data sharing, data basing and variant interpretation expertise, resources and technical facilities. The HVP ASEAN Regional Node also provides help with capacity building and training, especially to less well-resourced countries in the South East Asian region, for example Cambodia, Laos, and Myanmar. The HVP ASEAN Regional Node was launched in 2013 at Universiti Sains Malaysia, Kota Bharu, Malaysia. Representatives from Thailand, Vietnam, Singapore, Brunei, Indonesia, Philippines and the international Human Variome Project were represented. This presentation will provide an in-depth overview of the HVP ASEAN Regional Node and its progress to date.

Keywords: Human Variome Project (HVP); genetic variation; data; clinical genomics; human health

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AB053. Role of inflammation in the mucopolysaccharidoses & review of recent therapies

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Abstract: The mucopolysaccharidoses (MPS) comprise a group of 11 related lysosomal storage disorders due to inherited deficiencies of enzymes involved in glycosaminoglycan (GAG) degradation. Each disorder is characterized by the accumulation of specific GAGs and GAG fragments, mostly in connective tissue cells and tissues, resulting in an array of clinical findings that include abnormal bone growth, joint and skull deformities, tracheal abnormalities and other connective tissue disease. Involvement of the central nervous system occurs in some patients, as does involvement of liver, spleen, lung and other organ systems. Enzyme replacement therapy (ERT) is available for four MPS types (I, II, IV and VI) and under development for several others. Bone marrow transplantation also may be undertaken in patients for whom ERT is not available, and gene therapies are being considered for several of the disorders. While each of these therapies may provide substantial clinical benefit, there are limited effects on the bones, cartilage and brain, indicating a need for new research and treatment options. The common clinical presentation of the different MPS disorders suggests that common underlying disease mechanisms are likely to be responsible, and that new drugs targeting these pathways may be of benefit to multiple MPS types. One such drug is pentosan polysulfate (PPS), which is being "re-purposed" to reduce inflammation and GAG storage in MPS. Two small proof-of-concept clinical trials of PPS in MPS patients have recently been completed. This lecture will review the pathophysiology and genetics of the MPS disorders, the current state of MPS treatment, and prospects for future treatments.

Keywords: Mucopolysaccharidoses (MPS); disorder; glycosaminoglycan (GAG); pathophysiology; genetics

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AB054. Overview of multi-gene panels for hereditary cancer

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Background and objective: Genetic testing for several hereditary cancer genes, such as *BRCA1* and *BRCA2*, has been available in the United States since the 1990's. Historically, many patients and families with histories suggestive of hereditary cancer predisposition tested negative for mutations in these well-described genes. Multi-gene panel tests can provide an answer for a proportion of these families, and have been available in the United States since 2012. There is an ongoing discussion among providers regarding the benefits and limitations of panel tests. The identification of a causative mutation allows patients and families to be aware of additional cancer risks, and therefore pursue appropriate management for risk reduction. Additionally, panel testing is a more time and cost-effective approach. However, many of the genes on the panels do not have published management guidelines or cancer risk estimates for mutation carriers, which can lead to difficulty in recommending appropriate screening for patients and families. We aim to briefly describe the overall panel results, and discuss the utility of multi-gene testing for hereditary cancer.

Methods: Ambry Genetics offers ten cancer panels, from a 5 gene high risk breast cancer panel, to a 49 gene pan-cancer panel. We assessed the overall results of these panels from March 2012 to March 2015.

Results: Over 50,000 individuals underwent testing through a multi-gene panel at Ambry Genetics. The overall positive rate is 8.6%, and the overall inconclusive rate is 17.92% across all panels. The two most frequently ordered panels are BRCAPlus, a panel assessing 5 genes associated with a high risk for breast cancer, and BreastNext, an expanded panel assessing 17 genes associated with moderate to high breast cancer risk. Without accounting for the total cases tested for each gene, most mutations were identified in *BRCA1*, *BRCA2*, *CHEK2*, and *ATM*, respectively. The vast majority of patients tested were Caucasian, while 3.4% were Asian.

Conclusions: Based on our review of a large number of cases submitted for panel testing, we found several genes that are commonly mutated that may provide an explanation

for a portion of hereditary cancer families. However, testing more genes increases the number of variants of uncertain significance, which can potentially lead to confusion and anxiety in patients. Appropriate genetic counseling and education for patients about the substantial benefits and limitations of multi-gene panel testing is necessary so that patients and their providers can make informed choices about the most appropriate testing option.

Keywords: Oncology; hereditary; panel

Cite this abstract as: Dalton E, Thompson J. Overview of multi-gene panels for hereditary cancer. Ann Transl Med 2015;3(S2):AB054. doi: 10.3978/j.issn.2305-5839.2015.AB054

AB055. The new meaning of translational genomics & developing consensus on best practices for areas critical to enabling precision medicine

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Abstract: Paradigm shifts are occurring at all stages of the path to translational medicine, complicating the process while creating exciting opportunities. Translational genomics is no longer simply a nebulous bridge from bench research to bedside care with research only conducted in a top down fashion involving studies are done in secrecy with little reporting back to research participants. Patients are contributing more to as well as demanding more from their clinical encounters and genetic and genomic data is more easily flowing between greater and less resourced institutions. The opportunity to develop high impact solutions based on research and focused on greater relevance to human health is also greater than ever before. At the same time, there is no consensus as to what are truly 'best practices' to deal with issues of data management/integration, ethics, patient empowerment roles, provider knowledge, etc. A shared understanding of how best to proceed forward is key to establishing viable translational and precision medicine. In this talk I will present the interests and concerns that need to be considered regarding translational research. I will then actively engage participants to share their understanding of these issues in the context of local and national considerations, and how they are addressing them, towards the shared goal of achieving a better regional, if not international, understanding of successful strategies for enabling translational medicine. I intend to write up the results of this discussion for the APHGS and publication in the *Journal of Applied & Translational Genomics*. I invite participants to connect and encourage those who want to be actively involved in creating a statement to attend this session and fully participate.

Keywords: The new translational genomics; data management/interpretation; ethics; patient empowerment; provider knowledge

Cite this abstract as: Barash CI. The new meaning of translational genomics & developing consensus on best practices for areas critical to enabling precision medicine. Ann Transl Med 2015;3(S2):AB055. doi: 10.3978/j.issn.2305-5839.2015.AB055

AB056. Establishing the procedure for detection of gr/gr deletions on the Y chromosome in Vietnamese infertile men

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Background and objective: About 2-10% cases of both azoospermia or cryptospermia have a micro Y deletion. These deletions mainly locate at AZFa, b, or c which containing several crucial genes for normal spermatogenesis. Among various category of deletions is gr/gr deletions on AZFc. The influence on spermatogenesis mostly depend on characteristics of the Y chromosome which may vary among different ethnicity and geographical locations. In Vietnam, there is no study about gr/gr deletions. (I) To establish an approachable procedure to detect gr/gr deletions on the Y chromosome, which is applicable for hospitals and clinics in Vietnam; (II) to determine the percentage of gr/gr deletions carriers among infertile men (azoospermia and severe oligozoospermia).

Methods: Blood samples were collected for DNA extraction. Based on inclusive and exclusive criteria, 3 fertile and 32 infertile men (azoospermia and cryptozoospermia) were recruited to our study with informed consent. Sequence tagged sites (STSs) và primers were designed by using design software (Ape, PrimerPlex2, Oligoanalyzer IDT) based on the information of NCBI. DNA was extracted *in silico* from blood sample and applied to Multiplex PCR to detect gr/gr deletions. To ensure the true positive result of gr/gr deletions, MLPA was subjected to perform.

Results and discussion: With our designed primers, an approachable procedure was successfully established to detect gr/gr deletions that resulted at a high level of sensitivity (at 0.5ng DNA sample/reaction) and high level of stability. The percentage of gr/gr-deletion carriers among infertile men was 12.5% (4 positive samples), higher than 10.6% in an Asian population from a current study. All positive samples are confirmed significantly by MLPA technique.

Conclusions: An approachable procedure was successfully established to detect gr/gr deletions that resulted at a high level of sensitivity (at 0.5 ng DNA sample/reaction) and high level of stability. The percentage of gr/gr-deletion carriers among azoospermia/ severe oligozoospermia men was 12.5%.

Keywords: gr/gr deletions; multiplex PCR; infertile men

Cite this abstract as: Nguyễn TB, Nguyễn NA, Phạm TT, Nguyễn TT. Establishing the procedure for detection of gr/gr deletions on the Y chromosome in Vietnamese infertile men. Ann Transl Med 2015;3(S2):AB056. doi: 10.3978/j.issn.2305-5839.2015.AB056

AB057. Wilson disease in children clinical and laboratory manifestations

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Background and objective: Wilson disease (WD) is disorder of copper metabolism caused by an autosomal recessive mutation 13q14.3 in *ATP7B* gene. WD's clinical manifestations are injured at liver, brain, eyes, kidneys, joints, bones, etc. which accounts for 40-50% of liver damage. The diagnosis and prompt treatment will help WD's patients limit the complications of the disease and improve quality of life for them. The aim is to describe clinical characteristics, laboratory finding and follow up of WD patients after treament.

Methods: The retrospective description.

Results: A total of 46 patients (28 males:18 females) were diagnosed WD based on Leipzig 2001 standard at Hepatology Deparment in NHP from 12/2013 to 1/2015. The average age at diagnosis: 12±0.25 years old. Symptoms of onset was persistent transaminase increase (78.7%), liver failure, decrease ceruloplasmin <0.2 mg/dL (97.5%), copper in the urine increased >100 µg/24 h (100%). All of patients were treated with D-penicillamine, zinc and supportive treatment. Results of follow up: 89.1% patients improved liver function after treatment. Mortality rate during follow-up (12 months) is only 0.02%.

Conclusions: WD in children usually manifests as chronic liver injury. Do not overlook WD in a patient who has persistent transaminase elevations or hepatic failure unknown the origin. The follow up results showed 89.1% of WD's patients responded to treatment with D-penicillamine and zinc.

Keywords: Wilson disease (WD); chronic liver disease

Cite this abstract as: Anh HT, Hoa NP. Wilson disease in children clinical and laboratory manifestations. Ann Transl Med 2015;3(S2):AB057. doi: 10.3978/j.issn.2305-5839.2015.AB057

AB058. Newborn screening in preterm babies at the Newborn Screening Center-National Institutes of Health, Manila: impact, implications, and outcomes on its first year of implementation

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Background and objective: Preterm, low birth weight and sick newborns are at risk of missed or unreliable testing due to many factors such as infant condition, treatment, and maternal status. In June 2014, a newborn screening testing protocol which recommends performing the newborn screening test on babies less than 37 weeks age of gestation immediately after 24 h of birth and a second screening test at 28 days of age was implemented. This study is being undertaken to evaluate the impact, implications, and outcomes of the new testing protocol in the screening of preterm babies.

Methods: This is a descriptive study which includes one year screening data (June 2014-May 2015) of babies less than 37 weeks of age at the Newborn Screening Center, National Institutes of Health (NSC-NIH), Manila. Data extracted include laboratory number, date of birth, date of collection, age at collection, birth weight, age of gestation, initial result, date of repeat collection, age at repeat collection, repeat test result, and confirmatory tests results. The completed data will be analyzed further under the 2 age groups, namely, the <35 weeks age group and the 35 weeks to <37 weeks age group.

Results: Preliminary data show that there were 11,297 babies less than 37 weeks age of gestation who had newborn screening at the NSC-NIH, 10,089 of them had normal results on initial screen. Of the 10,089 babies who had normal initial screening result, only 180 babies had repeat testing at 28 days of age and beyond as of May 22,2015. Of these 180 babies, 2 turned out to be positive for congenital hypothyroidism and were confirmed through thyroid function testing, and 3 turned out to be positive for glucose-6-phosphate dehydrogenase (G6PD) deficiency.

Conclusions: Uptake of the protocol on its first year of implementation is still very low. Nevertheless, even with this low availment for repeat tests at 28 days of age, 5 out of 10,089 babies who initially had normal screening results had repeat testing which turned out to be positive and confirmed for either congenital hypothyroidism or G6PD deficiency. The results of this study could be used as basis to justify the need for additional testing at 28 days of age in preterm babies. Further analysis under different age groups will help in the determination of appropriate age of gestation cut-offs for the current protocol.

Keywords: Newborn screening; preterm; protocol

Cite this abstract as: Dion-Berboso AG, Cabic AG, Carluen-Nario I, Valeza G, Alcausin MM. Newborn screening in preterm babies at the Newborn Screening Center-National Institutes of Health, Manila: impact, implications, and outcomes on its first year of implementation. Ann Transl Med 2015;3(S2):AB058. doi: 10.3978/j.issn.2305-5839.2015.AB058

AB059. The clinical profile and factors influencing loss to follow-up on the use of repeat otoacoustic emissions (OAE) and auditory brainstem response (ABR) among infants with refer results on newborn hearing screening at Cebu Doctors' University Hospital

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Objective: To determine the clinical profile and factors influencing loss to follow-up on the use of repeat otoacoustic emissions (OAE) and auditory brainstem response (ABR) among infants who obtained REFER results in newborn

hearing screening using OAE born in Cebu Doctors' University Hospital from January 2012-December 2013.

Methods and materials: Design: retrospective descriptive along with a structured interview. Setting: private tertiary hospital. Patients/participants: infants with REFER results in the newborn hearing screening were the subjects of this study. The mothers of these infants were then interviewed. Sixty-seven infants had REFER results. Only 43 (64%) of the subjects were recalled. Main outcome measure(s): factors influencing loss to follow-up.

Results: Majority of the babies who did not follow up after a failed screening test were term and of normal birth weight. The top three reasons given for failure to follow up were: the belief that their baby could hear (52%), no advice given (20%), and living far from the hospital (16%). For those who did not proceed with the ABR, the reasons for non-compliance were the assumption that their baby could hear (75%) and that they could not afford the testing fee (25%).

Conclusions: The top three reasons for poor compliance were the belief that their baby can hear, no advice given and living far from the hospital.

Keywords: Universal newborn hearing screening; otoacoustic emissions; loss to follow-up

Cite this abstract as: Dominguez AN, Cavan BC. The clinical profile and factors influencing loss to follow-up on the use of repeat otoacoustic emissions (OAE) and auditory brainstem response (ABR) among infants with refer results on newborn hearing screening at Cebu Doctors' University Hospital. Ann Transl Med 2015;3(S2):AB059. doi: 10.3978/j.issn.2305-5839.2015.AB059

AB060. A4164G alteration of mitochondrial *MT-ND1* gene in a Vietnamese patient group with colorectal cancer

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Background and objective: Colorectal cancer (CRC) is one of the most common cancers which is increasing all over the world and in Vietnam. Many causes of disease have been identified, including variations in nuclear genes and mitochondrial genes. The *MT-ND1* gene is located in the heavy strand of mitochondrial DNA and encodes NADH dehydrogenase 1 protein. Some mutations were detected in mitochondrial DNA of CRC patients such as T3394C, T4216C and C3497T. These mutations occur in high conservative region, thus can effect on structure and function of the NADH dehydrogenase 1. In this study, we investigated the incidence of A4164G and T4216C alterations of mitochondrial *MT-ND1* gene in Vietnamese CRC patients and whether these alterations might be associated with some pathological characteristics of CRC.

Methods: A total of 107 Vietnamese CRC patients and 100 controls were determined for A4164G and T4216C alterations by using PCR-RFLP and sequencing methods. Relationship between the genotype and pathological characteristics of CRC patients was calculated by using χ^2 test. Odds ratio and 95% confidence interval were calculated as an estimate of the relative risk.

Results: The results showed that there were 14.95% CRC tissue samples, 10.53% cancer blood samples and 9% blood control samples with the A4164G alteration in the *MT-ND1* gene. T4216C mutation was not found in those samples. There was no difference of A4164G distribution in subgroups of age, gender, size of tumor ($P>0.05$), but difference in site of tumor and TNM (lymph-node-metastasis) stage ($P<0.05$). The A4164G alteration was only found in 22.22% blood samples of CRC patients which had A4164G alteration in the tissue samples. So A4164G alteration can be a somatic mutation in these CRC patients.

Conclusions: The difference of A4164G alteration between tumor tissue, adjacent tissue and blood of the same patient in some CRC cases can be considered as an evidence of somatic mutation in these CRC patients in Vietnam.

Keywords: A4164G alteration; colorectal cancer (CRC); *MT-ND1*; PCR-RFLP; sequencing

Cite this abstract as: Bich PT, Chang HT, Ha DM, To TV, Thai TH. A4164G alteration of mitochondrial *MT-ND1* gene in a Vietnamese patient group with colorectal cancer. Ann Transl Med 2015;3(S2):AB060. doi: 10.3978/j.issn.2305-5839.2015.AB060

AB061. Screening of thalassemia in the Philippines

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Background and objective: The thalassemias are autosomal recessive disorders which result in reduced production of one or more subunits of hemoglobin. They are a growing public health concern. Prognosis is improving, however, there is a lack of estimates of the affected population, resources for prevention, control and management in the country. In light of recent findings on the severity and genetics of this inherited disorder, the authors aimed to summarize information on the epidemiology, screening, diagnostic procedures such as high performance liquid chromatography and genetic analysis of thalassemias in the Philippines.

Methods: Patients referred by hematologists from different parts of the country from 2008 to 2014 were included in the study. Peripheral blood extracted from the subjects were hemolyzed and subjected to Variant™ high performance liquid chromatography (HPLC) for detection of thalassemias and hemoglobinopathies. Genomic DNA extracted in 55 clinically diagnosed beta thalassemic patients subsequently underwent GAP PCR and direct sequencing. In an ongoing study, extracted genomic DNA from 20 alpha thalassemic patients was analyzed using alpha thalassemia strip assay and direct sequencing.

Results: HPLC results showed that majority of the patients were beta thalassemics (47%) followed by alpha-thalassemics (15%). HbE disease was also found in 1% of the population. Interestingly, thalassemia and hemoglobinopathy interactions such as beta thalassemia with HbE interaction (2.2%) and alpha-beta thalassemia (0.4%) have also been reported. Of the 55 clinically diagnosed beta thalassemics who underwent molecular analysis, 10 published beta globin gene defects were observed in 35 patients. The FIL deletion was found to be the most prevalent mutation among the alpha thalassemic patients.

Conclusions: Preliminary data on the thalassemias suggest that these disorders deserve priority in the country's health agenda. The Philippines still faces many challenges in the provision of basic care for thalassemic patients. The prevention of severe alpha and beta thalassemia is dependent on the availability of molecular characterization, supported by genetic counselling and targeted public awareness programs. This will reduce the economic burden and comprehensive and effective management of this problem in our country will be better achieved.

Keywords: Alpha-thalassemia; beta-thalassemia; Philippines

Cite this abstract as: Silao CL, Fabella T, Yuson E, Naranjo ML, Padilla C. Screening of thalassemia in the Philippines. Ann Transl Med 2015;3(S2):AB061. doi: 10.3978/j.issn.2305-5839.2015.AB061

AB062. Identification of disease susceptibility genes in Filipino SSPE patients

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Background: Subacute sclerosing panencephalitis (SSPE) is a fatal neurodegenerative disease resulting from long-term persistence of the measles virus in the brain. Host genetic factors contributing to SSPE predisposition were investigated in the study.

Methods: Microarray technology, genotyping of candidate genes and whole genome expression profiling were done to determine the genes and pathways that are associated to the development of SSPE in Filipinos.

Results: Significant association was observed in MXA variants. Polymorphisms tested in the *IL-4*, *IL-10*, *IFN- γ* and *IRF-1* genes did not show significant association. Differential expression analysis between patients and controls showed altered expression in 851 probes majority of which were underexpressed in SSPE patients. Functional annotation of the genes showed that many are immunity-

related and are particularly involved in viral response. Comparison of expression levels between patients in remission (stage 1) and diseased patients (stages 2, 3, and 4) revealed 496 differentially expressed probes, 138 were downregulated in diseased patients. Most of these genes have immune response function particularly involved in viral response. More importantly, significant differentially expressed genes with no a priori association with SSPE were identified in the pathway analysis.

Conclusions: Results of the study provided additional evidence that the host immune response has a key role in SSPE etiology. It is recommended that further investigation be done to test for association of the differentially expressed genes with SSPE risk either by genotyping or functional analysis.

Keywords: Subacute sclerosing panencephalitis (SSPE); measles; neurodegenerative; microarray

Cite this abstract as: Silao CL, Lukban M, Salonga A, Sanchez-Gan B, Lu M, Pipo-Deveza J, Nevado J. Identification of disease susceptibility genes in Filipino SSPE patients. Ann Transl Med 2015;3(S2):AB062. doi: 10.3978/j.issn.2305-5839.2015.AB062

AB063. Prevalence of thalassemias and hemoglobinopathies detected via high performance liquid chromatography in Filipinos

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Background: The thalassemias and hemoglobinopathies are groups of autosomal recessive inherited blood disorders affecting the quantity and quality of hemoglobin. Patients with the disease can vary from clinically asymptomatic to transfusion dependent individuals. It is considered prevalent in many parts of the world particularly in Southeast Asia. Thus, screening and prevalence determination of these diseases are important in the Filipino population. The study aims to determine the prevalence of thalassemias and hemoglobinopathies in the Philippines using high performance liquid chromatography.

Methods: Referred patients by hematologists from different parts of the country from October 2008 to December 2014 were included. Peripheral blood extracted from the subjects, were hemolyzed and screened for thalassemias and hemoglobinopathies using Variant^{TM} HPLC. Data interpretations were based on levels of the HbA2 Fetal Hb and HbA detected.

Results: Majority of the patients were beta thalassemics followed by alpha-thalassemics. Hemoglobin E was found in 1% of the population tested while 2% of the patients have beta thalassemia with HbE interaction.

Conclusions: A significant proportion of thalassemias and hemoglobinopathies were determined from the 6-year screening of Filipinos using Variant^{TM} HPLC. The results of this study provide not only confirmation of the occurrence and prevalence of these growing and diverse groups of genetic blood diseases but also suggest that the use of HPLC is a useful screening tool.

Keywords: Thalassemias; hemoglobinopathies; HPLC; Filipinos

Cite this abstract as: Silao CL, Fabella TD, Yuson E, Naranjo ML, Padilla C. Prevalence of thalassemias and hemoglobinopathies detected via high performance liquid chromatography in Filipinos. Ann Transl Med 2015;3(S2):AB063. doi: 10.3978/j.issn.2305-5839.2015.AB063

AB064. Autosomal recessive diseases caused by a rare mechanism: uniparental disomy

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Abstract: Uniparental disomy (UPD) represents an

imbalance in the distribution of paternal and maternal chromosomes in the offspring. It is defined as a condition in which both homologues of a chromosome are inherited from only one parent. UPD for some chromosomes does not exert any adverse effect on an individual. However, for certain chromosomes, it can result in abnormality through aberrant genomic imprinting, when the chromosomes contain imprinted genes. In isodisomy, not only is there a risk for a disturbance due to imprinting, but there is also risk that the two pairs of homologs are identical creating homozygosity for a large region of a certain chromosome, with an associated increased risk for recessive disorders. In this report, we will demonstrate two rare autosomal recessive diseases, argininosuccinic aciduria and recessive congenital methemoglobinemia, were caused by uniparental isodisomy or uniparental heterodisomy with segmental isodisomy. The molecular studies of this mechanism and literatures reviewed will be presented in this report.

Keywords: Uniparental disomy (UPD); recessive congenital methemoglobinemia; argininosuccinic aciduria; microsatellite genotyping

Cite this abstract as: Chen YC, Hsiel SC, Pai JS, Chu TH, Niu DM. Autosomal recessive diseases caused by a rare mechanism: uniparental disomy. Ann Transl Med 2015;3(S2):AB064. doi: 10.3978/j.issn.2305-5839.2015.AB064

AB065. MDM2 SNP309 polymorphism and lung cancer risk

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Abstract: Lung cancer is the most common type of cancer with high incidence rate and leading cancer cause death worldwide. Murine double minute 2 (MDM2) SNP309 polymorphisms have been reported to influence the risk of lung cancer. This study was conducted to determine the associate of the MDM2 SNP309 polymorphisms and lung cancer risk. One hundred and fifty patients were diagnosed with lung cancer in Respiratory Center, Nuclear Medicine and Oncology Center-BachMai Hospital and 173 controls who were selected to analyze clinical features and MDM2-SNP309 associated with lung cancer risk. The 309 GG genotype was associated with increased lung cancer risk in recessive model (OR =1.82; 95% CI, 1.09-3.05; P=0.02).

Keywords: Lung cancer; SNP309 polymorphisms; murine double minute 2 gene (*MDM2* gene)

Cite this abstract as: Chi TK, Thinh TH, Ha NT, Khanh TV, Van TT. MDM2 SNP309 polymorphism and lung cancer risk. Ann Transl Med 2015;3(S2):AB065. doi: 10.3978/j.issn.2305-5839.2015.AB065

AB066. Pseudohomozygous familial hypercholesterolemia has better outcome than homozygous familial hypercholesterolemia

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Background: Homozygous familial hypercholesterolemia (HoFH), due to the *LDLR* gene defect, is very rare. Drugs are largely not effective enough for HoFH patients except LDL apheresis or liver transplantation combined with cholesterol-lowering drugs. However, pseudohomozygous familial hypercholesterolemia, also known as sitosterolemia, is a recessively inherited disorder that results from mutations in either ABCG5 or G8 proteins, with hyperabsorption of dietary sterols and decreased hepatic excretion of plant sterols and cholesterol. Ezetimibe appears to reduce plasma plant sterol concentrations in patients with sitosterolemia in previous studies. In this study, we compare the clinical manifestations and treatment outcome of these two diseases.
Methods: We conducted a retrospective review of 10 patients diagnosed with HoFH and five patients with pseudohomozygous familial hypercholesterolemia in our pediatric endocrinology department. HoFH and sitosterolemia were diagnosed by molecular study of these

patients and their parents. Lipid profile before and after treatment was analyzed.

Results: Seven HoFH patients showed a reduction of more than 50% of the total cholesterol levels in response to conventional drug therapy (high-dose statin with ezetimibe). The low-density lipoprotein—cholesterol levels of three HoFH patients decreased to lower than 160 mg/dL. None of HoFH patient's treatment results meet the current treatment target endpoint. Sitosterolemia patients were on ezetimibe therapy and had satisfactory total serum cholesterol levels, though their plant sterol levels were still higher than normal.

Conclusions: Pseudohomozygous familial hypercholesterolemia must be distinguished from homozygous familial hypercholesterolemia, as these two disease mandates different treatments and has a different prognosis. Despite aggressive therapy, HoFH patients are not well-controlled; atherosclerosis may progress. In contrast, total cholesterol levels can be well-controlled after ezetimibe treatment in sitosterolemia patients.

Keywords: Homozygous familial hypercholesterolemia (HoFH); *LDLR* gene; pseudohomozygous familial hypercholesterolemia; atherosclerosis; sitosterolemia

Cite this abstract as: Yen CY, Chu TH, Yang CF, Niu DM. Pseudohomozygous familial hypercholesterolemia has better outcome than homozygous familial hypercholesterolemia. Ann Transl Med 2015;3(S2):AB066. doi: 10.3978/j.issn.2305-5839.2015.AB066

AB067. Glucose tetrasaccharide (Glc4) level in urine sample as a biomarker for Pompe patients

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Abstract: Pompe disease is a lysosomal storage disease caused by an accumulation of glycogen in the lysosome due to deficiency of the lysosomal acid alpha-glucosidase (GAA) enzyme. Our team has started newborn screening of Pompe disease since 2008. Until now, around 800,000 newborns have been screening. Rapid diagnostic criteria for the newborns with infantile-onset Pompe disease (IOPD) has been established by our team. Through this effective program, IOPD infants can receive their first ERT within 4 hours of admission and at an average of 11.56±3.4 days of age. However, for late onset Pompe disease (LOPD) newborns, it is very difficult to distinguish them from the newborns with low GAA enzyme activity caused pseudo-deficiency allele (G576S) by clinical manifestations or enzyme activity. Therefore, we try to find a reliable biomarker to distinguish LOPD newborns from pseudo-deficiency. In this study, we analyzed urinary Glc4 levels of the newborns who have pseudo-deficiency (G576S), suspected LOPD (suspected by their genotypes), or IOPD. We found that all the pseudo-deficiency newborns and suspected LOPD newborns had normal urinary Glc4 level, while IOPD had very high urinary Glc4 level before they got ERT. The urinary Glc4 level decreased rapidly after ERT in these IOPD newborns. For long-term ERT follow up, the urinary Glc4 level elevated gradually after longer-term follow-up in some patients, especially in who received ERT later. We also found that urinary Glc4 level was correlated with serum creatine kinase (CK) level in our patients. Our data suggested that urinary Glc4 level is a good biomarker for IOPD newborns, but not LOPD, especially for the pre-symptomatic LOPD patients.

Keywords: Pompe disease; glucose tetrasaccharide (Glc4); infantile-onset Pompe disease (IOPD); late onset Pompe disease (LOPD); pseudo-deficiency

Cite this abstract as: Huang CK, Liao HC, Hsieh YP, Chen YC, Yang CF, Niu DM. Glucose tetrasaccharide (Glc4) level in urine sample as a biomarker for Pompe patients. Ann Transl Med 2015;3(S2):AB067. doi: 10.3978/j.issn.2305-5839.2015.AB067

AB068. Association between MTHFR C677T and carotid intima medial thickness progression in post-ischemic stroke patient

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Background and objective: Substitution of c.677C > T in methylenetetrahydrofolate reductase (*MTHFR*) gene contributes to increase blood level of homocysteine (Hcy). Hyperhomocysteinemia is believed to have association with vascular damage leads to atherosclerosis. Defect MTHFR may influence vascular progression in post ischemic stroke. Carotid intima media thickness (c-IMT) has been known as vascular marker for atherosclerosis and predictor for ischemic stroke. The study aims to determine association between MTHFR C677T and c-IMT progression in post-ischemic stroke patients.

Methods: Seventy one of post-ischemic stroke patients were included in epidemiological prospective observational cohort study. Genotyping *MTHFR* gene polymorphism was done using PCR-RFLP with HinfI restriction enzyme. Blood Hcy level was determined using enzyme immunoassay (EIA) method. Carotid duplex ultrasound was used to evaluate c-IMT in 1st, 6th, and 12th month after the onset of stroke.

Results: The genotype distribution of MTHFR C677T in samples was CC (81.9%), CT (13.9%) and TT (4.2%). No significant differences in mean Hcy levels between genotype TT and others (CT and CC) were identified (P=0.250). Mean c-IMT showed no significant differences between genotype TT and others at evaluation in 1st month (P=0.979), 6th month (P=0.670) and 12th month (P=0.770). All samples with genotype TT were observed to have increase c-IMT level at evaluation in 1st, 6th and 12th month.

Conclusions: The presence of homozygote TT of MTHFR C677T may contribute to increase c-IMT level. However, this study found no association between MTHFR C677T with hyperhomocysteinemia as well as an increase in c-IMT in post-ischemic stroke patients.

Keywords: Methylenetetrahydrofolate reductase (MTHFR); homocysteine (Hcy); c-IMT; post-ischemic stroke

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AB069. Effect of osteogenesis imperfecta on children and their families

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Abstract: Osteogenesis imperfecta (OI) is a heterogeneous genetic disorder, with features that include increased bone fragility, pathological fractures, blue sclera, dentinogenesis imperfecta and conductive or mixed hearing loss. Clinical variability is wide from children with few fractures and normal stature to children with multiple fractures, long bone deformity, scoliosis and extreme short stature. Although there is no curative treatment, there are several therapeutic tools capable of improving the course of the condition and patient quality of life. We aim to evaluate the effect of OI on the well-being of children with the disorder and their families through a family-centered questionnaire. Sixty children with OI from the Vietnam National Hospital of Pediatrics and/or their parent(s), who attended the first annual family support group in 2011, completed a child and parent questionnaire. Sixty patients participated, 26 female and 34 male. The median age was 6.0 years [interquartile range (IQR), 0.25-18 years]. Of these, 36 (60%) had dentinogenesis imperfect and 23 (38.3%) had a scoliosis. The median number of fractures

was 6.0 (IQR 0-30) and median number of hospitalizations due to OI was 5.0 (IQR 0-30). Among patients of school age, 9 (15%) could not go to school due to OI. Almost all parents (93.7%) worried about school social communication of the patients. Among these parents, 100% fear of inferiority with friends and 98.3% fear of broken bones. Most parents (76.2%) were significantly concerned about their child's health. The parents' themselves reported psychological concerns, with feelings of desperation (58.4%), anxiety (81.7%) and depression (56.7%). OI appeared to have a significant deleterious effect on the life of the patients and their families. These data provide a baseline from which to evaluate the effectiveness of interventions to improve the medical and psychological needs of this cohort and their families.

Keywords: Osteogenesis imperfecta (OI); children; parents; interventions; psychological concerns

Cite this abstract as: Dung VC, Armstrong K, Ngoc CT, Thao BP, Khanh NN, Trang NT, Hoan NT, Dat NP, Munns C. Effect of osteogenesis imperfecta on children and their families. Ann Transl Med 2015;3(S2):AB069. doi: 10.3978/j.issn.2305-5839.2015.AB069

AB070. Mutations of SRD5A2 in Vietnamese patients: phenotype and genotype

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Abstract: A rare form of the 46,XY disorders of sex development (DSD), 5α-reductase deficiency was first described in patients with pseudovaginal perineoscrotal hypospadias, microphallus, and cryptorchid testes in 1974 by Imperato-McGinley *et al*. and Walsh *et al*. This undervirilization in the male is due to an alteration in the 5α-reductase type 2 gene (*SRD5A2*), which encodes for 5α-reductase activity. Our registry of 750 patients with DSD showed no definitive diagnosis in 80% of cases with 46,XY DSD. Our aim is to identify mutations in *SRD5A2* gene and to describe phenotype of detected mutative cases. Mutation analysis was performed for genomic DNA extracted from WBC of 10 patients with 46,XY DSD using PCR and direct sequencing. We identified mutations of *SRD5A2* gene in two cases. The first case presented with isolated micropenis at birth, two palpable testes in the normal scrotum. Pelvic ultrasound showed no ovaries and uterus, karyotype was 46,XY and SRY was positive. Serum FSH level was 2.4 UI/L; LH level was 0.9 UI/L and testosterone level was 0.4 nmol/L at 8 years of age. A homozygous missense mutation (p.R237G) was identified in the *SRD5A2* gene. The second case presented with microphallus, penoscrotal hypospadias, and gonad bilateral in labioscrotal folds. No uterus and ovaries were found by pelvic ultrasound. Karyotype was 46,XY and SRY was positive. A novel homozygous missense mutation (c.659C>T; p.S220L) was identified in the *SRD5A2* gene. Mutation analysis of *SRD5A2* gene helps to make definitive diagnosis for patients with 46,XY DSD.

Keywords: Disorders of sex development (DSD); *SRD5A2* gene; phenotype; genotype

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AB071. Mutations of *AR* gene in Vietnamese patients: genotype and phenotype

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Abstract: Androgen insensitivity syndrome (AIS) is the most common specific cause of 46,XY disorder in sex development. The androgen signaling pathway is complex but so far, the only gene linked with AIS is the androgen

receptor (AR). Mutations in the *AR* are found in most subjects with complete AIS but in partial AIS, the rate has varied 28-73%, depending on the case selection. More than over 800 entries of mutations causing AIS, representing over 500 different *AR* mutations from more than 850 patients with AIS have been reported. We aim to describe clinical manifestations and to identify mutation of *AR* in Vietnamese patients with AIS. This case series study included 12 patients from 9 unrelated families with AIS. The gonadal position and external genitalia were evaluated clinically and using ultrasound. The mutation analysis of *AR* was performed using PCR and direct sequencing. The age of diagnosis was 1 to 83 years old. 8/12 cases were complete androgen insensitivity syndrome (CAIS) (female external genitalia) and 4 cases were predominantly female partial AIS phenotype. Four cases had two labial testes, six cases had inguinal testes and two cases had abdominal testes. Five different mutations of *AR* were identified from seven cases of three unrelated families including three novel ones. The novel missense mutation p.L701F (c.2103G > T) was identified in a patient of 83 years of age. The novel missense mutation p.L705F (c.2113C > T) was identified in two sibs. The novel mutation p. W752S (c.2256G > T) was identified in a child with CAIS phenotype and had family history. The reported missense mutation p.V747M was identified in two sibs. The reported mutation p.V867M (c.2599G > A) was identified in a child with female phenotype. Our study identified three novel and two reported mutation in the *AR* gene that may provide us new insights into the molecular mechanisms of AIS. The expanded database of these mutations should benefit patients in the diagnosis and treatment of this syndrome.

Keywords: Androgen insensitivity syndrome (AIS); 46,XY disorder; sex development; *AR* gene

Cite this abstract as: Dung VC, Fukami M, Ngoc CT, Thao BP, Khanh NN, Nga PT, Dat NP, Ogata T. Mutations of *AR* gene in Vietnamese patients: genotype and phenotype. Ann Transl Med 2015;3(S2):AB071. doi: 10.3978/j.issn.2305-5839.2015.AB071

AB072. Novel mutation in the hepatocyte nuclear factor 1b/maturity-onset diabetes of the young type 5 gene—unreported Vietnamese case

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Abstract: Maturity-onset diabetes of the young type 5 (MODY5), a type of dominantly inherited diabetes mellitus and nephropathy, has been associated with mutations of the hepatocyte nuclear factor-1 (*HNF-1β*) gene, mostly generating truncated protein. Various phenotypes are related to *HNF-1β* mutations. Our aim to describe clinical and genetic findings in the unreported Vietnamese case identified with *HNF-1β* mutations. The proband with kidney failure from 7.5 years of age and diabetes diagnosed at 13.5 years of age who were described. Case report included information: characteristics of diabetes, renal function and structure, pancreas structure. Genomic DNA was extracted from WBC of whole blood and *HNF-1β* mutation was performed using PCR and direct sequencing. The proband is heterozygous for a novel *HNF-1β* missense mutation (c.505T > C; p.Y169H). This mutation results in the substitution of the amino acid histidine (charged polar) for tyrosine (uncharged polar) at codon 169. The tyrosine residue is conserved across species and it is therefore likely that the p.Y169H mutation is pathogenic. This result is consistent with a diagnosis of renal cysts and diabetes syndrome (RCAD). Testing was done for proband's parents and no mutation was found in *HNF-1β*. It is therefore likely that the p.Y169H mutation has arisen *de novo*. Kidney MRI showed right kidney atrophy and pancreas MRI showed only tissue of head of pancreas. Investigations at 14.5 years of age—diagnosed diabetes showed: plasma urea 10.1 mmol/L; creatinine 250 micrommol/L; HbA1C 13.6%. He was given insulin of 0.8 UI/kg/day and HbA1C was 6.8% after 1 year of treatment with insulin injection. Maturity-onset diabetes of the young type 5 encompasses a wide clinical spectrum. Analysis for mutations of *HNF-1β* is warranted, even without a family history of diabetes, in nonobese patients with diabetes and slowly progressive non

diabetic nephropathy, particularly when pancreatic atrophy.

Keywords: Maturity-onset diabetes of the young type 5 (MODY5); hepatocyte nuclear factor-1 (*HNF-1β*) gene; mutations; kidney failure; diabetes

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AB073. Mutations in the type II 3β-hydroxysteroid dehydrogenase gene caused primary adrenal insufficiency & 46,XY disorders of sex development

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Abstract: Congenital adrenal hyperplasia (CAH) is one of the most common inherited metabolic disorders. It includes a group of autosomal recessive disorders caused by the deficiency of one of the enzymes involved in one of the various steps of adrenal steroid synthesis. 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency is a rare cause of CAH caused by inactivating mutations in the *HSD3B2* gene. Most mutations are located within domains regarded crucial for enzyme function. Our aim is to describe phenotype and to identify mutations of *HSD3B2* in two classic β-HSD deficient patients belonging to two apparently unrelated pedigrees. This is a case series study. Family history and clinical manifestations were described. Genomic DNA from these patients was extracted using standard procedures from the peripheral blood leukocytes. Mutation analysis of *HSD3B2* was performed using polymerase chain reaction (PCR) and DNA direct sequencing. Vietnamese 46,XY newborn referred at 2.5th month of life with salt loss associated with hyponatremia (123 nmol/L) and hyperpigmentation. The testes were palpable in the scrotum but associated with a severe hypospadias (micropenis 0.5 cm; posterior). At 4 months of age, a second adrenal crisis has occurred with hyponatremia 127 nmol/L and increased 17OH-Progesterone (26.8 ng/mL) in this 46,XY DSD. This clinical and biological data associated with a sibling with female phenotype deceased at 18 months old after adrenal crisis (1st occurred at 7 days of life) suggest the diagnosis of 3β-HSD deficiency. The sequencing of *HSD3B2* confirms the diagnosis because he is homozygous for a missense mutation, pAla161Pro. This mutation affects an amino acid conserved in all species and is located in one two alpha-helix involved in the dimerization of the two sub-units of the enzyme. The changing from Alanine to proline could break the alpha-helix. The same mutation has been found in the other Vietnamese family. The 46,XY newborn referred at 3th month of life with severe dehydration associated with hyponatremia (93 nmol/L) and hyperpigmentation. The testes were palpable in the scrotum but associated with a severe hypospadias (micropenis 0.5 cm; posterior). Clinical presentation and increased 17OH-progesterone (9.7 ng/mL) in this 46,XY DSD suggest the diagnosis of 3β-HSD deficiency. The sequencing of *HSD3B2* also confirms the diagnosis because he is homozygous for a missense mutation, pAla161Pro. The severity of this mutation correlates well with the phenotype in these patients. Parents of two unrelated pedigrees are not consanguinity. This study contributes to a better understanding of the molecular defects of 3β-HSD and of the phenotypic heterogeneity of CAH related to 3β-HSD deficiency.

Keywords: Congenital adrenal hyperplasia (CAH); 3β-hydroxysteroid dehydrogenase (3β-HSD) deficiency; *HSD3B2* gene; phenotype; mutation

Cite this abstract as: Dung VC, Thao BP, Khanh NN, Ngoc CT, Morel Y. Mutations in the type II 3β-hydroxysteroid dehydrogenase gene caused primary adrenal insufficiency & 46,XY disorders of sex development. Ann Transl Med 2015;3(S2):AB073. doi: 10.3978/j.issn.2305-5839.2015.AB073

AB074. Registry of congenital adrenal hyperplasia at the north pediatric referral centre of Vietnam during 15 years

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Abstract: The National Hospital of Pediatrics (NHP), Hanoi, Vietnam is a 1,200 bed tertiary referral centre servicing approximately 30 million people from northern provinces of Vietnam. This audit was undertaken to analyze anecdotal reports of increasing patient numbers. Retrospective review of all congenital adrenal hyperplasia (CAH) patients registered at NHP from 1999-2014. Ethical clearance was granted by the NHP Directorate. At the start of 1999 there were 90 children with CAH managed at NHP. By May 2014 this increased to 715 including 375 (52%) male patients and 340 (48%) female patients. Number of cases with 21α-hydroxylase deficiency (21-OHD), 11β-hydroxylase deficiency and 3β-hydroxysteroid dehydrogenase deficiency was 703 (98.3%); 9 (1.3%) and 3 (0.4%), respectively. Among cases with 21-OHD, 72% were salt wasting and 28% were simple virilisation). Total number of cases represents a more than seven folds increase over 14 years. Number of new cases doubled from 30 to 67 in 2013. Most children (85%) were diagnosed at less than 12 months of age (55% at less than 1 month of age); 70% of all children were younger than 10 years. Formal mortality figures were low (seven known deaths). The caseload of CAH at NHP has increased since 1999 and additional capacity is needed for patient care. Introduction of NBS would enable more accurate estimation of CAH incidence, reduce infant mortality and minimize trauma to affected infants and their families.

Keywords: Congenital adrenal hyperplasia (CAH); pediatrics; Vietnam

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AB075. Mutations of *WT1* gene caused 46,XY disorder of sex development and Wilms' tumor

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Abstract: The Wilms' tumor suppressor gene (*WT1*) is a transcription factor that plays a major role in development of the gonads and kidneys. It is expressed even earlier than sex-determining region of the Y chromosome in the urogenital ridge from which the gonads and kidneys are derived. *WT1* mutations will impair gonadal and urinary tract development and have been demonstrated to cause syndromes of WAGR, Denys-Drash and Fraiser. In this study, our aim is to identify mutation in *WT1* gene and to describe clinical features of a Vietnamese patient with 46,XY disorder of sex development (DSD) associated with Wilms' tumor. DNA was extracted from WBC and mutation analysis of *WT1* gene was performed using PCR and direct sequencing. A 5-day newborn presented with penoscrotal hypospadias, microphallus, right testis in the right inguinal and no left testis was found. Karyotype was 46,XY and no ovaries and uterus were found using pelvic ultrasound. Wilms' tumor was detected at 13 months of age by abdominal ultrasound and CT scan. Mutation analysis was identified a heterozygous missense mutation (c.1390G > A; p.D464N) in exon 9 of *WT1* gene. In conclusions, WT1 analysis should be performed in newborns with complex hypospadias with at least one cryptorchid testis and in isolated 46,XY partial to complete gonadal dysgenesis. WT1 analysis is mandatory in all 46,XY DSD with associated kidney disease. Patients with *WT1* mutations should be followed up closely because the risk of developing a Wilms' tumor, nephropathy.

Keywords: Wilms' tumor suppressor gene (*WT1* gene); 46,XY disorder; sex development; nephropathy; Wilms' tumor

Cite this abstract as: Dung VC, Thao BP, Hai LT, Fukami M. Mutations of *WT1* gene caused 46,XY disorder of sex development and Wilms' tumor. Ann Transl Med 2015;3(S2):AB075. doi: 10.3978/j.issn.2305-5839.2015.AB075

AB076. Congenital hyperinsulinism due to mutation of *HNF4A*: a case report

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Background: Hyperinsulinemic hypoglycemia (HH) is the commonest cause of persistent hypoglycemia in the neonatal and infancy periods. *HNF4A* mutations are the third most common cause of diazoxide responsive congenital hyperinsulinism. Individuals carrying *HNF4A* mutations that result in familial monogenic diabetes later in life can present in early infancy with hyperinsulinism. The study was to describe a child associated with HH due to mutations in the *HNF4A* gene.
Methods: Clinical data were obtained from chart review. Gene sequencing of *HNF4A* was performed.
Results: At the National Hospital of Pediatric from January 2007 to April 2015. We have 68 cases with congenital hyperinsulinism diagnosed and molecular analysis. Mutation *HNF4A* gene found 1/68 cases (1.47%). A boy born large for gestational age (birth weight 4,700 g with 40 gestation weeks) after an uneventful pregnancy, presented 24 h after birth with lethargy, poor feeding, cyanosis, no seizure caused by severe hypoglycaemia (serum glucose 0.5 mmol/L). Hypoglycaemia was treated with 2 mL/kg 10% glucose i.v. followed by a continuous i.v. glucose infusion (9.57 mg/kg/min) to maintain serum glucose above 3 mmol/L. Laboratory investigations at the time of hypoglycaemia showed inadequate suppression insulinemia (57.9 pmol/L) and C-peptide (0.38 nmol/L), negative ketone bodies, absent urine ketone bodies, hyperammonemia (365.1 µg/dL), hypofattyacidaemic. His mother had three pregnancies [the first boy born with 35 gestational weeks (birth weight 2,800 g), died 2 days after birth with cyanosis unknown causes, the second 28 gestational weeks boy, died some hours after birth with cyanosis unknown causes]. A diagnosis of 'hyperinsulinism' was made. Molecular analysis showed he is heterozygous for a novel *HNF4A* missense mutation on location Exon 6, protein description p.Leu220Pro (p.L220P) from his mother. The leucine residue at codon 220 is highly conserved across species and it is therefore likely, although not certain, that the p.L220P mutation is pathogenic. Treatment was started with diazoxide (10 mg/kg per day in three doses) and had good response and stopped diazoxide after 2 weeks with normal glucose level and normal. Up to now, he is 5 years old with normal growth and development, normal glucose level.
Conclusions: Babies who inherit the *HNF4A* gene often have a high birth weight (over 4 kg) and may have low blood sugars early in life (neonatal hypoglycaemia) needing treatment immediately after birth. *HNF4A* mutations responses well to diazoxide.

Keywords: Hyperinsulinism; *HNF4A*

Cite this abstract as: Duong DA, Dung VC, Dat NP, Ngoc CT, Thao BP, Khanh NN, Dien TM. Congenital hyperinsulinism due to mutation of *HNF4A*: a case report. Ann Transl Med 2015;3(S2):AB076. doi: 10.3978/j.issn.2305-5839.2015.AB076

AB077. Clinical symptoms, molecular genetics, genotype and phenotype correlations of children with congenital hyperinsulinism

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Background and objective: Congenital hyperinsulinism (HI) causes severe hypoglycemia in neonates and infants. Molecular genetic results is very important which help clinicians will have suitable treatment. The study aims to describe clinical symptoms, signs of HI patients and to identify mutations in the *ABCC8* and *KCNJ11*, *HNF4A* and *GLUD* genes, genotype and phenotype correlations of children with HI.
Methods: A prospective study was conducted on 68 cases with congenital HI diagnosed and treated in National Hospital of Pediatric from January 2007 to April 2015. Patients were selected by using inclusion criteria of Hussain K [2008]. During the work-up clinical, biochemal was collected. Genomic DNA was extracted from peripheral leukocytes using standard procedures. Single exon of *KCNJ11*; 39 exons of *ABCC8*; were amplified & sequenced. Sequencing reactions were analyzed on an ABI 3730 capillary sequencer & were compared to published sequences using Mutation Surveyor version 3.24.

Results: Major clinical symptoms, signs of HI patients when hypoglycemia are: lethargy (69.12%), poor feeding (66.2%), cyanosis (57.4%), ear hair (52.9%), seizure (42.6%), grunting (42.7%), apnea (23.5%), hypotonia (27.9%), diaphoresis (19.12%), unconsciousness (11.7%), hypothermia (2.9%). Glucose level on admission 0.99±0.94 mmol/L, insulin level and C-peptid when hypoglycemia are 214.2±190.6 pmol/L and 1.78±1.5 nmol/L. Gene mutations were detected in 64.29% of cases including mutation of genes *ABCC8* (88.89%), *KCNJ11* (8.33%), *HNF4A* (2.78%). Mutation of *ABCC8* included homozygous mutations (25%), compound heterozygous mutation (31.25%), one dominant mutation from father (40.63%), one dominant mutation from mother (3.13%). All cases with homozygous mutations, 83.3% of cases with compound heterozygous mutation and 83.3% of cases with one dominant mutation of *ABCC8* gene from father did not respond to diazoxide treatment and required 95% pancreatectomy. Other cases with non-mutation usual respond to diazoxide.

Conclusions: Children with congenital HI causes severe hypoglycemia in neonates and infants with clinical symptoms, signs of hypoglycemia are changeful and not specific for mutation or no mutation. So, children with HI should be analyzed for identifying mutations which helps in making diagnosis and suitable treatment decision.

Keywords: Hyperinsulinism (HI); hypoglycemia

Cite this abstract as: Duong DA, Dung VC, Dat NP, Ngoc CT, Thao BP, Khanh NN, Dien TM. Clinical symptoms, molecular genetics, genotype and phenotype correlations of children with congenital hyperinsulinism. Ann Transl Med 2015;3(S2):AB077. doi: 10.3978/j.issn.2305-5839.2015.AB077

AB078. Novel mutation of *ABCC8* and *KCNJ11* of children with congenital hyperinsulinism

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Background and objective: Congenital hyperinsulinism (HI) causes severe hypoglycemia in neonates and infants. To date, more than 350 mutations have been reported in HI patients. However, the genetic screening has failed to define the genetic basis of disease in more than 18% of the cases, demonstrating that pathogenic mechanisms of HI have not been completely elucidated. Patients with HI can have novel mutations that have been announced. The study aims to describe novel mutations of *ABCC8* and *KCNJ11* of children with HI.

Methods: A prospective study was conducted on 68 cases with HI diagnosed and treated in National Hospital of Pediatric from January 2007 to April 2015. Patients were selected by using inclusion criteria of Hussain K [2008]. Genomic DNA was extracted from peripheral leukocytes using standard procedures. Single exon of *KCNJ11*; 39 exons of *ABCC8*; were amplified & sequenced. Sequencing reactions were analyzed on an ABI 3730 capillary sequencer & were compared to published sequences using Mutation Surveyor version 3.24.

Results: In the group cases have *ABCC8* mutations (reported mutations are 81.25%, novel mutations are 18.75%), *KCNJ11* mutations (reported mutations are 33.33%, novel mutations are 66.67%).

Conclusions: Mutation of *ABCC8* and *KCNJ11* are common causes of HI. Children with congenital HI causes severe hypoglycemia in neonates and infants with clinical symptoms, signs of hypoglycemia are changeful and not specific for mutation or no mutation. So, children with HI should be analyzed for identifying mutations which helps in making diagnosis and suitable treatment decision.

Keywords: Hyperinsulinism (HI); hypoglycemia

Cite this abstract as: Duong DA, Dung VC, Dat NP, Ngoc CT, Thao BP, Khanh NN, Dien TM. Novel mutation of *ABCC8* and *KCNJ11* of children with congenital hyperinsulinism. Ann Transl Med 2015;3(S2):AB078. doi: 10.3978/j.issn.2305-5839.2015.AB078

AB079. Phenotype variation in untreated 46,XX congenital adrenal hyperplasia

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Abstract: Simple virilizing congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder characterized by 21 hydroxylase deficiency leading to excessive androgen production. In infants with 46,XX karyotype, prenatal exposure of androgen overproduction leads to a gradual virilization of the external genital. Consequently, babies are born with an ambiguous genital which complicates sex assignment. Genital virilization will be progressive if these babies remain untreated. In country where newborn screening is available, patients with CAH are identified soon after birth and receive medication afterward, unlike in Indonesia. Many parents and patients with CAH did not seek healthcare professionals due to lack of information about inheritance and clinical manifestation of CAH. In Indonesia, newborn screening has not been applied yet; management and therapy for CAH are only available in some big cities but glucocorticoid medications need to be imported. Diagnosis was established using karyotype, hormonal and gene mutation analysis. In this study, we reported 30 patients with different physical appearance i.e., big phallus, one or two ending perineum, very severe chordae, labioscrotal fusion, complete labial fusion with scrotalization, masculization, no breast development, severe hyperpigmentation, appears Adam's apple and short stature. Sex assignment is still a dilemma; some of the children were raised as males, females, or left undefined. The complex management of children and adults with CAH highlights the importance of raising awareness among medical personnels to promote early detection and treatment for CAH patients. Genetic counseling is essential for these families.

Keywords: Congenital adrenal hyperplasia (CAH); untreated; Indonesia

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AB080. Genetic findings provide insight of biliary atresia patient complexity

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Background: Biliary atresia (BA) is a rare complex disease with unknown etiology. Current treatment of BA is Kasai portoenterostomy but is ineffective. BA is now the most common cause of pediatric liver transplantation worldwide. Characterize the disease complexity and stratify patients for personalized medicine is necessary. Genetic variants underlie BA pathogenesis and yet comprehensive genotype-phenotype correlations are yet to be investigated.

Methods: We first reviewed the disease course of 89 isolated BA patients with long term follow up (median =17.2 years), whose blood DNA was genotyped on Affymetrix5.0. Copy number variants (CNVs) and single nucleotide polymorphisms (SNPs) were called. Meanwhile 23 BA patients' liver DNA was submitted to exome sequencing for discovery of *de novo* mutations. After wise we narrowed the genotype callings down to BA unique mutations, i.e., CNVs, SNV/INDELs, and BA-associated genes through gene-based association test of SNPs, then genotype-phenotype correlations were interrogated. Last, interconnectivity among the candidate genes were examined, topology of the molecular network was then interrogated correlating to the BA clinical complexity.

Results: Clinical revision revealed that 41.57% isolated BAs had chronic extra-hepatic diseases, with high prevalence of autoimmune-atopic diseases (22.47%) and glucose-6-phosphate dehydrogenase deficiency (14.29% in males). In

genotype data, we shortlisted 29 CNVs ≥100 kb private to BA and related sted 29 (I) *de novo* BA-CNVs, perturbing genes known to hepato-biliary diseases, associated with BA liver pathology; (II) three BA-CNVs encompassing genes known to immunity defects, correlated with comorbidities of those immune disorders in three carriers, and overall BA-CNVs intersected 'immunologically-important' genes (P=0.017). Biologically BA-CNVs are anchored to other BA candidate genes as interactions were observed between genes encompassed by BA-CNVs (N=102) and BA-associated genes tagged by SNPs (N=103) (empirical P=0.039). Additional SNV/INDELs associated with intrahepatic biliary anomalies were uncovered in exome-sequencing. All together the BA candidate genes converge into a molecular network with inflammatory regulators as the signalling hub, moreover, the network fell into multiple function modules, which coincides with the BA patients' clinical profile.

Conclusions: Genetic variants underpin BA clinical manifestations. The BA-associated common and rare genetic converge in a molecular network, which support the plausible associations of BA with a host of 'non-BA' diseases, as supported by observation in patients comorbidities with non-BA disease. We propose this 'diseasome' network approach that integrates clinical/epidemiological data and BA genetic findings to decode the phenotypic complexity of this rare disease.

Keywords: Rare complex disease; patient complexity; genetic screening; genotype-phenotype correlation; network

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AB081. Acute encephalopathy in Dravet syndrome: two case reports and discussion of risk factors

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Background: Acute encephalopathy has been sporadically reported in patients with Dravet syndrome. However, risk factors for this important complication in Dravet syndrome are not clear yet.

Methods: We describe two patients who had clinical diagnosis of Dravet syndrome and experienced acute encephalopathy initiated by refractory status epilepticus.

Results: SCN1A mutational analysis by direct sequencing and multiplex ligation-dependent probe amplification revealed a previously reported *de novo* heterozygous nonsense mutation in one patient and a novel *de novo* homozygous missense mutation in the other. The analysis of clinical features of our cases and previously published cases showed that an earlier age of onset (6 months of age or less) and a more typical phenotype of Dravet syndrome, including the presence of myoclonic seizures and status epilepticus, are possible phenotypic risk factors for Dravet syndrome patients to complicate acute encephalopathy.

Conclusions: We have identified, for the first time, a *de novo* homozygous missense mutation in *SCN1A* gene. We also review literature and discuss some possible phenotypic risk factors for patients with Dravet syndrome to develop acute encephalopathy.

Keywords: Dravet syndrome; acute encephalopathy; Vietnamese

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AB082. Phenotype and genotype of Vietnamese patients with mucopolysaccharidosis II: first case series report

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Background and objective: Mucopolysaccharidosis II (MPS II, Hunter syndrome, OMIM 309900) is an X-linked lysosomal storage disorder. MPS II is caused by a deficiency in the enzyme iduronate-2 sulfatase (I2S), leading to the accumulation of the glycosaminoglycans (GAGs) dermatan sulfate and heparan sulfate in lysosomes. Excessive storage of these GAGs causes a variety of clinical manifestations: coarse facies, hearing loss, cardiac valve disease, restrictive and obstructive airway disease, hepatosplenomegaly, skeletal abnormalities, joint contractures, short stature. The study aims to describe clinical characteristics and to identify mutations in the *IDS* gene in Vietnamese patients with MPS II.

Methods: This case series report including 18 cases with MPS II diagnosed and treated at the National Hospital of Pediatric, Vietnam from December 2012 to May 2015. We describe clinical manifestations, radiological, biochemical evaluations and identified mutations of *IDS* gene of the patients confirmed by enzyme assay. Nine exons and their intronic boundaries of the *IDS* gene were sequenced using genomic DNA from the patient. Subsequently, to identify the recombination event with pseudogene, PCR analysis was carried out.

Results: Mean age of diagnosis was 8.2±5.9 years. The clinical symptoms included coarsened facial features (100%); mental retardation and joint stiffness (88.89%); bone deformation (66.67%); hepatomegaly (33.33%); valvular heart disease (22.22%); hearing loss (16.67%); obstructive airway disease (100%). Mutations of *IDS* gene were identified in 14/18 of cases (77.8%) including six cases (33.33%) had recombination event. Three reported causative mutations were identified: c.120-122del (p.L41del); c.1001A > G (p.D334G); c.879G > C (p.Q293H); and five novel one were identified in this study: c.166dup (p.D56Gfs*2); c.1124-1128dup (p.L377Gfs*10); c.473del (p.Y158fs); c.814C > T (p.Q272*) and c.1048A > T (p.N350Y).

Conclusions: Description of clinical characteristics to predict severity of phenotype and identification of mutations in the *IDS* gene help in making diagnosis and suitable treatment decision.

Keywords: Mucopolysaccharidosis II (MPS II); *IDS* gene

Cite this abstract as: Hang le TT, Dung VC, Tomatsu S, Yen NT, Hung TT, Ngoc CT, Nguyen NK, Hwu WL, Ki GH, Yoo HW. Phenotype and genotype of Vietnamese patients with mucopolysaccharidosis II: first case series report. Ann Transl Med 2015;3(S2):AB082. doi: 10.3978/j.issn.2305-5839.2015.AB082

AB083. A cause of cholestasis and hepatic failure in children: neonatal intrahepatic cholestasis cause by citrin deficiency

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Background and objective: Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) is rare disease. It is a novel metabolism disease which caused by deficiency of citrin, a liver-type mitochondrial aspartate-glutamate carrier encoded by the *SLC25A13* gene. Citrin deficiency causes NICCD and adult-onset type II citrullinemia (CTLN2) with severe hepatic-neurology syndrome. The study presents some clinical features, laboratory finding, results molecular analysis and following process of 96 NICCD.

Methods: Prospective description study.

Results: Two hundred and thirty-six patients, who had

hepatic troubles and were diagnosed *SLC25A13* mutations by PCR/PCR-RFLP enrolled in this study. There were 96 in 236 patients were diagnosed NICCD by molecular analysis. Some NICCD clinical manifestations include: Jaundice (95.8%), hepatomegaly (31.3%), steatorrhea (89.6%), chubby face (88.5%), splenomegaly (29.4%), and faint (2.1%). Laboratory finding: Hyperbilirubinemia (95.8%), increase AST (100%), ALT (88.5%), AST/ALT ratio >2.5 (89.6%), coagulation disorder (87.5%), hypoproteinemia (82.3%), hypoalbuminemia (84.4%), hyperammonemia (92.7%), 100% patients had elevation of AFP and 70.8% had increase of citrullin. DNA analysis of *SLC25A13* revealed combinations of 851del4, 1638ins23, IVS6 + 5G > A and IVS16ins3kb with 5 genotypes, 89 homozygous and 7 compound heterozygous. No relation between phenotype and genotype has been found. With supportive treatment and nutritional manipulation most of patients in group recovered completely by the age 18 months. However, there were eight patients had died of uncompensative hepatic failure.

Conclusions: NICCD should be considered in the differential diagnosis of cholestatic and hepatic failure infants. Phenotype of NICCD is very polymorphic and not always benign. All NICCD should be long term followed up.

Keywords: Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD); *SLC25A13*; cholestasis; hepatic failure

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AB084. Cause of death and clinical characteristics of 34 mortality patients with mucopolysaccharidosis II in Taiwan, 1995-2012

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Background and objective: Mucopolysaccharidosis type II (MPS II; Hunter syndrome; OMIM +309900) is an X-linked recessive, multisystemic lysosomal storage disorder caused by iduronate-2-sulfatase (I2S) deficiency, which catalyzes a step in the catabolism of glycosaminoglycans (GAGs). It leads to accumulation of GAGs in the lysosomes of many organs and tissues, causing progressive cellular dysfunction. MPS II has a variable age of onset and variable rate of progression. In Asian countries, there is a relatively higher incidence of MPS II compared to other types of MPS. The study aims to delineate the cause of death and natural history of Taiwanese patients with MPS II.

Methods: A retrospective analysis was carried out of 34 Taiwanese MPS II patients who died between 1995 and 2012. The clinical characteristics, medical records, age at death, and cause of death were evaluated to better

understand the natural progression of this disease.

Results: Among these 34 mortality patients, 31 were severe form with significant cognitive impairment, two were mild form without cognitive involvement, and one was intermediate form. The mean age at death was 14.2±4.2 years. The mean ages at onset of symptoms and confirmed diagnosis were 2.5±2.1 and 4.8±3.1 years, respectively (n=32). The mean gestational age and birth weight were 39.2±1.8 weeks and 3,522±581 grams, respectively (n=24). The mean age at death of 31 severe form was 13.2±3.2 years, compared with 22.6±4.3 years of three patients with mild or intermediate forms (P<0.001). Among the 27 patients with available records of primary cause of death, respiratory failure was the leading cause (70%), followed by cardiac failure (22%), post-traumatic organ failure (4%), and infection (sepsis) (4%). Age at onset of symptoms was positively correlated with life expectancy (P<0.01). The longevity also increased gradually over time between 1995 and 2012 (P<0.05).

Conclusions: The life expectancy of Taiwanese MPS II patients has improved in recent decades. With the implementation of National Health Insurance in Taiwan since 1995, it is possible that referral of patients to specialist and improvement in multidisciplinary care underlie this trend. These findings can be used to develop quality of care strategies for these patients.

Keywords: Cause of death; Hunter syndrome; mortality; mucopolysaccharidosis II (MPS II); Taiwan

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AB085. Imprinting mutation of CDKN1C in Beckwith-Wiedemann Syndrome: inheritance, genetic counselling and surveillance

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Background: Beckwith-Wiedemann Syndrome (BWS), a genetic overgrowth disorder is typified by exomphalos, macroglossia and neonatal gigantism. The molecular basis is known in approximately 80% of patients and is heterogeneous involving epigenetic and genetic changes at chromosome 11p15.5. An uncommon cause is a point mutation at CDKN1C found in approximately 5% of cases. When found, 1/3 of CDKN1C mutation is familial. We describe the first Malaysian family with CDKN1C mutation c.232C > T (Q78X), their clinical features, issues related to genetic counselling and subsequent follow-up.

Case presentation: Fifteen children fulfilling the clinical criteria for the diagnosis of BWS were included in a research study to uncover their genotype. One patient was found to carry the CDKN1C mutation c.232C > T (Q78X). This patient was the first child born to unrelated parents at 30+6/40 gestation. He was large for gestational age with a birth weight of 2.21 kg. He had an exomphalos, bilateral dysplastic kidneys and facial dysmorphism consistent with BWS. After a stormy neonatal period, he succumbed on day 17 of life. Before his molecular analysis was completed, his mother gave birth to a girl at 37+1/40 gestation; birth weight was 3.4 kg. This child was antenatally diagnosed with exomphalos and amniocentesis revealed normal karyotype. At birth, she had facial features of BWS, cleft palate and normal kidneys. Her exomphalos was surgically corrected on day 3 of life, after which she progressed well albeit with mild developmental delay. Their mother is phenotypically normal and carries the said pathogenic CDKN1C mutation. She is currently pregnant with her third child. Genetic counselling was provided and she fully comprehends the recurrence risk of 50% in this pregnancy

as well as the availability of prenatal diagnostic testing. Prenatal testing was declined.

Discussion and conclusions: The diagnosis of BWS can be confidently achieved with well-established clinical criteria. However, molecular diagnosis is of utmost importance for accurate genetic counselling because of the high recurrence risk of maternally inherited CDKN1C point mutation. Surveillance on follow-up can also be tailored with the knowledge of molecular diagnosis as CDKN1C mutation is associated with the lowest risk of embryonal tumours commonly associated with the other genotype. In this report, we have shown a phenotypically unaffected mother with a pathogenic CDKN1C mutation. This mutation could have occurred *de novo* or inherited from her father as it is silent in the paternal allele. In the latter scenario, genetic counselling should be offered to all her sisters so that they may make informed choices with regards to their reproduction.

Keywords: CDKN1C; imprinting; overgrowth

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AB086. Chromosomal microarray analysis—detection of both duplication and deletion in patients with multiple congenital anomalies and/or developmental delay

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Background and objective: Chromosomal microarray analysis (CMA) is recommended as first-tier genetic testing for patients with multiple congenital anomalies, developmental delay/intellectual disability and/or autism spectrum disorder. It detects chromosomal imbalance at a higher resolution than conventional chromosomal analysis. CMA diagnostic service was launched in our hospital in February 2014. The aim of this report is to review the incidence of detecting both duplication and deletion in patients referred for this test.

Methods: DNA was extracted using Gentra Puregene Blood Kit. CMA was performed using the Agilent 4×180 K CGH + SNP array and analysed with Agilent CytoGenomics. G-banding analysis was carried out on stimulated lymphocytes culture. Targeted fluorescence in-situ hybridization (FISH) was performed using locus specific probes.

Results: From 1 February 2014 to 31 May 2015, a total of 205 patients were tested. Seven (3.4%) were identified to have both duplication and deletion of chromosomal segments that were pathogenic [5] or of uncertain clinical significance [2]. We present a case of a 1-day-old Chinese girl with oligohydramnios, prematurity (35+5 weeks) and multiple congenital anomalies including heart defect, cleft palate, ear anomalies, microcephaly, vaginal skin tag, bilateral clinodactyly and wide anterior fontanelle. Karyotyping and FISH analysis for 22q11 deletion were normal. CMA revealed a pathogenic gain of 2.143 Mb at 16p13.3 and a pathogenic loss of 0.271 Mb at 16q24.2q24.3. The gain at 16p13.3 affects 67 genes including CREBBP. The 16p13.3 duplication syndrome is a contiguous gene syndrome characterized by normal to moderate intellectual disability, normal growth, mild arthrogryposis, frequently small and proximally implanted thumbs, characteristic facial features and occasionally, developmental defects of the heart, genitalia, palate or eyes. The 0.271 Mb deletion at 16q24.3 affects four genes including ANKRD11 and *CDH15*. The clinical features of 16q24.3 microdeletion syndrome include facial dysmorphisms, cognitive impairment, autism, structural anomalies of the brain and seizures. The patient's reported phenotypes overlap with clinical features seen in both the 16p13.3 duplication syndrome and the 16q24.3 microdeletion syndrome.

Conclusions: CMA helps to identify clinically significant chromosome anomalies that are too small to be detected by karyotyping. Of the seven cases reported with both duplication and deletion, six would not have been picked up by karyotyping. Through CMA, the hospital care team is able to make an accurate genetic diagnosis for the patients.

Keywords: Chromosomal microarray analysis (CMA); 16p13.3 duplication; 16q24.3 microdeletion

Cite this abstract as: Ee HJ, Yon HY, Tan ML, Roch R, Brett M, Yong MH, Law HY, Lai A. Chromosomal microarray analysis—detection of both duplication and deletion in patients with multiple congenital anomalies and/or developmental delay. Ann Transl Med 2015;3(S2):AB086. doi: 10.3978/j.issn.2305-5839.2015.AB086

AB087. Most common *SLC25A13* mutation in 695 Vietnamese patients with NICCD

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Background and objective: Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) which resulted from mutation in *SLC25A13* gene can present transient intrahepatic cholestasis, low birth weight, growth retardation, hypoproteinemia, prolong jaundice, chronic liver disease and so on. This study aimed to identify four mutations of *SLC25A13*.
Methods: Four common mutations termed as 851del4, IVS6 + 5G > A, 1638ins23, IVS16ins3kb in the NICCD patients were detected by DNA analysis.
Results: 851del4 was accounting for 90.5% in mutant allele. One hundred and sixty-nine patients have identified mutations, including 93 patients were 851del4 homozygotes, 62 patients were 851del4 heterozygotes, three patients was heterozygotes of single mutation 1638ins23, one patient was heterozygotes of single mutation IVS6+5G > A and two patients was compound heterozygotes of 1638ins23+851del4, two patients were compound heterozygotes (851del4 + IVS6 + 5G > A), six patients were compound heterozygotes (851del4 + IVS16ins3kb). Genotype of NICCD in Vietnam were 851del/851del4, 851del/1638ins23, 851del/IVS6 + 5G > A. 851del/IVS16ins3kb.
Conclusions: 851del4 was the most common mutation type and 851del/851del4 is the major genotype. We recommend that this mutation should be firstly screen for Vietnamese patients suspected NICCD caused by citrin deficiency.

Keywords: Citrin deficiency; neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD); citrullinemia; Vietnam

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AB088. Mutation analysis of 16 Vietnamses Wilson patients

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Background: Wilson disease (WD) is an autosomal recessive disorder of copper transport, which is caused by mutation in copper-transporting P-type ATPase (*ATP7B*).
Objective: The aim of this study was to detect mutations in hot-spot region of *ATP7B* gene, including exon 2b, 8, 11, 12, and 13. Sixteen unrelated WD patients were selected for this study.
Methods: Direct DNA sequencing was used to identify the mutation in *ATP7B* gene.
Results and conclusions: The results showed that 10/16 (62.5%) patients have been found with six different known mutations. Mutation detection rate of exon 2b (25%) is the highest, including six patients having c.314C > A (TCG > TAG, S105X) mutation and one patient having c.525insA (V176S-frameshift). The remaining mutations are c.2333G > T (CGG > CTG, R778L); c.2828G > A (GGT > GAT, G943D), c2954G > A (TGC > TAC, C985T) và c.3029A > C (AAG > ACG, K1010T). The most common mutation

is S105X in exon 2b, accounting for 21.9%. We strongly recommend that exon 2b should be screened firstly on Vietnamese Wilson patient, before sequencing analysis the whole gene.

Keywords: Wilson disease (WD); *ATP7B* gene; mutation analysis, Vietnam

Cite this abstract as: Nguyen TM, Ngoc ND, Nguyen TP, Hoa NP, Khánh TV, Văn TT, Van Chi P. Mutation analysis of 16 Vietnamses Wilson patients. Ann Transl Med 2015;3(S2):AB088. doi: 10.3978/j.issn.2305-5839.2015.AB088

AB089. Postnatal and prenatal diagnosis for neonatal intrahepatic cholestasis caused by citrin deficiency

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Background and objective: Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD) which resulted from mutation in *SLC25A13* gene can present transient intrahepatic cholestasis, low birth weight, growth retardation, hypoproteinemia, prolong jaundice, chronic liver disease and so on. This study aimed to identify mutations of *SLC25A13* for 649 NICCD and 46 siblings, from September 2009 to December 2014 and prenatal diagnosis for pregnancies with high risk of NICCD.
Methods: Detection four common termed as 851del4, IVS6 + 5G > A, 1638ins23, IVS16ins3kb for NICCD patients by DNA analysis (PCR-RFLP) and confirm by directly sequencing. The NICCD parents who had fetus then enrolled in DNA testing for the carrier. Prenatal diagnosis for their fetus was performed by PCR-RFLP following by amniocentesis and amniocyte culture.
Results: In 695 patients, 169 (24.3%) patients have identified mutations, of which 93 patients were 851del4 homozygote, 62 patients were 851del4 heterozygote, three patients were heterozygote of single mutation 1638ins23, one patient was heterozygote of single mutation IVS6 + 5G > A and two patients were compound heterozygote of 1638ins23 + 851del4, two patients were compound heterozygote (851del4 + IVS6 + 5G > A), six patients were compound heterozygote of (851del4 + IVS16ins3kb); 851del4 was the major mutation type, accounting for 76.3% in mutant allele, followed by c.1638ins23 (1.5%), IVS16ins3kb (1.8%), and IVS6 + 5G > A (0.9%); out of 10 couple enrolled DNA testing, two fathers revealed that they were homozygous mutation without any clinical figure and the others were carrier. Among four pregnancies having prenatal diagnosis, two fetuses were homozygote, one fetu was heterozygote and the remaining was normal.
Conclusions: 851del4 was the most common mutation in Vietnamese NICCD patients. Moreover, citrin deficiency prenatal diagnosis might open a novel area of clinical management for citrin deficiency in Vietnam.

Keywords: Neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD); *SLC25A13* gene; mutation; DNA analysis; citrin deficiency; Vietnam

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AB090. Molecular diagnosis outcome of Duchenne muscular dystrophy gene after 10 years in Vietnam

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Background: Duchenne muscular dystrophy (*DMD*) is the most common allelic X-linked muscular disorder caused by mutation in the dystrophin gene.
Objective: The aims of this study are to investigate

mutation rate and characteristic spectrum mutation in dystrophin gene of 564 patients, from January 2005 to December 2015.

Methods: Deletion mutations in the hotspot gene were detected by Multiplex Polymerase Chain Reaction (MPCR). The negative patients with 25 hotspot-exon deletion would be identified mutation using Multiplex Ligation dependent Probe Amplification (MLPA).

Results and conclusions: The rate of mutation is 28.7%. Among 162 mutation patients, 156 patients showed deletions in 25 hotspot regions, accounting for 27.7% of overall mutation ratio. The negative remaining had 6-patient deletion and 10-duplication mutation in the gene fragment analysed. Most of mutation located on the hotspot of the dystrophin gene, including mutations in exon 45-60 (54.4%) and exon 1-19 (28.4%), following is the remaining of the gene (14.3% respectively). All of deletion mutation in hotspot region in Vietnamese *DMD* patients were accordance with investigated other publications in the word. Distribution and frequency of the most common deletion in Vietnamese *DMD* patients using MPCR method is demonstrated. MLPA can detect some additional mutations that had been missed by MPCR were detected. But, it is necessary for further analysis for the samples which did not detect any mutation caused of their clinically positive.

Keywords: Duchenne muscular dystrophy (*DMD*) gene; Multiplex Polymerase Chain Reaction (MPCR); deletion; duplication; Vietnamese Duchenne muscular dystrophy

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AB091. Analysis of LA2 as a functional candidate gene for Emery Dreifuss muscular dystrophy and dilated cardiomyopathy

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Abstract: Laminopathies are rare genetic disorders caused by mutations in genes encoding lamins or lamin-interacting proteins. *LMNA*, *EDM* and to a lower measure the *Nesprin* genes have been associated with Emery Dreifuss muscular dystrophy (EDMD), characterized by contractures of the Achilles tendons, progressive skeletal muscle weakness and heart rhythm disturbances leading to dilated cardiomyopathy (DCM) and sudden cardiac death. Since ~60% EDMD patients are not associated to known genes, we used a functional candidate-gene approach to identify additional EDMD disease genes. Based on reported interactions of lamina associated polypeptide 2 (LAP2) with nucleoplasmic lamin A/C and the association of the LAP2alpha isoform to DCM (Matthew R.G Taylor *et al*. 2005), 111 EDMD and 87 DCM patients were investigated for DNA variations in *LAP* (encoding six LAP2 isoforms) using heteroduplex analysis and direct sequencing. Among ten variations found, four changes—p.P426L (c.1481C>T) in LAP2alpha, p.D271E (c.1054T>G) in LAP2beta, and p.V423L (c.1058G>C) and p.M381I (c.1387 G>A) in LAP2gamma—were unique for EDMD patients, but segregation analysis indicated only p.P426L LAP2alpha as a mutation potentially associated to EDMD. P426L-LAP2 alpha localized to the nucleoplasm in skin fibroblasts, like wild-type protein and did not affect the loalization of lamin A. However, in mutant fibroblast cultures phosphorylated retinoblastoma protein (Rb) levels were reduced compared to wild-type cultures, sugesting that the mutated protein may affect cell cycle progression in agreement with previous studies implicating LAP2alpha-lamin A in Rb-mediated cell cycle control. The present study shows that LAP2alpha mutations might add to the pathology of EDMD in ~1% of EDMD patients.

Keywords: Laminopathies; LAP2alpha; mutations; Emery

Dreifuss muscular dystrophy (EDMD)

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AB092. Regional IBD analysis (RIA): a new method for linkage analysis in extended pedigrees using genome-wide SNP data

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Background: Recent shift of focus in genetic studies to rare variants has revived interest in linkage analysis, which can ascertain effects of a locus regardless of allelic heterogeneity. However, exact calculations for traditional linkage analysis are computationally impractical in large, extended pedigrees, which are often encountered in studies of complex, low-penetrance diseases. Although simulation-based methods can be used in such circumstances, they require significant computational work and are not exact. We propose regional IBD analysis (RIA), a non-parametric linkage method based on comparison of locally and globally estimated identity by descent (IBD) sharing in affected relative pairs (ARPs) for use in these circumstances.

Methods: In RIA, genome-wide SNP data are used to calculate the "global" expected IBD sharing probabilities specific to each ARP, against which a "local" set of IBD sharing probabilities, estimated using SNP data within a window of pre-specified width, can be compared. These IBD sharing probabilities can be estimated using a variety of programs. We used PLINK and KING in this study. The global and local IBD sharing probabilities can be used to construct a non-parametric maximum likelihood statistic (MLS)-like test of linkage in each window. We illustrate the use of our method to detect linkage signals in real nuclear-family data from a study of primary vesicoureteral reflux and in simulated data based on large extended pedigrees from a Brazilian study of visceral leishmaniasis, and compared the results with those obtained from "traditional" methods including the Kong and Cox exponential model LOD score from Merlin and the MCMC-based lm_ibdtests from MORGAN.

Results: RIA successfully detected the linkage signals in these data sets, but with significant reduction in computational time (e.g., 2 *vs.* 66 h on a dedicated server on a data set consisting of 3,626 individuals from 308 extended families, 357 of whom were affected, genotyped at 545,433 SNPs) and resources compared with the traditional methods.

Conclusions: The proposed method should be useful in studies involving large extended families, in which traditional linkage analysis is not feasible. Additionally, because it does not rely on any prior knowledge about familial relatedness, the method has an additional advantage of being robust to pedigree misspecification and can be used even in absence of pedigree information. RIA is available at www.staff.ncl.ac.uk/richard.howey/ria/.

Keywords: Regional IBD analysis (RIA); non-parametric linkage analysis; identity by descent (IBD); affected-relative-pair method; extended family

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AB093. A case of exogenous C5-acylcarnitine giving rise to a false positive result in newborn screening (NBS)

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Background and objective: NBS Screening by MS/MS

is considered an effective screening test. However, the technique cannot distinguish between isobaric compounds, thus contributing to some false positive results. One such compound is C5-acylcarnitine in the in the identification of isovaleric acidemia (IVA) in the MS/MS profile. To report and contrast the findings of two newborns with C5-acylcarnitine elevations in newborn screening (NBS).

Methods: Blood collected on Guthrie card from newborns between 24-72 hours of life is analyzed by MS/MS. C5-acylcarnitine and its related ratios are measured in DBS sample to identify at risk newborn.

Results: Newborn A: DBS sample C5: 7.96 µmol/L (normal <0.50), C5/C0: 0.99 (normal <0.025), C5/C3: 16.1 (normal <0.40); Plasma acylcarnitines profile: C5: 9.42 µmol/L (normal 0.06-0.29). Urine organic acid profiles showed marked elevations of isovalerylglycine (IVG), ketone bodies and lactate. This profile is consistent with a patient presenting with a diagnosis of IVA. Newborn B: DBS sample C5: 0.84 µmol/L (normal <0.50), C5/C0: 0.041 (normal <0.025), C5/C3: 0.46 (normal <0.40); Despite the abnormal plasma acylcarnitines profile (C5: 4.38 µmol/L, normal 0.06-0.29), the urine acylglycine profile was normal [IVG: 1.02 mg/g Cr (normal 0.3-14.3 mg/g); 2-MBG: 0.16 mg/g Cr (normal: 0.3-7.5 mg/g)]. A 2nd plasma acylcarnitine showed a lower C5 level (1.49 µmol/L) and a repeat urine organic acid profile was normal; no IVG and 2-MBG detected. Mother's (Newborn B) plasma acylcarnitines and urine organic acid profiles were normal, ruling out a possible maternal condition. Moreover, it was confirmed that mother and newborn were not on any antibiotics or steroids, which have been previously reported as the causal agents of falsely elevated C5-acylcarnitine. Further investigation revealed mom was using Mustela Nursing Comfort Balm which contained neopentanoate, a compound demonstrated by Boemer *et al*. [2014] as the causal agent for the false elevation of C5-acylcarnitine in NBS. The elevated C5 levels in the newborn's plasma samples appear to correspond to the timing of the feed with the blood draw (i.e., the high C5 plasma acylcarnitine result (4.38 µmol/L) corresponds to a blood sample taken within minutes of a feed and the second sample was collected several hours (>2 hours) after the feed (C5: 1.49 µmol/L). Withdrawal of Comfort Balm use eliminated the biochemical derangements in Newborn B and he is clinically well at 10 months.

Conclusions: Neopentanoate in the form of an emollient can cause a C5 false positive result in NBS.

Keywords: Newborn screening (NBS); C5-acylcarnitine; isovaleric academia (IVA)

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AB094. Efficacy of combined preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) cycles—early results

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Abstract: Preimplantation genetic diagnosis (PGD) using PCR allows couples where one or both carry a hereditary single gene disorder to avoid having a child with that disorder. It can also be an effective therapeutic tool in curing an existing affected sibling through tissue matched cord blood stem cell transplant. However early preimplantation embryos have significant levels of chromosomal aneuploidy increasing with maternal age. Recent PGS technologies such as comparative genome hybridization (CGH) allow screening of all 24 chromosomes in the early embryo, allowing selective single embryo transfer (eSET) with significantly increased IVF implantation rates and significantly decreased miscarriage rates. We discuss early results on the efficacy of using PGD-PCR in combination with PGS-CGH (combined cycle) in couples who present for PGD for hereditary single gene disorders. PGD-PCR patients have a family specific test established, with the test components multiplexed and checked for reliability on single maternal cumulus cells. Patients having combined cycle had the individual test components checked on existing whole genome amplification (WGA) products and, if unreliable, reverted back to a standard PGD-PCR test/cycle only. Couples had an ovarian

stimulation cycle, harvested eggs were fertilized using intracytoplasmic sperm injection (ICSI), and resultant normally fertilized embryos cultured to day 5 and day 6 blastocyst stage. Suitable blastocysts were biopsied with assistance of a near-infra-red laser. The 1-6 cells obtained had their DNA extracted and either PCR amplified using the established multiplexed PGD-PCR test (PGD-PCR cycle) or WGA amplified (combined cycle). From 2007-2014, 109 couples presented for PGD-PCR for 16 different familial single gene disorders, predominantly beta-thalassemia (61/109) or alpha-thalassemia (25/109). In 2012 we introduced PGS-CGH for 24 chromosome screening of infertility couples, and soon after offered PGD-PCR patients the option of a combined PGS-CGH and PGD-PCR cycle; to date 19 patients had requested the combined cycle. For PGD-PCR only, 97 patients had 154 cycles with 85 embryo transfers (114 embryos). 57/85 (67%) were clinically pregnant with an implantation rate of 50%. For requested combined cycles, 5/19 patients (all alpha-thalassemia) failed the WGA check and reverted to PGD-PCR test/cycle only. 11/14 had 14 cycles with 8/14 cycles freeze-all (with no transfers to date) and 4 embryo transfers (5 embryos). 4/4 (100%) were clinically pregnant with an implantation rate of 80%. Early results, while low numbers, indicate offering patients presenting with a hereditary single gene disorder the option of having all 24 chromosomes screened prior to implantation may significantly increase their chance of a healthy pregnancy.

Keywords: Preimplantation genetic diagnosis (PGD); preimplantation genetic screening (PGS); array comparative genome hybridization (aCGH)

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AB095. Comparison pregnancy of day 6 fresh blastocyst and day 5 frozen-thawed blastocyst transfer following array comparative genome hybridization (aCGH)

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Abstract: Advances in assisted reproductive technologies (ART) have benefitted many infertile couples. However while many modern technologies were applied in ART, pregnancy rates remained lower than expected. Some studies have suggested that successful embryo implantation depends on many factors including genetic anomalies such as aneuploidy. While pre-implantation genetic screening (PGS) using fluorescent in situ hybridization (FISH) was introduced around 20 years ago to screen for aneuploidy in selected subsets of chromosomes, it failed to improve pregnancy rates and reduce miscarriage rates. FISH had technical limitations, some inaccuracies, and could only screen up to 8-11 chromosomes. Recent more modern technology, array comparative genome hybridization (aCGH), has been shown to significantly improve pregnancy rates and decrease miscarriage rates by allowing the detection of aneuploidy in all 23 pairs of chromosomes, and allowing the transfer of euploid embryos. Couples have an ovarian stimulation, eggs are collected and fertilized using intracytoplasmic sperm injection (ICSI), and any normally fertilized embryos are cultured to the blastocyst stage. Suitable blastocysts are biopsied on either day 5 or day 6 of embryo culture with the assistance of a near-infra-red laser, and the removed cells amplified in a whole genome amplification (WGA), fluorescently labelled, hybridized and scanned using the BlueGnome (Illumina) 24Sure CGH microarray system. Advances in aCGH means the total process from biopsy to result can be done overnight, allowing for a suitable embryo from a day 5 biopsy to have potential fresh embryo transfer on day 6 of culture. Alternatively, following biopsy, embryos can be frozen immediately and euploid embryos transferred in a subsequent frozen-thaw cycle. We retrospectively compared pregnancy outcomes of good quality blastocysts biopsied and analysed using aCGH following by fresh embryo transfer on day 6 (n=50) versus frozen embryo transfer of

embryos biopsied and frozen on day 5 (n=61). The average age of patients having a fresh embryo transfer on day 6 is 32±3.2 and having frozen embryo transfer is 30±3.7 years old. The results showed that pregnancy rates were not significantly different between frozen embryo transfer and fresh embryo transfer (59% *vs.* 52% respectively, P value >0.05). Nevertheless, as well as indicating that not only is frozen embryo transfer as good as or better than fresh embryo transfer, frozen embryo transfer can also have advantages in in-vitro fertilization in allowing optimal embryo transfer planning for couples.

Keywords: Array comparative genome hybridization (aCGH); *in vitro* fertilization, fresh blastocyst transfer; frozen-thawed blastocyst transfer

Cite this abstract as: Thaijaroen P, Benjaponwattana P, Jiaranai P, Marshall J, Tiewsiri K. Comparison pregnancy of day 6 fresh blastocyst and day 5 frozen-thawed blastocyst transfer following array comparative genome hybridization (aCGH). Ann Transl Med 2015;3(S2):AB095. doi: 10.3978/j.issn.2305-5839.2015.AB095

AB096. Pharmaco-genetic guided personalized medicine: discovery of a maturity onset diabetes of the young (MODY2) novel mutation [S441W in glucose kinase (GCK) gene] by next generation sequencing (NGS)

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Background and objective: Monogenic diabetes or maturity onset diabetes of the young (MODY) is characterized by young-onset (<45 years old), non-insulin dependence and a strong family history (autosomal dominant mode of inheritance). The major candidate genes include HNF4α (MODY1), glucose kinase (GCK) (MODY2) and HNF1α (MODY3). MODY is an attractive model for pharmaco-genetics because an accurate diagnosis may inform specific choice of anti-hyperglycemic therapy for better clinical outcome. We report a slim lady (BMI 22.4 kg/m^2) with Type 1 diabetes diagnosed based on abnormal fasting glucose and oral glucose tolerance test at age 21. She was started on multiple daily insulin injections (total daily dose 18-22 units/day) with good glycemic control (HBA1c 6.2%). Glutamic acid decarboxylase (GAD) autoantibody was negative. On occasions when she ran out of insulin supply, there was no incidences of diabetic ketoacidosis. We aim to identify proband with monogenic diabetes phenotype to perform high through-put exonic mutation screening using next-generation sequencing (NGS) on an extended panel of candidate genes (i.e., beyond GCK, HNF1A and HNF4A) for these individuals. We will also recruit other members within the pedigree for segregation analysis to strengthen causality of discovered variant (this is necessary primarily because more variants-of-unknown-significance are expected to be observed in an extended gene panel).

Methods: DNA from peripheral blood cells was subjected to high-throughput targeted nucleotide sequencing for all 16 known MODY candidate genes including exons, un-translated (UTRs) and promoter regions using the Ampliseq kit (Life Technologies).

Results: We discovered a novel non-synonymous mutation (c.1322C>G, p.Ser441Trp) in the GCK gene, which was confirmed by bi-directional Sanger's sequencing. The mutation is predicted to be functionally deleterious using multiple bioinformatics tools (e.g., SIFT and PolyPhen-2). In accordance with clinical practice guideline, insulin replacement was successfully discontinued with no deterioration of glycemic control.

Conclusions: The successful treatment-conversion based on genotype exemplifies how pharmaco-genetics can improve disease-stratify to inform diagnosis and treatment. This can translate into improved clinical outcome and quality of life.

Keywords: Diabetes; maturity onset diabetes of the young (MODY); mutation; sequencing

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AB097. Clinical and molecular characterization of patients with 6p25 deletion syndrome

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Objective: Chromosomal imbalances and rearrangements have been implicated in the etiology of intellectual disability and congenital anomalies. Many of these imbalances are caused by submicroscopic deletions or duplications not detected through conventional cytogenetic analysis. The advances in technology for detecting copy number changes, most notably chromosomal microarray analysis (CMA) has allowed the detection of these submicroscopic deletions or duplications. Submicroscopic 6p25 deletion is now recognized as a clinically identifiable syndrome. Clinical features in this syndrome include intellectual disability, developmental delay, hypotonia, sensorineural hearing loss, midface hypoplasia, ocular anomalies, cardiac defects and varying central nervous system anomalies. The aim of this report is to describe the phenotypic range of individuals with 6p25 deletion syndrome in the South East Asian population.

Methods: We reviewed the records of patients who are follow up in the Genetics clinic at KK Women's and Children's Hospital (KKH) and have CMA carried out using the Agilent 4×400 K and 4×180 K CGH+SNP catalogue array at KK Research Centre and DNA Diagnostic & Research Laboratory, respectively.

Results: We provide detailed molecular cytogenetic descriptions and clinical presentation of four unrelated patients with submicroscopic 6p25 deletion syndrome. Patient 1 has 5.1 Mb deletion (chr6: 224,712-5,352,662 hg19), while Patient 2 has 2 Mb deletion (chr6: 381,537-2,408,671 hg19). Patient 3 has 1.2 Mb deletion (chr6:1,486,461-2,692,219 hg19) and Patient 4 has 4.1 Mb deletion (chr6: 206,749-4,320,368 hg19). All of these patients have congenital heart defects, developmental delay, dysmorphic features and additional phenotypic abnormalities: Patient 1 has sensorineural hearing loss, hernia, bilateral undescended testes and buried penis, while Patient 2 has mild intellectual disability, bilateral mixed hearing loss, microphthalmia and submucous cleft palate. Patient 3 has congenital glaucoma, corneal clouding, hydrocephalus and ventriculomegaly, while Patient 4 has congenital glaucoma, micropenis, hearing loss and hypotonia.

Conclusions: Our result supports the notion that the genes responsible for the physical phenotype reside in the 6p25.1 region. Our results also reiterate the benefits of CMA in identifying these submicroscopic copy number variants, establishing new phenotype-genotype correlation in known syndromes and refining previously established ones.

Keywords: 6p25 deletion syndrome; chromosomal microarray analysis (CMA); developmental delay; KK Women's and Children's Hospital (KKH)

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AB098. The mutation spectrum of the *phenylalanine hydroxylase* (*PAH*) gene in Taiwanese population

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Abstract: Phenylalanine hydroxylase (PAH) deficiency is responsible for most cases of phenylketonuria (PKU). A total of 71 PAH-deficient Taiwanese families were included for *PAH* gene analysis. A total 34 different mutations, including 20 missense mutations, 4 nonsense mutations, 4 deletion/insertion within structural gene, 1 deletion in enhancer region and 5 affecting splicing were identified. The most prevalent mutations in Taiwan are R241C, R408Q and Ex6- 96A4G accounting for 23.2%, 12.0%

and 9.2% of the 142 mutant chromosomes, respectively. A total of 18 patients were regular follow up in our clinics and good responsive to high dose of BH4 (10 mg/kg/day). Their genotypes and phenotypes were analysis and the correlation between the proposed mutant PAH structures and functions regarding BH4 responsiveness are suggested.

Keywords: Phenylalanine hydroxylase (PAH); mutation; phenylketonuria (PKU); BH4; structure

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AB099. Beta ketothiolase deficiency: phenotype and genotype in Vietnam population

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Background and objective: Beta ketothiolase (T2) deficiency is rare inherited metabolic disease worldwide. But it is the most common organic aciduria in Vietnam with 35 cases. To describe phenotypes, genotypes of T2 deficiency.
Methods: Descriptive study of 35 patients with T2 deficiency at National Hospital of Pediatrics-Hanoi-Vietnam from 2005 to 2012. A total of 21 cases from 19 families were analyzed of T2 gene.
Results: A total of 35 patients were born to 30 unrelated families and unconsanguinity parents. And 33/35 patients presented crisis of ketone acidosis. One case was diagnosed without symptom at 3 days of age but developed the crisis at 6 months of age. One case was asymptomatic until now (5 years old). Mean onset of the first crisis was 13.1 months. Clinical features of the crises were dehydration, tarchypnea, lethargy/coma and triggered by infections. Laboratory of the crises showed 100 % severe ketone metabolic acidosis (pH: 6.5-7.05), leukocytosis. 94% cases recovered from the 1st crisis and 79% cases had normal development. A total of 20/35 patients had recurrent crisis. And 21/21 patients were found homozygous/compound heterozygous mutations of T2 gene. Five different mutations have been identified: R208X; IVS10-1g>c; A410V; 163_167del5ins2; c.1032-103 *INS* A. And R208X is the most common mutation in Vietnam (70% of mutant allele) as well as in the world.
Conclusions: The biggest T2 deficiency patients were identified in a Vietnam center (account 1/3 total cases in the world). Worldwide, there are no common mutations of T2 gene found except common R208X mutation in Vietnam.

Keywords: Beta ketothiolase deficiency; mitochondrial acetoacetyl CoA thiolase deficiency; T2 deficiency

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AB100. Phenotypes of primary hyperlipidemia in a Vietnamese referral center

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Background and objective: Primary hyperlipidemia is genetic dyslipoproteinemia. Without any intervention, cardiovascular diseases and acute pancreatitis may be occurred. Detection and appropriate management of pediatric hyperlipidemia can have a significant impact upon the disease course and can prevent complications. The article aims to describe the clinical and biochemical characteristics of hyperlipidemia in Vietnamese children and to evaluate outcome of treatment.
Patients and methods: From 2007 to 2013, 30 children were diagnosed with primary hyperlipidemia using included and excluded criteria and were treated with diet and/or

lipid-lowering drug therapy.

Results: Among 30 cases from 28 families, 8 patients were mixed hyperlipidemia (MHL), 13 patients were hypertriglyceridemia (HT) and 9 patients were hypercholesterolemia (HC). Mean age of diagnosis was 5.5 years (1 months-16 years). The rate of male/female was 13/17. Clinical manifestations included hepatomegaly (4 cases), xanthemas in the knees and elbows (5 cases), "creamy" blood (21 cases). Twenty cases were asymptomatic. A total of 8/28 patients had family history with hyperlipidemia and cardiovascular diseases. Serum cholesterol level of HC group was 9.2±4 mmol/L. Serum triglyceride level of HT group was 23.6±9.9 mmol/L. MHL group had hypercholesterolemia (12.1±4.5 mmol/L) and HT (20.3±10.5 mmol/L). After interventions, HT group had the best result with serum triglyceride level was 10.1±4.6 mmol/L, next to MHL group with serum cholesterol level was 5.8±1.8 mmol/L, and serum triglyceride level was 9.5±5.2 mmol/L; finally, serum cholesterol level of HC group was 12.4±5.5 mmol/L. Five infants with HT had the best results of treatment: serum triglyceride level decreased from 19-57.6 to 5-10 mmol/L. Two patients with HC had the worsen results (unchanged blood lipid level).

Conclusions: Primary hyperlipidemia had poor clinical manifestations and good results of treatment. Screening for primary hyperlipidemia help to prevent premature cardiovascular diseases.

Keywords: Primary hyperlipidemia; primary hyperlipoproteinemia; family hypercholesterolemia; hypertriglyceridemia (HT)

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AB101. Neonatal form of Isovaleric acidemia in Vietnamese patients: clinical history and outcomes

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Background and objective: Isovaleric academia (IVA) is an autosomal recessive inborn error of leucine metabolism caused by a deficiency of the mitochondrial enzyme isovaleryl-CoA dehydrogenase (IVD). The clinical presentation of IVA appears to be highly variable ranging from severely affected to asymptomatic subjects. This is the first report of Vietnamese patients with IVA. Describe clinical features and outcome of Vietnamese patients with IVA.

Methods: Case series report including three Vietnamese patients who were diagnosed IVA with the criteria of elevation of C5 and urinary isovalerylglycines, 3-OH-isovalerate using Tandem Mass and GC/MS.

Results: Three probands (2 female and 1 male) from three unrelated families were born to non-consanguinity parents. The age of onset was within the first 2 weeks of age (8, 9 and 10 days). The male patient had older brother died due to unknown comma at 18 days old. The initial symptom was poor feeding (3/3). After 1-2 days of the onset, they appeared lethargy/comma (3/3), convulsion (1/3) and respiratory failure required mechanical ventilation in one case. The investigations revealed metabolic acidosis (PH: 7.2-7.3) in 3 cases, thrombopenia in 3 cases; ketonuria in 3 cases and hyperammonemia in 3 cases. Management for acute crisis and long-term follow up of IVA was started after 2-3 days of the onset: coma was released after 2-3 days of treatment. All patients have normal development at 16 months of age; at 15 months of age and at 10 months of age, respectively.

Conclusions: Three Vietnamese patients with IVA presented early initial symptoms within 2 weeks of age and have good outcome.

Keywords: Isovaleric academia (IVA)

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AB102. Vietnamese patient with Tyrosinemia type 1: a case report

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Abstract: Tyrosinemia type 1 is an error of metabolism of tyrosine amino acid due to deficiency of the enzyme fumarylacetoacetate hydrolase (FAH). The incidence is 1 in 100,000 to 120,000 births. Symptoms include liver and kidney disturbances and mental retardation. Case report: the 2.5 months old girl admitted with chief complains of poor feeding, vomiting, fever and distended abdomen. She was the 5th child of the family and normal vaginal delivery, birth weight was 4.2 kg and normal development. Two sisters died with same symptoms at 1 and 1.5 months of age, respectively. Three days before admission, she presented with poor feeding, vomiting, fever, distended abdomen, blood and black stool. On admission, she presented with irritability, fever, edema, cold extremities, distended abdomen, and hepato-spleenomegaly. The routine investigations revealed coagulation disorder with prothrombin time (PT) of 22%, hypoalbuminemia (21.5 g/L), hyperlactatemia (7 mmol/L), increased AFP and increased infectious markers (CRP: 69 mg/dL). Plasma amino acids analysis and urinary GC/MS showed elevated plasma serin, lysine, tyrosine and urinary succinylacetone, 4-OH-phenyllactic, 4-OH-phenylpyruvic, phenyllactic and N-acetyltyrosine. She was managed with plasma infusion, vitamin K, glucose infusion, antibiotic. Her situation became better but PT and AFP was still low and too high, respectively, 3 weeks of treatment. This is the first Vietnamese patient diagnosed tyrosinemia type 1. The prognosis depends on treatment with nitisinone.

Keywords: Tyrosinemia type 1

Cite this abstract as: Nguyen KN, Vu DC, Can NT, Bui TP, Nguyen MC, Yamaguchi S. Vietnamese patient with Tyrosinemia type 1: a case report. Ann Transl Med 2015;3(S2):AB102. doi: 10.3978/j.issn.2305-5839.2015.AB102

AB103. Phenotype and genotype of urea cycle defect in a Vietnamese referral center

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Background and objective: Urea cycle disorders (UCDs) are inborn errors of ammonia detoxification/arginine synthesis due to defects affecting the catalysts of the Krebs-Henseleit cycle. Clinical spectrum of UCDs is variant from mild to severe form. To describe clinical spectrum and genotype of UCDs in Vietnamese patients.

Methods: Case series report of 20 patients with UCDs from 2005 to 2014 at National Hospital of Pediatrics, Hanoi, Vietnam.

Results: During 10 years, we have 20 cases with UCDs including 2 case of argininosuccinic ciduria; 6 cases of citrullinemia type 1; 12 cases with ornithine transcarbamylase deficiency (OTC). Ten cases (two with argininosuccinic aciduria, five cases with citrullinemia type 1 and three cases with OTC) were newborn onset form. The remaining 10 cases were late onset form. All of newborn onset patients

presented with poor feeding, irritability, then coma and dyspnea with hyperammonemia (415-1,254 µg/dL). Clinical symptoms of late onset patients were variant: acute encephalopathy (6/10), recurrent vomiting (5/10), convulsion (4/10), development delay (4/10), hemiplegia (3/10), elevated transaminase (10/10), coagulation disorders (10/10), hyperammonemia (200-1,200 µg/dL) with onset age from 4 to 18 months. Only one case of newborn onset form is survival with normal development (OTC deficiency—20 months old boy), 9/10 cases of late onset form is survival with normal development and mild development delay. Mutation Analysis was identified three causative hemizygote mutations c.77G > A (p.R26Q), c.298+5G>C (IVS3+5G > C) and c.422G > A (p.R141Q) of OTC in three probands and a nonsense homozygous mutation in exon 14 c.1030C > T (p.R344X) of ASS gene in one proband.

Conclusions: UCDs can occur at any age with non-specific neurological, hepatic—gastrointestinal, psychiatric symptoms. UCDs had good prognosis if early diagnosis and adequate management

Keywords: Urea cycle disorder (UCD); ornithine transcarbamylase deficiency (OTC); Citrullinemia type 1; argininosuccinic aciduria

Cite this abstract as: Nguyen KN, Bui HT, Vu DC, Can NT, Bui TP, Nguyen MC, Daut HV, Dang TA, Yamaguchi S, Kim GH, Yoo HW. Phenotype and genotype of urea cycle defect in a Vietnamese referral center. Ann Transl Med 2015;3(S2):AB103. doi: 10.3978/j.issn.2305-5839.2015.AB103

AB104. Glucose-6 phospate dehydrogenase deficiency among mongolian neonates

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Background and objective: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme deficiency in humans, affecting 400 million people worldwide and a high prevalence in persons of African, Middle Asian countries. The most common clinical manifestations are neonatal jaundice and acute hemolytic anemia, which is caused by the impairment of erythrocyte's ability to remove harmful oxidative stress triggered by exogenous agents such as drugs, infection, or fava bean ingestion. Neonatal hyperbilirubinemia caused by G6PD is strongly associated with mortality and long-term neurodevelopmental impairment. The study aims to determine a level of G6PD in healthy neonates.

Methods: We obtained blood spot samples from 268 infants around 24-72 hours in their age who has unsuspected intranatal and neonatal disorders. Glucose 6 phosphate dehydrogenase "Perkin Elmer, Finland" level is determined by Victor 2D Fluorometer assay, developing of neonatal jaundice is examined by recall.

Results: The76.5% of all participants (n=205) was assessed 4.36±1.15 Ug/Hb in normal reference range of G6PD, other 23.5% (n=63) was 0.96±0.51 Ug/Hb with G6PD deficiency. In the both sex, 51.5% of male 0.88±0.46 Ug/Hb (n=33) and 47.6% of female (n=30) 0.97±0.55 Ug/Hb was assessed with G6PD deficiency. Developing Jaundice period in number of 63 neonates with G6PD deficiency, 86% of neonates (n=54) was in 1-4 days, 4% of neonates (n=3) was in 5-7 days and there is no sign of jaundice in 9% (n=6). Therefore neonates with G6PD deficiency, 53.9% (n=34) continued jaundice more than two weeks.

Conclusions: G6PD deficiency was determined in male neonates (51.5%) more than female (47.6%). The 76.5% of all participants (n=205) was assessed 4.36±1.15 Ug/Hb in normal reference range of G6PDH other 23.5% (n=63) of all participants was 0.96±0.51 Ug/Hb with G6PD deficiency. It shows that G6PD might be one potential risk of neonatal jaundice and hyperbilirubinemia in neonates in Mongolia.

Keywords: Neonatal jaundice; enzyme deficiency; newborn screening; neonatal hemolysis

Cite this abstract as: Batjargal K, Nansal G, Zagd G, Ganbaatar E. Glucose-6 phospate dehydrogenase deficiency among mongolian neonates. Ann Transl Med 2015;3(S2):AB104. doi: 10.3978/j.issn.2305-5839.2015.AB104

AB105. Novel large mitochondrial DNA deletions in pediatric patients with clinical features of mitochondrial disorders

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Background and objective: So far, over 100 large mitochondrial DNA (mtDNA) deletions have been identified. Those large deletions can lead to a broad spectrum of clinical features including mild mitochondrial myopathies (MM), progressive external ophthalmoplegia (PEO), Kearns-Sayre syndrome (KSS) and Pearson syndrome (PS). Pediatric patients have been paid much attention because mitochondrial disorders in children are diverse and many clinical features are difficult to distinguish. The study aims to investigate the large mtDNA deletions in the Vietnamese pediatric patients with clinical features of mitochondrial disorders.

Methods: Total DNAs were extracted from blood samples of 62 pediatric patients with clinical features of mitochondrial disorders and 19 pediatric patients without clinical features of mitochondrial disorders collected at Vietnam National Hospital of Pediatrics. The large mtDNA deletions were determined by using nested PCR and PCR-sequencing of the deletion junctions.

Results: Using nested PCR, the large mtDNA deletions in pediatric patients with clinical features of mitochondrial disorders were identified with 72.58% (45/62 cases) carrying 4,977 bp deletion and 20.97% (13/62 cases) carrying multiple large mtDNA deletions. Using PCR-sequencing of deletion junctions, some novel large mtDNA deletions were also detected, including: 4,443, 4,701, 4,732, 4,814, 4,860, 4,969, 4,994, 5,122, 5,135 and 5,144 bp deletions. The large mtDNA deletions in pediatric patients without clinical features of mitochondrial disorders were also detected with 89.47% (17/19 cases) carrying 4,977 bp deletion and only 5.26% (1/19 cases) carrying the multiple large mtDNA deletions. Therefore, further studies have been being conducted to determine the multiple large mtDNA deletions in Vietnamese pediatric patients.

Conclusions: The 4,977 bp deletion is a common mtDNA deletion in Vietnamese pediatric patients. In 20.97% of pediatric patients with clinical features of mitochondrial disorders harboring the multiple large mtDNA deletions, some novel large mtDNA deletions have been found.

Keywords: Large mitochondrial DNA deletions; nested PCR; Vietnamese pediatric patients

Cite this abstract as: Phuong LL, Anh PT, Trang LH, Sen NT, Anh LN, Hung CV, Thai TH. Novel large mitochondrial DNA deletions in pediatric patients with clinical features of mitochondrial disorders. Ann Transl Med 2015;3(S2):AB105. doi: 10.3978/j.issn.2305-5839.2015.AB105

AB106. The role of apolipoprotein E polymorphism in dyslipidemic obese adolescents who received the intervention of physical exercise and National Cholesterol Education Program step II

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Background and objective: Lifestyle changes including physical exercise and diet is the management of dyslipidemia before considering the blood lipid-lowering drugs. Genetics factor is often regarded as the cause of the management's failure in dyslipidemic subjects who had been doing physical exercise obediently and good diet consumption. This article aims: (I) to create the algorithms of dyslipidemia management in obese adolescents including apolipoprotein (apo) E polymorphism with 28 days of physical exercise and National Cholesterol Education Program (NCEP) step II diet interventions in order to start giving the blood-lipid lowering drugs; (II) To determine the apo E genotype's profiles in subjects who has improved and unimproved lipid

profile levels; and (III) To determine the apo E alleles' roles in improving the lipid profile levels.

Methods: The study designs were cross-sectional and one group pretest and posttest study. Sixty dyslipidemic obese adolescents aged 10-18 years participated in the study. Subjects who met the inclusion criteria received the physical exercise and NCEP step II diet intervention for 28 days, as well as the apo E genotyping.

Results: Total cholesterol, triglycerides, LDL-chol, and HDL-chol level improvements were 51 (85%), 28 (46.7%), 40 (66.7%), and 11 (18.3%). Apo E3/E3 genotype was the largest proportion of genotypes in all subjects who had improved and unimproved lipid profile levels after the interventions. The total cholesterol, triglycerides, and LDL-chol mean levels before and after the intervention showed (I) were not significantly different in the apo E2 allele ($P>0.05$); and (II) significantly different in apo E3 allele ($P<0.001$). The total cholesterol and LDL-chol mean levels were significantly different ($P<0.001$), whereas the triglycerides mean levels was not significantly different ($P>0.05$) in apo E4 allele.

Conclusions: The profile of apo E genotypes was not evident in dyslipidemic obese adolescents who had improved or unimproved lipid profile levels after receiving the interventions, and apo E3/E3 genotype was the largest proportion of genotypes in both of subjects' groups. Physical exercise and NCEP step II diet had no role in apo E2 allele, but they played a role in apo E3 allele in improving the total cholesterol, triglycerides, and LDL-chol levels. The interventions also played a role in improving the total cholesterol and LDL-chol levels, however, they did not have a role in improving the triglycerides levels in apo E4 allele. The algorithm of dyslipidemia management could be made in order to treat the dyslipidemia condition in obese adolescents.

Keywords: Physical exercise; National Cholesterol Education Program step II diet (NCEP step II diet); apo E; dyslipidemia

Cite this abstract as: Gultom LC, Hadinegoro SR, Sjarif DR, Sudoyo HA, Immanuel S, Mansyur M, Setiawati M. The role of apolipoprotein E polymorphism in dyslipidemic obese adolescents who received the intervention of physical exercise and National Cholesterol Education Program step II. Ann Transl Med 2015;3(S2):AB106. doi: 10.3978/j.issn.2305-5839.2015.AB106

AB107. Challenges in the management of patients with maple syrup urine disease diagnosed by newborn screening in a developing country

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Abstract: Maple syrup urine disease (MSUD) is a rare inborn error of metabolism resulting from a deficiency in the branched-chain alpha-ketoacid dehydrogenase complex. MSUD has been reported to be the most common inborn error of metabolism in the Philippines. This study describes all patients with MSUD patients diagnosed through newborn screening during its first two years of implementation and the challenges encountered during their medical management. We reviewed the medical records of all patients diagnosed with MSUD by newborn screening in the Philippines from its initiation in July 2012 to June 2014. There were 24 patients diagnosed with MSUD by newborn screening for the two-year period. The mean age at newborn screening is 4 days. All patients needed hospital admission. The most common complication during hospital admission was infection, needing intravenous antibiotics which were given to 21 of the patients. Out of the 24 diagnosed, 16 (66.67%) of patients are alive, while 8 (33.33%) have died. Several neurologic and non-neurologic complications have been observed during the follow-up of the patients. The common challenges of MSUD diagnosis and management in a low-resource setting identified in this study were late diagnosis, lack of access to metabolic specialists and medical supplies, nosocomial septicaemia, and protein deficiency. Aside from early properly-timed collection, improvement in other logistical concerns such as an efficient system of sending and delivery of samples will also help in earlier diagnosis. Mechanisms of transfer of critically ill patients, albeit challenging in our setting due to geographical concerns, must be improved. Hospitals in difficult-to-reach areas must be equipped to handle critical metabolic cases when transfers are not possible. Newborn screening has been proven to improve outcome in patients diagnosed to have MSUD but the success of the newborn screening program in preventing disability is also dependent

on improvements in other aspects of healthcare.

Keywords: Maple syrup urine disease (MSUD); newborn screening

Cite this abstract as: De Castro-Hamoy L, Chiong MA, Estrada S, Cordero C. Challenges in the management of patients with maple syrup urine disease diagnosed by newborn screening in a developing country. Ann Transl Med 2015;3(S2):AB107. doi: 10.3978/j.issn.2305-5839.2015.AB107

AB108. The appliance of Bio-Plex immunoassay using dried blood spots for mucopolysaccharidosis IVA newborn screening in Taiwan—a pilot study

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Background: Mucopolysaccharidosis (MPS) IVA is an autosomal recessive lysosomal storage disorder caused by the deficiency of N-acetylgalactosamine-6-sulfatase (GALNS) resulting in excessive lysosomal storage of keratan sulfate. This excessive storage causes a systemic skeletal dysplasia, short stature, and joint abnormalities. Treatments for MPS IVA are available. Better outcomes are associated with early treatment, which highlights a need for newborn screening for MPS IVA.
Methods: We have conducted a newborn screening pilot program for MPS IVA since December 1, 2013. Screening involved measuring the quantity of GALNS in dried blood spots on filter paper (DBFP) from newborns using a Bio-Plex immunoassay. The amounts of fluorescence sorting detected by YAG laser with wavelengths of 532 (exciting) and 580 nm (emission) is proportional to the quantity of GALNS protein.
Results: More than 5,657 neonates have been analyzed, in those, 132 newborns had GALNS quantification less than the cut-off value (48.64 ρg/mL) at the first screening test. Most of them (n=124) were exclusive and only eight had been recalled for a second DBFP collection and GALNS quantity rechecked. The reference values were 48.64-552.4 ρg/mL. For the confirmed MPS IV patients without enzyme replacement therapy (n=11), the GALNS quantities were far less than 5% of the normal population, and ranged from 0.00 to 4.02 ρg/mL. The GALNS quantities of the carriers (n=2) were significantly reduced comparing with those of the normal values.
Conclusions: The Bio-Plex immunoassay has the potential to be adopted for newborn screening of MPS IVA. This method is reliable, sensitive, validated, simple, and cost-effective in measuring GALNS enzyme in DBFP.

Keywords: Bio-Plex immunoassay; keratan sulfate; Morquio A syndrome; N-acetylgalactosamine-6-sulfatase (GALNS); newborn screen

Cite this abstract as: Lin CH, Chuang CK, Lin HY, Wang TJ, Tsai CC, Lin SP. The appliance of Bio-Plex immunoassay using dried blood spots for mucopolysaccharidosis IVA newborn screening in Taiwan—a pilot study. Ann Transl Med 2015;3(S2):AB108. doi: 10.3978/j.issn.2305-5839.2015.AB108

AB109. Noninvasive prenatal testing (NIPT): differences in testing indications between the US and Southeast Asia

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Background and objective: Cell-free DNA-based noninvasive prenatal testing (NIPT) has been validated as a screening test for certain fetal aneuploidy in high-risk women. High-risk indications include advanced maternal age (AMA), positive serum screen result, abnormal fetal ultrasound findings, and a previous affected pregnancy. However, recent studies show the effectiveness of NIPT in all pregnant women, regardless of risk. Determine if there are global differences in NIPT implementation between centers in Southeast Asia and the United States (US).
Methods: We queried the Illumina laboratory database for NIPT samples originating from Southeast Asian

countries and the United States. The ordering providers had the option of specifying clinical indications for testing on the test requisition form (TRF). Indications included: AMA, abnormal ultrasound, positive serum screen, history of increased risk, or other. Samples were reviewed and classified into one of the five indications listed above or classified as "multiple indications" if more than one indication was selected; samples without an indication selected were excluded. Test indications were compared between the Southeast Asian and US cohorts.

Results: There was a significant difference in the test indications of the two cohorts (P<0.001). The majority of samples from the US had AMA as the indication (70.1%) *vs.* 47.0% in Southeast Asia samples. A positive serum screen was a more common indication in Southeast Asia (31.5%) than in the US (8.4%). More US (11.6%) than Southeast Asian (3.7%) samples specified an abnormal ultrasound as a test indication. "Other", indicated in 7.4% of Southeast Asian samples and 0.6% of US samples, covered a range of indications detailed by providers, including maternal anxiety, borderline risk on serum screening, and late entry to prenatal care. Interestingly, maternal anxiety was more commonly listed for Southeast Asia (4.6%) than US (0.1%) samples.

Conclusions: This study suggests differences in testing indications between the US and Southeast Asia. Centers in Southeast Asia appear to be using NIPT more often as a secondary screen, following a positive screen, whereas US centers appear to be using NIPT as a primary screen, particularly in AMA women. A potential explanation for differences in test indications could be access to care and reimbursement for testing. Further studies are needed to better understand the global differences in NIPT implementation.

Keywords: Noninvasive prenatal testing (NIPT); indications; Southeast Asia; United States

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AB110. Novel alteration of mitochondrial tRNA^{Trp} in a group of Vietnamese breast cancer patients

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Background and objective: Mitochondria are organelles that generate ATP—required energy for all activities of the cell. Mitochondria have their own genome which encodes for 22 transfer RNAs (tRNAs), 2 ribosomal RNAs (rRNAs) and 13 proteins of oxidative phosphorylation system in the inner membrane. Previous studies have shown that the alterations of mitochondrial DNA were associated with various cancers including breast cancer. In particular, the alterations of mt-tRNA genes were focused because they directly affected the tRNA molecules and the translation, many proteins and different processes. In this study, we analyzed the alterations of some mitochondrial tRNA genes in a group of Vietnamese breast cancer patients to investigate the variants of mt-tRNA genes and their role in breast cancer.

Methods: Total DNA was extracted from pairs of tumor and adjacent tissues (2-3 cm from the tumor) of 30 breast cancer patients collected at Vietnam National Cancer Hospital. The alterations of mt-tRNA genes were determined by using PCR - RFLP and sequencing methods. Bioinformatics tools were used to predict the changes in secondary structure of mutated tRNAs. Data were analyzed by statistical methods.

Results: We did not identify the mutations m.3243A > G of mt-tRNA$^{Leu(UUR)}$ and m.12300G > A of mt-tRNA$^{Leu(CUN)}$ in studied samples. However, the sequencing results revealed two variants m.5591G > A and m.5536A > T of mt-tRNA^{Ala} and mt-tRNA^{Trp} respectively, in which m.5536A > T (1/30 cases) was a novel alteration that has not been reported so far. Besides, m.5536A > T does alter the structure of tRNA^{Trp} predicted by bioinformatics tools and affects its function. Moreover, PCR-RPLP analysis also indicated the

heteroplasmy of m.5536A > T variant. Further studies have been being conducted to determine the heteroplasmic levels related to this alteration in breast cancer tissues.

Conclusions: It is the first time that alteration m.5536A > T of mt-tRNA^{Trp} gene has been reported in patients with breast cancer. This alteration may change the normal structure of the tRNA^{Trp} and affect the function of the molecule.

Keywords: Breast cancer; RCR-RFLP; tRNA^{Ala}; tRNA^{Trp}

Cite this abstract as: Nguyen LT, Quach MT, Do DT, Do HM, Ta TV, Trinh TH. Novel alteration of mitochondrial tRNA^{Trp} in a group of Vietnamese breast cancer patients. Ann Transl Med 2015;3(S2):AB110. doi: 10.3978/j.issn.2305-5839.2015.AB110

AB111. HBB: c. -78A>G/nt-28(A>G) associated with Cd 26(A-G) HbE, beta thalassemia variant causes thalassemia intermedia

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Abstract: β-thalassemia is the most common single gene disorder worldwide and in Vietnam. In the present study we report in members of a family from North Vietnam, the mother compound heterozygous thalassemia intermedia presenting mutation of hemoglobin HBB: c. -78A>G/nt-28(A>G) with Cd 26(A-G) HbE. The father, heterozygous for Cd71/72(+A), β^+ beta thalassemia. To our knowledge, this is the first report of -28(A>G) in trans with beta thalassemia variant Cd 26(A-G) HbE leading to beta thalassemia intermedia. Our data highlight the necessity of deep molecular characterization of subjects presenting normal HbA2 level associated with abnormal red cell indices. It's necessary for accurate diagnosis and improved genetic counseling.

Keywords: Beta thalassemia intermediate; Cd 26(A-G) HbE; c. -78A>G/nt-28(A>G)

Cite this abstract as: Ly TT, Ngo ND, Ngo NT, Nguyen MT, Nguyen HT, Duong TB. HBB: c. -78A>G/nt-28(A>G) associated with Cd 26(A-G) HbE, beta thalassemia variant causes thalassemia intermedia. Ann Transl Med 2015;3(S2):AB111. doi: 10.3978/j.issn.2305-5839.2015.AB111

AB112. Detection of human sperm DNA fragmentation by alkaline comet and neutral comet improved by research center for genetics and reproductive health (CGRH)

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Objective: To optimize the specificity and sensitivity of protocols to detect single-strand and double-strand DNA fragmentation of human sperm by using Neutral Comet and Alkaline Comet assay respectively. In both assays, the same conditions of lysis solution and gel electrophoresis were applied.

Materials and methods: Thirty samples of semen were collected to assess DNA fragmentation. The inclusion criteria were 18-40-year-old men and the exclusion criteria was azoospermia.

Results: The sample was prepared on lame at the concentration of 1×106 sperm/mL. The duration of cell lysis was successfully decrease to 30 minutes using the optimized solutions of 1.5 M NaCl and 1 mM DTT. After lysing and removing saline solutions, the gel electrophoresis was run at 20 V/70 mA in 10 minutes. The positive control sample was well-prepared by using 2% H_2O_2 to detect specificity and sensitivity of lysing and electrophoresis. In assay of Alkaline Comet, the sample was covered in alkaline solution (pH>13), 4 ℃ in 5 minutes after lysing, then moved into gel electrophoresis. The sample was dyed with SYBR and observed the sperm DNA fragmentation

under fluorescent microscope. Four hundred of sperms were randomly counted in every sample. The images were well-captured in terms of detection of fragmented and nonfragmented sperms.

Conclusions: The optimized protocol allowed to detect the single- and double-strand DNA fragmentation in human sperms by only using the same conditions of lysis solutions and gel electrophoresis; moreover, to reduce the duration of lab performance and the cost. The protocols could be easily applied in andrology labs to provide for useful information together with semen analysis (WHO, 2010). This is the first result in Vietnam to detect DNA fragmentation by using Comet assays.

Keywords: Alkaline Comet

Cite this abstract as: Mai MP, An NT, Thai Ha NT, Tram NB, Bao NH, Tuong HM. Detection of human sperm DNA fragmentation by alkaline comet and neutral comet improved by research center for genetics and reproductive health (CGRH). Ann Transl Med 2015;3(S2):AB112. doi: 10.3978/j.issn.2305-5839.2015.AB112

AB113. The first genetic study on congenital choledochal dilatation (CCD) implicates extracellular matrix proteins

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Abstract: Congenital choledochal dilatation (CCD) or paediatric choledochal cyst refers to the congenital dilatation of the choledochs (bile ducts) which leads to the obstruction of the ducts and bile retention. Symptoms include cholestatic jaundice, abdominal pain and liver enlargement complicated with cholangitis and pancreatitis. New-borns undergo surgery otherwise the liver could be permanently damaged. CCD is rare, mostly sporadic with variable population incidence, the highest being in Asia (1/1,000 in Asians; 1/150,000 in Caucasians). Its aetiology implicates congenital structural anomalies reflecting a failure in the hepatobiliary-pancreatic development. Thirty-one CCD trios were exome sequenced. Gene/pathway-set enrichment analyses grouped genes with at least one damaging allele into focal adhesion and extracellular matrix-receptor interaction pathways. Pathogenic mechanisms considered included *de novo* germ-line mutations and/or recessive inherited mutations in homozygosis, compound heterozygosis (CH) or as "di-genic/oligogenic" model of inheritance whereby variants in genes of related pathways coexist in a patient through parental inheritance. Fifteen gene members of those pathways were recurrently mutated and had variants at different sites (more than one damaging allele per gene). These alleles were in CH or co-existing with a mutated functional gene-partner in the same individual. Patients' genetic profiling revealed CCD as not only genetically heterogeneous but with di/oligogenic inheritance. Yet, the relevant mutated genes are functionally convergent. Data are consistent with the sporadic presentation of CCD. Incidentally, the cholangiocarcinoma rate in Asians is also the highest world-wide. We are also aiming at finding possible links between these choledochal disorders and at explaining their high incidence in Asia.

Keywords: Congenital choledochal dilatation (CCD); exome sequencing

Cite this abstract as: Garcia-Barceló MM, Wong JK, Ngo ND, Tran NS, Nguyen TL, Nguyen L, Sham P, Cherny S, Tam P. The first genetic study on congenital choledochal dilatation (CCD) implicates extracellular matrix proteins. Ann Transl Med 2015;3(S2):AB113. doi: 10.3978/j.issn.2305-5839.2015.AB113

AB114. Change in bone mineral density of patients with osteogenesis imperfecta after 6 months of pamidronate therapy in the philippine general hospital: a retrospective review

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Background: Osteogenesis imperfecta is a heritable disorder due to a collagen gene mutation causing a structural abnormality leading to brittle bones and osteopenia. To address the osteopenia, intravenous bisphosphonates (pamidronate) act by temporarily halting the action of osteoclasts giving time for osteoblasts to build bone. To date, there has been no local data regarding the improvement in bone mineral density of Filipino patients with osteogenesis imperfecta following treatment.
Methods: This study is a retrospective review that included six patients aged 1 year and 10 months to 9 years and 9 months old at the Philippine General Hospital with moderate to severe osteogenesis imperfecta who have undergone 6 months of pamidronate infusions at 1 mg/kg/dose monthly or a total dose of 6 mg/kg. Chart review was done. Hand radiographs taken at baseline and after 6 months of therapy were reviewed by a radiologist who was blinded, to determine metacarpal indices.
Results: There was an increasing trend in the metacarpal index from baseline to 6 months post-treatment with a mean difference of 0.053 mm (CI, –0.0112 to 0.117). However, the increase was not statistically significant (P value 0.0874) when analyzed using the paired *t*-test at a 95% confidence interval. No adverse events were noted and only one patient reported a fracture after starting therapy.
Conclusions: Bisphosphonate infusions among the six pediatric patients with moderate to severe osteogenesis imperfecta are well tolerated and although the increase in the metacarpal index from baseline after six months of treatment is not statistically significant, the trend shows improvement of the osteopenia from baseline.

Keywords: Osteogenesis imperfecta; bisphosphonate; bone mineral density

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AB115. Plasma amino acid and urine organic acid profiles of Filipino patients with maple syrup urine disease (MSUD) and correlation with their neurologic features

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Background and objective: Maple syrup urine disease (MSUD) is the most common inborn error of metabolism in the country. The main cause of the neuropathology is still not well established although the accumulation of branched chain amino acids (BCAA) and alteration in large neutral amino acids (LNAA) as well as energy deprivation have been suggested. It is the aim of the study to determine the plasma amino acid and urine organic acid profiles of Filipino patients with MSUD and correlate the findings with their neurologic features.
Methods: Twenty six Filipino patients confirmed to have MSUD were studied in terms of their plasma amino acid and urine organic acid profiles. Their results were compared with 26 age and sex matched controls. Their neurologic features were reviewed and correlated with the results of their plasma amino acid and urine organic acid profiles.
Results: Majority of the patients with MSUD had developmental delay/intellectual disability (88%), speech

delay (69%) and seizures (65%). The amino acid profile of MSUD patients revealed low glutamine and alanine with high levels of leucine, isoleucine, phenylalanine, threonine and alloisoleucine compared to controls (P<0.05). The urine organic acids showed significantly elevated excretion of the branched chain ketoacids and succinate (P<0.05), however other Krebs cycle metabolites that would indicate possible energy perturbation were not found in significant amounts. There were also no metabolite markers in the plasma amino acids or urine organic acids that correlated significantly with the neurologic features. The most remarkable finding in this study was the discriminant analysis done on 7 clinically and statistically significant important amino acids in the plasma wherein elevations in leucine, isoleucine, alloisoleucine, phenylalanine and threonine, and decreased levels of glutamine and alanine clearly defined the boundary between an MSUD case and control.

Conclusions: The findings suggest that there could be altered LNAA metabolism among patients with MSUD when the BCAAs are elevated in plasma. A set of plasma amino profile comprising of 7 amino acids may be suggestive of MSUD if altogether present. The urine organic acid analysis showed elevated excretions of the branched chain ketoacids and succinate. However, the above biochemical findings were not significantly correlated with the neurologic features of patients with MSUD, thus, there could be other unknown factors that cause the neurologic impairments in MSUD other than the elevated BCAAs and their corresponding ketoacids or the elevated LNAAs.

Keywords: Maple syrup urine disease (MSUD); branched chain amino acids (BCAA); large neutral amino acids (LNAA); organic acids; neurologic features

Cite this abstract as: Chiong MA, Cordero CP, Fodra EG, Manliguis JS, Lopez CP, Dalmacio LM. Plasma amino acid and urine organic acid profiles of Filipino patients with maple syrup urine disease (MSUD) and correlation with their neurologic features. Ann Transl Med 2015;3(S2):AB115. doi: 10.3978/j.issn.2305-5839.2015.AB115

AB116. Germline mutations of *Syk* gene associated with breast cancer pathogenesis

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Abstract: Spleen tyrosine kinase (*Syk*) gene encodes a non-receptor type tyrosine kinase which is widely expressed in many cell types and is a known tumor suppressor for human breast carcinomas. Studies have reported that mutations in *Syk* gene are associated with cell invasion and an increased risk of cancer development. In this study the mutational status of the entire coding region of *Syk* was analysed in breast cancer patients in Brunei Darussalam. By using Sanger sequencing method, the germ-line mutations of *Syk* gene in breast cancer patients were identified. Analysis of sequencing result revealed seven previously unreported single nucleotide alterations in the coding region of the *Syk* gene of which five mutations were observed to be silent mutations. Two silent mutations, c.267C > Y and c.384G > R, were observed in exon 2, while one mutation, c.1320C > Y, was observed in sample exon 9. The remaining 2 silent mutations were c.1752T > Y and c.1800T > Y, observed on exon 11. Two missense variations, namely c.1807G > R and c.1834A > M, were identified in exon 11 of the *Syk* gene which encodes part of kinase domain of the *Syk* gene. All of these mutations are reported for the first time in this study. Additionally, substitution and deletion mutations were also detected in the non-coding region of the *Syk* gene. The importance of these mutations in breast cancer pathogenesis at this stage is not clear and requires further work.

Keywords: Spleen tyrosine kinase (*Syk*); germline; mutation; breast cancer

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AB117. An exploration of Australasian genetic counsellors' attitudes towards compassion fatigue, mindfulness and genetic counselling

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Abstract: Genetic counselling is a caring profession. It has been known for some time that genetic counsellors are susceptible to clinical burnout and/or compassion fatigue. Recent studies have shown that mindfulness may help health care professionals with their experience of burnout. It is hypothesised that mindful awareness may be useful in ameliorating these symptoms of burnout in genetic counsellors. The present study aims to collect information about the experiences of Australasian genetic counsellors in relation to compassion fatigue and mindfulness. This study is an online questionnaire open to practicing Australasian genetic counsellors. The survey is in three parts. The first part collects demographic information about the genetic counsellor completing the questionnaire. The second part of the survey is the Professional Quality of Life Scale, Compassion Satisfaction and Fatigue Subscales-Revision IV. The final part of the questionnaire is the Mindful Attention Awareness Scale. Both scales are validated. Descriptive analyses will generate frequency data to elicit a description of participants and the responses obtained. Analysis of categorical measures will be undertaken using χ^2 (chi-squared) analysis to determine if there are any differences in responses. For continuous variables, differences in means between groups will be assessed using *t*-tests. Qualitative content analysis (inductive approach) will be utilised to analyse open ended responses. The results of this questionnaire will provide important data about clinical burnout and compassion fatigue among genetic counsellors and will enable recommendations about the use of mindfulness to minimise the impact of these on those in this profession.

Keywords: Genetic counsellors; clinical burnout; compassion fatigue

Cite this abstract as: Burgess M, Tai G, Martinek N, Menezes M, Delatycki M. An exploration of Australasian genetic counsellors' attitudes towards compassion fatigue, mindfulness and genetic counselling. Ann Transl Med 2015;3(S2):AB117. doi: 10.3978/j.issn.2305-5839.2015.AB117

AB118. Validation of next generation sequencing by Sanger sequencing

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Background and objective: Development of the next generation sequencing (NGS) platform was driven by the completion of the Human Genome Project in 2003. With the availability of NGS, the time taken for sequencing of humongous genomic regions was greatly reduced and data generated per unit DNA was also significantly increased. Though the cost to use NGS in a clinically setting is far from ideal, economically speaking, there is a significant decrease in the average cost per sequenced base. To validate findings of NGS on mutation detected for *FBN1*, *TGFBR2*, *RAF1*, *RTEL1*, *LMNA*, *MID2*, *KCNK9*, *DMD*, *SMARCA2* and *IQSEC2* by using gold standard, Sanger Sequencing.
Methods: The coordinate of the mutation identified by NGS was used to retrieve the adjacent genomic sequence in UCSC Genome Browser (Available from URL: https://genome.ucsc.edu/). Targeted primers were designed with Primer 3 software (Available from URL: http://primer3.ut.ee/) based on the genomic sequence obtained from UCSC. The following step involves the optimization of a Polymerase Chain Reaction (PCR) with the designed primers to amplify the desired DNA template for the targeted region. Upon optimization, the template is purified and subjected to dye terminator sequencing to generate

multiple DNA fragments of varying sizes. Lastly, the DNA fragments will be purified and analysed with an automated sequencer. The sequencer separates the DNA fragments based on their size by carrying out capillary electrophoresis.
Results: A total of 28 cases were validated with Sanger sequencing. Of them, 25 (89.3%) cases concur with the findings from NGS and 3 (10.7%) cases were false-positive calls.
Conclusions: NGS shows promise in the future molecular diagnostic regime, however, at the present moment, it needs to be done concurrently with Sanger sequencing for clinical applications.

Keywords: Next generation sequencing (NGS); Sanger sequencing

Cite this abstract as: Low MH, Lai HM, Jamuar SS, Law HY. Validation of next generation sequencing by Sanger sequencing. Ann Transl Med 2015;3(S2):AB118. doi: 10.3978/j.issn.2305-5839.2015.AB118

AB119. Induction of suppressor of cytokine signaling-3 in *FLT3*-ITD positive MV4-11 acute myeloid leukemia cells in response to 5-Azacytidine and Trichostatin A

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Background and objective: Suppressor of cytokine signaling-3 (*SOCS-3*) has been shown to be an important candidate in molecular therapeutic strategies in management of acute myeloid leukemia (AML), particularly in patients carrying *FLT3*-ITD mutation. *SOCS-3* suppresses cytokine signalling by inhibiting the activity of Janus Kinase-2 (JAK-2), and by competing with signal transducer and activator of transcription (STAT) molecules that leads to underexpression. The study aims to determine the epigenetically silence genes in AML cells carrying a *FLT3*-ITD mutation and epigenetically expressed genes afer treatment with demethylating agent and histone deacetylase inhibitor.
Methods: MV4-11, a *FLT3*-ITD positive AML cell line was treated with epigenetic modulating agents; 5-azacytidine (5-Aza, a DNA demethylating agent) and Trichostatin A (TSA, a histone deacetylase inhibitor) at IC_{50} concentrations. One-Color Microarray-based expression analysis (Agilent SurePrint Technology) was utilized and the data was collected and analyzed by Genespring 12.6 software. The gene expression datasets were subjected to pathway analysis by online DAVID tool (http://david.abcc.ncifcrf.gov/) using KEGG pathway database. The microarray results were validated by quantitative real-time PCR to determine the relative quantification (RQ) values.
Results: Microarray analysis detected 1,291 expressed genes related to drug interactions. Pathway analysis by KEGG database revealed that the 1,291 genes were: 21 genes from MAPK pathway, 19 genes from pathways in cancers, 17 genes from cytokine-cytokine receptor interaction, 12 from focal adhesion, 12 from regulation of action cytoskeleton, 10 genes from JAK/STAT pathway, 10 genes from Calcium signalling and several other pathways with less than 10 genes involved. Among the 10 genes in JAK/STAT pathway, *SOCS-3* was highly expressed in 5-Aza and TSA with 66.24 and 147.43 folds (Genespring analysis, Benjamini Hochberg, $P<0.05$), respectively compared to untreated cells. Whereas, *STAT6* was down regulated by –8.57 and –2.28 folds, respectively. Validation of microarray result showed RQ of *SOCS-3* gene was upregulated by 3.7 and 18.2 folds, whereas *STAT6* by 0.7 and 0.1 folds in 5-Aza and TSA respectively. *SOCS-3* over expression reduces *STAT6* activities and thus induces cell death in AML cells.
Conclusions: *SOCS-3* was epigenetically silenced in AML cells and re-expressed after 5-Aza and TSA treatments. Whereas, *STAT6* plays a role in a negative feedback loop. The finding suggests that, *SOCS-3* expression is associated with pathogenesis of AML and can be served as prognosis marker in molecular targeted therapy of AML.

Keywords: Suppressor of cytokine signaling-3 (*SOCS-3*); MV4-11; acute myeloid leukemia (AML); 5-Aza; Trichostatin A (TSA)

Cite this abstract as: Johan MF, Jusoh SA. Induction of suppressor of cytokine signaling-3 in *FLT3*-ITD positive MV4-11 acute myeloid leukemia cells in response to 5-Azacytidine and Trichostatin A. Ann Transl Med 2015;3(S2):AB119. doi: 10.3978/j.issn.2305-5839.2015.AB119

AB120. Correlation of genotype with biochemical profile in patients with Glutaric Acidemia type I: a study from India

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Background and objective: Mutations in glutaryl-CoA dehydrogenase gene causes Glutaric Acidemia type I (GA-I), an autosomal recessive, metabolic disorder which leads to accumulation of Glutaric Acid, 3-Hydroxyglutaric acid, Glutaconic Acid and Glutarylcarnitine (C5DC). There are no studies that correlate the genotype with biochemical profile in patients with GA-I from India. The objective of this study was to screen Indian patients with GA-I, for common mutations such as R402W, A421V, A293T, R227P and V400M and to correlate the genotype profile of the patients with C5DC levels.

Materials and methods: The study was approved by the institutional Human Ethics Committee. Fifty confirmed GA-I patients from unrelated families were recruited based on clinical, biochemical and neuroimaging studies. Informed consent was obtained before taking blood spots on the filter paper. The dried blood spots were used for measuring C5DC levels by tandem mass spectroscopy and for screening mutations by RFLP or by direct sequencing of PCR products. Mutations screened by RFLP were further confirmed by sequencing. Those patients with mutant homozygous alleles were considered for correlation studies.

Results: The mutation R402W was found in 11 (22%) patients, among them, 7 (14%) were homozygous and 5 (8%) were heterozygous. During genotyping, known mutation such as F236L was found in 1 (2%) and R313W in 1 (2%). Novel mutations, P286S was found in 2 (4%), W225X in 1 (2%), H403Y in 1 (2%), Y295Y in 1 (2%) and 1606 G>T at 3'UTR in 1 (2%). Conversely, none of the GA-I patients had A421V, A293T, R227P and V400M mutations. Patients homozygous for R402W, W225X, H403Y and g.13670 G>T at 3'UTR were considered for correlation studies. The mean C5DC levels, in patients with R402W mutation was found to be 1.83 µmol/L, patients with 3'UTR g.13670 G>T, W225X and H403Y mutations were found to have 1.74, 1.28 and 1.21 µmol/L respectively.

Conclusions: In conclusion, R402W and novel P286S are the most prevalent mutations among Indian patients with GA-I. Patients with R402W were found to have highest elevated levels of C5DC as compared to other homozygous mutations in our study. Mutation such as A421V, A293T, R227P and V400M were found to be absent in our population.

Keywords: Glutaric Acidemia type I (GA-I); Glutaryl carnitine; genotyping

Cite this abstract as: Shaik M, Vedumurthy AB, Kruthika-Vinod TP. Correlation of genotype with biochemical profile in patients with Glutaric Acidemia type I: a study from India. Ann Transl Med 2015;3(S2):AB120. doi: 10.3978/j.issn.2305-5839.2015.AB120

AB121. *NAT2* sequence polymorphisms and acetylation profiles in Indians

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Abstract: Evolutionary study of genes and genomes are helpful in decoding the role played by various evolutionary forces including footprints of natural selection. These forces change the architecture of genes for their best adaption in a particular environment. Inter-individual variations in drug response are responsible for adverse drug reactions, therapeutic drug failure and susceptibility to other diseases, thus understanding the genetic basis of drug metabolization serves as a key to inter-individual drug responses. To this respect, human *N-acetyltransferase 2* (*NAT2*) is a drug metabolizing gene that helps in the metabolism of wide variety of drugs used for treating different diseases. The effective metabolization of exogenous chemicals by *NAT2* gene classifies individuals into two acetylator phenotypes; fast (rapid) acetylator and slow acetylator. In order to determine which of the two acetylation phenotypes are prevalent in Indian population, we sequenced *NAT2* 873 bp coding region in 250 Indian populations collected from six geographical zones (North

India, West India, South India, Central India, East India and North East India) and three tribal populations residing in Odisha (East India). On the basis of seven common *NAT2* SNPs found in worldwide population, the individuals can be categorized as fast acetylator and slow acetylator. Thus, evolutionary analysis of drug metabolizing gene helps in understanding the metabolism of different therapeutic drugs and exogenous chemicals present in diet. Furthermore, the polymorphisms present in *NAT2* gene will be used to understand how the variability of gene has evolved and how it affects the drug response mechanism in Indian populations.

Keywords: Indian population; drug metabolizing gene; polymorphisms; *N-acetyltransferase 2* gene (*NAT2* gene); evolutionary analysis

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AB122. Profiling the serine threonine kinase phosphorylation of TGF-β1 stimulated fibroblast using peptide microarray

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Background: Transforming growth factor (TGF)-β is involved in various physiological roles from cell growth, cell differentiation, apoptosis, and some disease development. The TGF-β is tightly regulated and involves a myriad of molecules. Moreover, signal cross talk is prominent in the pathway, making it very complex and very difficult to study. Serine-threonine kinases play important role in TGF-β signaling. TGF-β ligand activates its serine-threonine kinase receptors (TGF-βRI and TGF-βRII). The activated receptor phosphorylates R-Smad proteins Smad2/3 and recruits CoSmad4. The Smad complexes are then translocated into the nucleus to regulate the transcription of the target genes in cooperation with other co-factors. How the TGF-β signaling, particularly the Smad signaling, can control different physiological roles remains poorly understood. It is not known whether TGF-β via its secondary messenger signaling molecules activates various physiological processes simultaneously or each process in an independent fashion activated by a previous process. We seek to further understand how TGF-β regulates various physiological phenomenons through the workings of its secondary messenger signaling using state-of-the-art serine-threonine kinase microarray.

Methods: Normal fibroblast cell lines were grown in Ham's F-10 Nut Mix and serum-starved overnight. The fibroblast were then separated into two groups, stimulated and non-stimulated. The stimulated group were added TGF-β1 and incubated at room temperature for 25 minutes. The cells were then lysed and processed using PamGene commercial serine-threonine kinase microarray and analyzed using the PamStation12 at 200 ms exposure. The serine-threonine kinase chip contains 144 peptides immobilized into a 12×12 porous aluminum oxide substrates. Functional readout of the chip was based on the phosphorylation occurring on the array using labeled anti-phospho-antibodies.

Results: Out of 144 peptides on the serine-threonine kinase microarray, 95 peptides were found to have higher phosphorylation level and 6 peptides were found to have lower phosphorylation level after stimulation. Stimulation with TGF-β1 activated various cellular processes simultaneously such as cell division, cell proliferation, cytoskeleton remodeling, ions transport channels, cell-to-cell adhesion, apoptosis, cellular metabolism, carbohydrate and lipid metabolism.

Conclusions: The ability to determine the function and involvement of ligands and their secondary messenger response during cellular process can help examine the regulation of certain changes induced not only by TGF-β ligands but also other ligands and helps understand their involvement in many diseases such as cancer and various genetic diseases. Current high-throughput methods for the analysis of protein interaction such as peptide microarrays can yield more information and are taking the modeling of signal transduction processes to a new level.

Keywords: Transforming growth factor-β; serine-threonine kinase; microarray; cellular process

Cite this abstract as: Donny N, Sultana F, Dimitra M, Erik S, Gerard P. Profiling the serine threonine kinase phosphorylation of TGF-β1 stimulated fibroblast using peptide microarray. Ann Transl Med 2015;3(S2):AB122. doi: 10.3978/j.issn.2305-5839.2015.AB122

AB123. Carrier screening and prenatal diagnosis for α- and β-thalassemia in pregnancies at risk in National Hospital of Pediatrics, Vietnam

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Background: Thalassaemia is the most common hereditary disease in Southeast Asia. In Vietnam, the carrier rate for β-thalassemia varies from 1.5% to 25% depending on the ethnic groups of the population.

Objective: To evaluate the effectiveness of the first screening program for control of α- and β-thalassemia in a group of high-risk pregnancy patients who attended our clinic from January 2012 to April 2015.

Methods: The identification of pregnancies at risk was done retrospectively and prospectively. A total of 944 women with reduced levels of mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) were referred to the Human Genetics Department where, together with their husbands, were screened using standard protocols. If both members of the couple were positive for these markers, then determination of the thalassemia carrier status by DNA analysis was considered and the pregnancy was considered at risk.

Results: Out of the 944 couples tested, 754 pregnant women and 385 husbands were positive. Among the 754 women, 493 (65.3%) were α-thal carriers; 204 (27.1%) β-thal carriers; 40 (5.3%) α- and β-thal carriers and 17 (2.2%) were positive for hemoglobin H disease (HbH). Among the men, 300 (77.9%) were α-thal carriers, 37 (9.6%) β-thal carriers; 37 (9.6%) α- and β-thal carriers and 11 (2.9%) were positive for HbH disease. In total, we identified 508 couples at risk, 306 prospectively (284/306 homozygous α^0-thal, 19/306 β-thal, 3/306 both α^0-β-thal) and 202 retrospectively (166/202 β-thal, 36/202 HbH disease). After genetic counseling, prenatal diagnosis by fetal DNA analysis was performed on 312/508 (61.5%) couples including 91 pregnancies at risk for homozygous α^0-thal, 36 at risk for HbH disease, and 185 at risk for β-thal major.

Keywords: Thalassaemia; pregnancy; screening program; Vietnam

Cite this abstract as: Ngo DN, Ly TT, Ngo TT, Nguyen TP, Tran TH, Duong BT, Tran DC, Le TT, Lê TH, Tran TT. Carrier screening and prenatal diagnosis for α- and β-thalassemia in pregnancies at risk in National Hospital of Pediatrics, Vietnam. Ann Transl Med 2015;3(S2):AB123. doi: 10.3978/j.issn.2305-5839.2015.AB123

AB124. Mucolipidosis type II: clinical features and laboratories

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Background: I-cell disease (Mucolipidosis II) is a rare lysosomal storage disorder caused by the deficiency of N-acetylglucosamine-l-phosphotransferase, an enzyme that transfers phosphate groups onto oligosaccharide units of lysosomal enzyme precursors. Due to the absence of transferase activity, the common phosphomannose recognition marker of acid hydrolases is not generated, and the enzymes are not targeted to the lysosomes I. As a consequence the enzymes are secreted into the extracellular space, and high activities can be found in the serum,

cerebrospinal fluid and urine of the patients, whereas inside the cells (fibroblasts) the enzyme levels are considerably reduced. Mucolipidosis is also known as I-cell disease because of the coarse granular cytoplasmic inclusions seen in cultured skin fibroblasts which are large lysosomes containing heterogeneous material.

Objective: To describe clinical features and enzyme activity of patients with mucolipidosis type II.

Methods: Clinical features, laboratory and plasma lysosom enzyme activity by four MU-Fluorometric assay was study.

Results and conclusions: Sixteen cases (seven girls and nine boys) onset at 5.93±4.28 years of age the onset age of 2.3±3.1 years (median 1.25) with the feature of joint stiffness and bone deformation. 100% cases admitted with the feature of joint stiffness, chest deformation and kyphoscoliosis, 93.3% coarse facial features. No patients had hepatosplenomegaly on ultrasound, 5/15 patients had heart valves disease. Enzyme assay showed α-Hexosaminidase of 1,885.98±338.7 nmoL/mg plasma/17 h, α-Iduronate sulfatase of 4,534.78±1,062.97 nmoL/mg plasma/4 h. Mucolipidosis has seriously affected the life of the patients.

Keywords: Mucolipidosis type II; I-cell disease

Cite this abstract as: Can NT, Vu DC, Bui TP, Nguyen KN, Hwu WL. Mucolipidosis type II: clinical features and laboratories. Ann Transl Med 2015;3(S2):AB124. doi: 10.3978/j.issn.2305-5839.2015.AB124

AB125. Neonatal diabetes mellitus due to insulin gene mutation

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Background and objective: *Insulin* (*INS*) gene mutations that cause permanent neonatal diabetes mellitus change single protein building blocks (amino acids) in the protein sequence. These mutations are believed to disrupt the cleavage of the proinsulin chain or the binding of the A and B chains to form insulin, leading to impaired blood sugar control. At least ten mutations in the *INS* gene have been identified in people with permanent neonatal diabetes mellitus. To describe clinical features and laboratory manifestations of patients with *INS* gene mutation and to evaluate outcome of management.

Methods: Clinical features, biochemical finding, mutation analysis and management outcome of six cases from six unrelated families were study. All exons of *INS* gene were amplified from genomic DNA and directly sequenced.

Results: Six cases (three girls and three boys) onset at 129.2±128.8 days of age (median 101.5 days) with gestation age of 37.3±3.0 weeks, birth weight of 2,816.6±767.8 g. Five out of six patients admitted with the feature of diabetic ketoacidosis with pH of 7.04±0.22; plasma glucose levels were 34.3±12.7 mmoL/L, HbA1C of 9.75%±3.5%. Mutation analysis of the *INS* gene showed: heterozygous for a novel missense mutation (c.127T > A; C43S) in exon 2 in one case; heterozygous for a splicing mutation c.188-31G > A in intron 2 in two cases; heterozygous for a missense mutation c.286T > C in exon 3 in one case; heterozygous for a missense mutation c.265C > T [p.Arg89Cys (p.R89C)] in exon 3 in two cases. After 19.2±13.4 months of insulin treatment, 4/5 patients have normal development with DQ 80-100%, HbA1C of 6.85%±0.49%, quite normal blood glucose levels. The case with c.127T > A mutation treated with insulin for 14 years has physical development delay, poor blood glucose control with HbA1C of 11.4%.

Conclusions: It is important to perform screening gene mutation for patients with diabetes diagnosed before 6 months of age to control blood glucose and follow up the patients.

Keywords: *Insulin* gene mutation (*INS* gene mutation)

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AB126. Enzyme replacement therapy in patient with mucopolysaccharidosis type I: a case report

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Background and objective: Mucopolysaccharidosis I (MPS I) is a rare, recessively inherited, lysosomal storage disorder caused by deficiency on the enzyme α-L-iduronidase. This defect results in accumulation of heparan and dermatan sulfate in different tissues and organs due to a deficiency in the catabolism of glycosaminoglycans. The overall incidence of MPS I is 0.99-1.99/100,000 live births. Enzyme replacement therapy (ERT) with recombinant α-L-iduronidase (laronidase) has shown to significantl improve the quality of life in children. To describe clinical characteristics, enzyme activity and genetic finding in the first Vietnamese patient with MPS type I with aldurazyme replacement therapy.

Methods: Clinical features, biochemical finding, enzyme activity, mutation analysis and management in a 4 years 6-month-old girl was study. Based on analysis of a patient's clinical symptoms associated with enzyme α-L-iduronidase activity measurement in leukocyte, the diagnosis of MPS type I was therefore made. Genomic DNAs were extracted from peripheral blood leukocytes from the patient and identify mutation of *IDUA* gene, 14 exons and their intronic boundaries of the IDUA gene were sequenced using genomic DNA from the patient. The patient has been treated with aldurazyme infusion every week with the dose of 0.58 mg/kg/week.

Results: A 4 years 6-month-old girl was presented with joint stiffness at 2 years old. She was admitted with the features of short status, coarse facial, corneal clouding, carpel tunnel syndrome and joint stiffness, kyphosis, abdominal distension, palpable liver at 3 cm below the costal margin, sleep disturbances/snoring. Laboratory showed: hearing lost at right ear in acoumetry, hepatosplenomegaly in ultrasound with right liver length of 117 mm, spleen length of 89 mm, a 6-minute walk test distance of 158.6 m, α-I-duronidase 0.43 nmoL/mg Prot/hrs (normal: 41.8±15.9), urine glycosaminoglycan (GAG) of 508.83 mg/g creatinine (normal: 10.74-112.02). PCR sequencing of *IDUA* gene showed a novel heterozygous sequence variant c.1046A>G (p.Asp349Gly). After 6 months: more active, decreasing of wheezing in sleep, a 6-minute walk test distance of 252 m, ultrasound showed normal live and spleen size, urine GAGs of 61.18 mg GAGs/g Creatinine.

Conclusions: Enzyme therapy can improve of clinical manifestation which will lead to improvements in life expectancy and quality of life in MPS I patients.

Keywords: Mucopolysaccharidosis type I; hurler; aldurazyme therapy in MPS I

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AB127. Enzyme replacement therapy in patient with mucopolysaccharidosis type II: a case report

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Background and objective: Mucopolysaccharidosis (MPS) type II (Hunter syndrome) is an X-linked lysosomal storage disorder due to the deficit of iduronate 2-sulfatase, an enzyme catalysing the degradation of the glycosaminoglycans (GAG) dermatan- and heparan-sulfate. Treatment of the disease is mainly performed by enzyme replacement therapy (ERT) with idursulfase. This article aims to describe clinical characteristics, enzyme activity and genetic finding in the first Vietnamese patient with MPS type II treated with idursulfase (Elaprase) replacement therapy.

Methods: Clinical features, biochemical finding, enzyme activity, mutation analysis and management in a 4 years 6-month-old girl was study. Based on analysis of a patient's clinical symptoms associated with enzyme iduronate-2-

sulphate sulphatase activity measurement in plasma, the diagnosis of MPS type II was therefore made. Genomic DNAs were extracted from peripheral blood leukocytes from the patient and identify mutation of *IDS* gene, nine exons and their intronic boundaries of the *IDS* gene were sequenced using genomic DNA from the patient. The patient has been treated with Elaprase infusion every week with the dose of 0.05 mg/kg/week.

Results: A 34-month-old boy was presented with coarse facial at 24 months of age. He was admitted with the features of coarse facial, with frontal bossing, prominent supraorbital ridge, large nose and flat nasal bridge, widely spaced teeth, thickened gingival mucosa, and macroglossia, broadly built of the body habitus with a short neck, broad chest, and protuberant abdomen and Mongolian spots at the back and breech, joint finger stiffness, abdominal distension, palpable liver at 3 cm below the costal margin, sleep disturbances/snoring, mental development delay. Laboratory showed: hepatosplenomegaly in ultrasound with right liver length of 127 mm, spleen length of 93 mm, a 6-minute walk test distance of 240 m, DQ 55%, α iduronate sulphate: 0 nmoL/4 h/mL plasma (normal: 600-1,616) , urine glycosaminoglycan (GAG) of 498.5 mg/g creatinine (normal: 10.74-112.02). PCR analysis for recombination showed abnormal recombination in proximal and distal regions, which means *IDS* gene is disrupted by recombination with *IDS2* gene. After 6 months of treatment: more active, decreasing of wheezing in sleep, a 6-minute walk test distance of 300 m, ultrasound showed the right live length of 111 mm, and spleen size of 89 mm, urine GAGs of 254.15 mg GAGs/g Creatinine.

Conclusions: Enzyme therapy can improve of clinical manifestation which will lead to improvements in life expectancy and quality of life in MPS II patients.

Keywords: Mucopolysaccharidosis type II; Hunter syndrome

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AB128. Neonatal diabetes mellitus: genotype, phenotype and outcome

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Background and objective: Neonatal diabetes mellitus (NDM) is a rare (1:300,000-400,000 newborns) but potentially devastating metabolic disorder characterized by hyperglycemia combined with low levels of insulin. Two main groups have been recognized on clinical grounds, transient NDM (TNDM) and permanent NDM (PNDM). This article aims to describe clinical features and laboratory manifestations of patient with NDM and evaluate outcome of management.

Methods and materials: Clinical features, biochemical finding, mutation analysis and management outcome of 24 cases 24 unrelated families were study. All exon of *KCNJ11*, *ABCC8* and *INS* genes were amplified from genomic DNA and directly sequenced. If the mutation of *KCNJ11*, *ABCC8* and *INS* has failed to detect, methylation—specific PCR will be done to detect the loss of methylated region on chromosome 6q24.

Results: Twenty-four cases (11 girls and 13 boys) onset at 67.3±44 days of age with gestation age of 38.79±2.2 weeks and birth weight of 2,720.8±571.7 g. 9/24 cases admitted with the feature of polydipsia, polyuria and 17 cases with diabetes keton acidosis with pH of 7.13±0.18, blood glucose of 34.8±10.0 mmoL/L, HbA1C of 7.9%±2.9%. Mutation analysis showed six patients with heterozygous for a *KCNJ11* missense mutation, seven patients with *ABCC8* mutations, four patients with abnormal of chromosom 6, six patients with *INS* mutation, one patient with EIF2AK3 mutation. The patients have been followed up during 54.4±46.6 months (4 months to 14 years). Five patients with TNDM stop insulin at 8.25±5.8 months of diagnosis: four cases have abnormal of 6q24, one case has *ABCC8* mutation. Now all cases have normoglycemic (blood glucose: 5.0 and 5.9 mmoL/L), one patient has mild development delay and four patients has normal development. Nineteen patients with PNDM: 13 cases successfully transferred onto sulfonylureas and did not

need insulin injections, six cases require insulin. In there, two cases with DEND syndrome have development delay, others cases have normal mental development.

Conclusions: It is important to perform screening gene mutation for patients with diabetes before 6 months of age to control blood glucose and follow up the patients.

Keywords: Neonatal diabetes mellitus (NDM); transient neonatal diabetes; permanent neonatal diabetes

Cite this abstract as: Can NT, Vu DC, Bui TP, Nguyen KN, Flanagan S, Ellard S, Craig M, Nguyen DP, Nguyen HT. Neonatal diabetes mellitus: genotype, phenotype and outcome. Ann Transl Med 2015;3(S2):AB128. doi: 10.3978/j.issn.2305-5839.2015.AB128

AB129. Osteogenesis imperfecta: clinical features and bisphosphonate treatment outcome

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Background and objective: Osteogenesis imperfecta (OI) comprises a group of disorders principally affecting type I collagen which result in increased bone fragility. Children with severe OI suffer recurrent fractures, resulting in severe deformity and growth stunting in many cases, with loss of independent ambulation by the teenage years in over 50% of cases. Recently, cyclical intravenous treatment with pamidronate has proven of benefit to children with severe forms of OI. This article aims to describle clinical features and laboratory manifestations of patient with OI and evaluate outcome of bisphosphonate management.

Methods: Clinical features, biochemical finding, and management outcome of 104 cases were study. The patients were classified into four major subtypes of Sillience *et al*. 1979. Patients with severe types were treatment with pamidronate (Aredia) used Rauch protocol 2003.

Results: Now we have 196 patients (87 females and 109 males) but we studied focus on 104 patients from 98 families (60 males, 44 females) onset at 2.1±3.0 years (median 0.35) with the average fracture bone of 5.9±4.4 times. In there, 17% type I, 8% type II, 63% type III, and 12% type IV. Clinical features include of intrauterine fracture visible on ultrasound 35%, bone deformation after birth 68%, triangle face 76%, long bone deformation 91%, chest deformation 46%, scoliosis 27%, short status 90%, blue sclera 83%, dentinogenesis imperfecta 20%, hearing loss 6%. Thirty patients have been treated with pamidronate at 3.2±3.7 years (4 months to 8 years) during 13±0.8 months (6-30 months). Fourteen patients had fracture bone after 6 months of treatment but no patients had fracture bone after 12 months. Seven patients had been treatment after 1.6±0.5 years, BMD increase from 0.39±0.311 to 0.79±0.105 g/cm^2 ($P<0.05$). One patient had fever reaction after first pamidronate infusion but controlled with standard antipyretic therapy, and do not recur in later treatments.

Conclusions: OI has seriously affected the life of the patients and bisphosphonate therapy has shown some real long-term promise in the treatment of young patients with OI. No significant side effects have been noted, which is of the utmost importance in therapy destined for children.

Keywords: Osteogenesis imperfecta (OI); bisphosphonate treatment

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AB130. Pseudoaldosteronism due to mutation of *SCNN1A* gene: a case report

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Background: Pseudohypoaldosteronism type 1 (PHA1) is a rare inherited disease characterized by resistance to the actions of aldosterone. It was first described in 1958 by Cheek and Perry, and common clinical manifestations include salt wasting, hyperkalaemia, metabolic acidosis and elevated plasma aldosterone levels in the neonatal period.
Objective: To describe clinical characteristics, laboratory features and management of one Vietnamese patient with pseudohypoaldosteron.
Methods: Clinical features, biochemical finding, mutation analysis and management in a 1 month-old-boy was studied. Based on analysis of this patient's clinical symptoms associated with biochemical examination, the urinary steroid metabolomics analysis was performed using gas chromatography spectrometry and mutation analysis of *SCNN1A* was performed using PCR & direct sequencing.
Results: Patient is the first child normal delivery with the gestation age of 41 weeks, birth weight of 3,200 g, and onset of the disease at 7 days of age. He presented with lost weight, dehydration without vomit, diarrhea or hyperpigmentation. He was admitted with the features of cyanosis, allorhythmic, electrolyte imbalance with sodium of 119 mmoL/L, potassium of 7.4 mmoL/L. Investigation show pH 7.26, PCO_2 34 mmHg, PO_2 110 mmHg, HCO_3^- 18 mmoL/L, BE -10, plasma 17OHP level: 2.4 ng/mL, testosterone level: 1.94 nmoL/L, Cortisol 8am: 2,662.8 pmoL/L, Ure 7.4 mmoL/L, Creatinine 44.2 µmoL/L, Glucose 4.8 mmoL/L. The urine steroid metabolomics analysis showed extensive excretion of aldosterone ID-ISTD1 of 1,157.41 µg/L. Novel homozygous mutation (c.1668C > A; p.S556R) of *SCNN1A* gene was identified in the proband. He was treated with florinef of 0.1 mg/kg/day for electrolyte balance. He had complication of intestinal perforation and died due to infection. In conclusions, PHA1 causes severe hyponatremia, metabolic acidosis, and life-threatening hyperkalemia, with normal 17-a-hydroxyprogesterone levels and high excretion of aldosterone levels.

Keywords: Pseudohypoaldosteronism type 1 (PHA1)

Cite this abstract as: Can NT, Vu DC, Bui TP, Nguyen KN, Zennaro MC, Wudy SA. Pseudoaldosteronism due to mutation of *SCNN1A* gene: a case report. Ann Transl Med 2015;3(S2):AB130. doi: 10.3978/j.issn.2305-5839.2015.AB130

AB131. Genotype, phenotype of transient neonatal diabetes mellitus

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Background and objective: Transient neonatal diabetes mellitus (TNDM) is a rare but remarkable form of diabetes which presents in infancy, resolves in the first months of life, but then frequently recurs in later life. It is caused by overexpression of the imprinted genes *PLAGL1* and *HYMAI* on human chromosome 6q24, *ABCC8* or *KCNJ11* mutation. Over half of patients with maternal hypomethylation at the TNDM1 locus have additional hypomethylation of other maternally methylated imprinted genes throughout the genome, and the majority of these patients havemutations in the transcription factor *ZFP57*. This article aims to describe clinical features and laboratory manifestations of patient with TNDM and evaluate outcome of management.
Methods: Clinical features, biochemical finding, mutation analysis and management outcome of five cases from five unrelated families were study. All exon of *KCNJ11*, *ABCC8* and *INS* genes were amplified from genomic DNA and directly sequenced. If the mutation of *KCNJ11*, *ABCC8* and *INS* genes has failed to detect, methylation—specific PCR will be done to detect the loss of methylated region on chromosome 6q24.
Results: Five cases (two girls and three boys) onset at 26.2±11.2 days of age with gestation age of 38.6±2.6 weeks, birth weight of 2,440±512 g. 4/5 patients admitted with the feature of polydipsia, polyuria, macroglossia and diabetes ketone acidosis with pH of 7.11±0.2, blood glucose of 36.64±10.9 mmoL/L, HbA1C of 7.02%±0.96%. Methylation—specific PCR of two patients showed heterozygous mutation in *ZFP57*; two patients has maternal hypomethylation at the TND differentially methylated region on chromosome 6q24, in there, one methylation signature is characteristic of patients with mutations in

ZFP57 in the process of carrying out *ZFP57*, one patient has heterozygous for the previously reported *ABCC8* missense mutation, p.R1183W. All patients stopped insulin after 8.25±5.8 months of treatment. After 32±23 months of insulin stopped, all of them have normal blood glucose and normal HbA1C, four cases have normal development, and one case has mild development delay.

Conclusions: It is important to perform screening gene mutation for patients with diabetes diagnosed before 6 months of age to control blood glucose and follow up the patients.

Keywords: Transient neonatal diabetes; *ZFP57* mutation

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AB132. Neonatal diabetes in Wolcott-Rallison syndrome: a case report

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Background: Wolcott-Rallison syndrome (WRS) is a rare autosomal recessive disorder characterized by the association of permanent neonatal or early-infancy insulin-dependent diabetes, multiple epiphyseal dysplasia and growth retardation, and other variable multisystem clinical manifestations.

Objective: To describe clinical characteristics and genetic finding in the first Vietnamese patient with EIF2AK3 mutation.

Methods: Clinical features, biochemical finding, mutation analysis and management in a 64-day-old girl was study. Based on analysis of a 64-day-old girl's clinical symptoms associated with biochemical examination, the diagnosis of WRS was therefore made. Genomic DNAs were extracted from peripheral blood leukocytes from the patient and her parents with their informed consent for genetic studies. The coding and flanking intronic regions of the *EIFAK3* gene was analysed by sequencing.

Results and conclusions: The patient had gestation age of 41 weeks, birth weight of 3,200 g, and onset of the disease at 64 days of age. She was admitted with the features of convulsion, anemia, jaundice, diabetic ketoacidosis with pH of 7.27, HCO_3^- of 17.8 mmoL/L, BE of –8 mmoL/L, blood glucose 42.46 mmoL/L, HbA1C 6.5%, total bilirubin 59.2 µmoL/L, direct bilirubin 29.7 µmoL/L, AST 3,742.2 U/L, ALT 1,927 U/L. PCR of CMV, EBV, HAV were negative. Abdominal ultrasound did not find any sign of cholestasis. Sequencing analysis of patient's *EIF2AK3* gene has identified a homozygous missense mutation, p.R632W. The parents are carriers of heterozygous EIF2AK3 missense mutation, p.R632W. Now she is 3 years and 3 months old, she has normal development, good blood glucose control with the insulin dose of 1.0 UI/kg/day, no jaundice, HbA1c 5.8% (normal range, 4-6%), AST 30.43 UI/L, ALT 17.09 UI/L, not yet skeletal symtoms. Combining mutation screening of *EIF2AK3* gene with clinical manifestations and effective examination may provide a reliable diagnostic method for patients.

Keywords: Wolcott-Rallison syndrome (WRS)

Cite this abstract as: Can NT, Vu DC, Flanagan S, Ellard S. Neonatal diabetes in Wolcott-Rallison syndrome: a case report. Ann Transl Med 2015;3(S2):AB132. doi: 10.3978/j.issn.2305-5839.2015.AB132

AB133. SmartLabs—a solution to healthcare

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Abstract: DNA sequencing is the most commonly used approach for mutation scanning and is widely regarded as the gold standard in diagnostics of rare diseases. Genetic and genomic tests significantly impact clinical care through better disease prevention, faster and more accurate diagnosis, and informed treatment selection. Multi-gene panel tests, for example, enable physicians to

identify all disease-associated genetic mutations and drug responses, facilitating faster diagnosis and treatment. Next generation sequencing (NGS), also described as "second generation", has replaced Sanger sequencing as the primary methodology employed by researchers to identify novel disease genes. The ability to simultaneously analyse multiple or very large genes at a cheaper cost per base makes next generation sequencing an attractive solution for clinical diagnostic testing to identify the disease-causing variation (or variations) in patients with genetically heterogeneous disorders. While the market for high impact genetic and genomic testing is poised for rapid growth, most hospitals and health systems outsource these tests to third party reference laboratories, due to lack of technology and expertise for analytics. The ability to offer high impact, cost effective genetic testing, and data analysis services will be a key differentiating factor for hospital systems. A SmartLab is a clinical NGS diagnostics laboratory customized to meet the customer's existing capabilities and needs in terms of infrastructure, equipment, disease-specific gene panels, data analysis solutions, personnel, training, clinical laboratory accreditations and regulatory approvals. SmartLab enhances the brand value of partner organizations, helps them differentiate themselves from their regional competitors, empowers their participation in personalized medicine clinical research programs or new clinical trials. A SmartLab is a complete laboratory and informatics solution to enable patient-centered genomic medicine from sample to reports. It provides healthcare organizations with the necessary capabilities to offer their patients in-house, NGS-based genetic and genomic tests in various disease areas. SmartLab enabled health organizations can leverage clinical and patient genomic data to improve patient care and health outcomes. Strand SmartLab is a turnkey solution that combines the clinical NGS laboratory blueprint (i.e., design) and Strand's enterprise bioinformatics solutions to equip and enable customers with the necessary infrastructure, capabilities, personnel, regulatory approvals and training to offer clinical NGS testing services to patients.

Keywords: SmartLab; next generation sequencing (NGS); genomics solutions

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AB134. Trimethylaminuria: report of two cases in Ramathibodi hospital

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Background: Trimethylaminuria (TMAU), or fish-odor syndrome, is a rare metabolic disorder with clinical characteristic of rotten fish-like body odor due to excessive trimethylamine excretion, caused by flavin-containing monooxygenase 3 (FMO3) deficiency leading to defective hepatic trimethylamine metabolism. About 200 cases have been reported world-wide, but only five have previously been reported in Thailand. Here, we report two further unrelated cases of trimethylaminuria presented in Ramathibodi hospital.

Case presentation: (I) The first case is a 55-year-old Thai female who presented with foul-smelling body odor, resembling rotten fish, for 20 years, resulting in self-embarrassment and social anxiety. This did not relate to any activity or condition. Her mother also experienced the same condition, but with milder severity. Sequencing of her *FMO3* gene showed heterozygous c.769G>A (p.Val257Met) variant, which could cause reduction in TMA N-oxygenation activity. Her symptom improved after treatment with activated charcoal. Her mother declined any diagnostic investigation for trimethylaminuria. (II) The second case is a 19-year-old woman, who suffered from rotten fish-like body and urine odor for 3 years. She has two younger brothers, none of whom experienced a similar problem. Sequencing of her *FMO3* gene showed heterozygous c.472G>A (p.Glu158Lys) and c.923A>G (p.Glu308Gly) mutations, which could also cause reduction in TMA N-oxygenation activity. She was advised to avoid food containing high choline and trimethylamine (eggs, legumes, Brassica vegetables, and marine fish). Her fishy body and urine odor improved after dietary restriction.

Conclusions: We report two cases of *FMO3* deficiency resulting in symptoms of primary trimethylaminuria, with identified *FMO3* gene mutation, which was successfully treated with activated charcoal in the first case, and dietary restriction in the second case. Although it is

rare, trimethylaminuria causes patients to suffer from psychosocial problems. Early diagnosis and appropriate treatment remain very important steps that could help patients suffering from trimethylaminuria, which should be suspected in patients who report fishy body odor. Treatment includes a combination of low precursor diet with intermittent antibiotics and sequestering agents (activated charcoal and copper chlorophyllin). Genetic counseling is essential, especially in primary trimethylaminuria caused by identified *FMO3* gene mutation.

Keywords: Trimethylaminuria (TMAU); flavin-containing monooxygenase 3 (FMO3); fish-odor syndrome

Cite this abstract as: Girdwichai N, Khemthong P, Tunteeratum A, Dejsuphong D, Eu-Ahsunthornwattana J, Sura T. Trimethylaminuria: report of two cases in Ramathibodi hospital. Ann Transl Med 2015;3(S2):AB134. doi: 10.3978/j.issn.2305-5839.2015.AB134

AB135. The numerical chromosomal abnormalities in prenatal screening and diagnosis by QF-PCR

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Background and objective: Currently, screening and prenatal diagnosis of chromosomal disorders has become popular in big hospitals in Vietnam. One of the most popular rapid tests in aneuploidy diagnostic is quantitative fluorescence PCR. Prenatal diagnosis is indicated for high-risk pregnancy screened by screening tests (combined test, triple test) or ultrasound (fetal anatomic defects, such as congenital heart defect or markers suggestive of fetal aneuploidy like a nuchal translucency, thickened nuchal fold, absent nasal bone, renal pyelectasis, or echogenic bowel…). However, research about the ratio of aneuploidy in these each screening test has not been carried in Vietnam. This study is evaluating the correlations between the screening test, ultrasonography results and results of chromosomal analysis on fetal cells.

Methods: Analyzing 5,557 QF-PCR results of amniotic fluid samples from pregnant women with high risk from Jan to Dec 2014.

Results: In our study, we identified 242 cases (4.35%) with aneuploidy (of which 132 cases of trisomy 21, 78 cases of trisomy 18, 11 cases of trisomy 13, 21 cases of other chromosomal abnormalities). The most cases of amniocentesis are pregnancy with high risk of biochemical test: 3,955/5,557 cases (71%). The ratio of high risk pregnancies screened by combined test is higher than this by triple test: 4.7% (107/2,275) compared to 2.44% (41/1,680). The ratio of trisomy 21 is highest in cases of absent nasal bone: 20.8% (40/192). Multiple congenital cases are the highest ratio of trisomy 18: 61% (11/18 cases).

Conclusions: This report confirms the importance of prenatal screening and diagnosis in detecting aneuploidy. It also helps obstetricians and geneticists in counseling and indicating invasive techniques for high risk pregnancies.

Keywords: Prenatal screening; diagnosis; aneuploidy; chromosome; high risk

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AB136. Arthrogryposis, renal dysfunction, cholestasis (ARC) syndrome

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Background: ARC (arthrogryposis, renal dysfunction, cholestasis) is a clinical syndrome with multisystem disorder, the major presentations are arthrogryposis, renal tubular dysfunction and cholestasis. It is a rare autosomal recessive syndrome which is caused by mutations in *VPS33B* gene on chromosome 15q26.1. ARC is a rare syndrome. Until now, there haven't had any reports on ARC syndrome in Vietnam.

Objective: Describle clinical, laboratory characteristics and follow up ARC patients.
Methods: The retrospective description.
Results and conclusions: In the time 1/2012-2/2014, at National Hospital of Pediatrics, we detected eight ARC cases. The major clinical signs: arthrogryposis, renal tubular dysfunction, cholestasis. Some other disorders: ichthyosis, failure to thrive, recurrent fever, diarrhea… mutations in *VPS33B*. The ARC patients have high mortality, inability to cure. The next pregnancy of woman, who have had ARC baby should be followed up and consulted carefully.

Keywords: Arthrogryposis, renal dysfunction, cholestasis syndrome (ARC syndrome); arthrogryposis; renal dysfunction; cholestasis; mutations on *VPS33B* gene

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AB137. Preimplantation genetic diagnosis for rare monogenic disorder: a lesson from pantothenate kinase-associated neurodegeneration

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Background and objective: Preimplantation genetic diagnosis (PGD) is a technique to identify the genetic defects in the embryos created through *in vitro* fertilization (IVF). The purpose of this technology is to assist reproduction in one or both biological parents carrying known genetic abnormalities. Herein, we report on a Thai couple having an experience on the loss of the first child affected by neurodegeneration and died at the age of 2. Brain MRI revealed the tiger-eye-sign, which was suspected for pantothenate kinase-associated neurodegeneration (PKAN). DNA sequencing of *PANK2* gene was performed in the whole family members and novel g.21738G>C at the splice site was identified as likely pathogenic variant, relying on autosomal recessive inheritance model. This article aims to develop PGD strategy for *PANK2* variant inherited in this family.
Methods: Genetic counseling for PGD was performed to the couples and the ethical clearance was done. IVF and intra cytoplamic sperm injection (ICSI) with PGD was performed. All of embryos were biopsied in the cleavage stage and subsequently performed for whole-genome amplification. Genetic status was diagnosed with the linkage analysis using family-specific short-tandem repeat markers and direct mutation testing using SNaPshot Mini-sequencing. The aneuploidy screening was performed by low-pass whole genome next-generation sequencing (NGS)-based strategy.
Results: Only single cycle of IVF-ICSI was processed. There were seven embryos from these couples: two likely affected, three likely being carriers, one likely unaffected and one failed in the target genome amplification. Aneuploidy screening was done before making decision of embryo transfer and only one unaffected embryo passed the screening. Thereafter, this embryo was transferred in frozen thawed cycle and the pregnancy was successful. The confirmation was done by amniocentesis, which showed the consistent result to PGD. At 38 weeks of gestational age, a healthy male baby was born.
Conclusions: PGD is currently established as a technology to prevent the recurrence of genetic disorders in the family. Here we report the first successful story of PGD for PKAN.

Keywords: Preimplantation genetic diagnosis (PGD); pantothenate kinase-associated neurodegeneration (PKAN); *PANK2*; *in vitro* fertilization (IVF); next-generation sequencing (NGS)

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AB138. Next-generation sequencing as a tool for molecular diagnosis of hypertrophic and dilated cardiomyopathies in Thai patients

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Background and objective: Familial hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) are the hereditary cardiac disorder that can cause early morbidity and mortality in young people without any cardiovascular risk factors. These particular conditions are genetically heterogenous. To date, genetic information of HCM and DCM in Thai population is limited. We aimed to develop the effective high-throughput molecular strategy to detect the pathogenic variants of HCM and DCM in Thai patients, mainly to support clinical cardiovascular services and genetic counseling.

Methods: Ramathibodi inherited cardiac disease (RICD) chip was developed using next-generation sequencing (NGS)-based technology (Ion PGM™). The chip contains 72 genes causing various types of cardiomyopathies and sudden cardiac death with total 3,280 amplicons within 1,696 targets. Using genomic DNA from the patients' samples, all exon and their flanking splice junctions of the filtering gene targets for HCM and DCM was sequenced. Bioinformatic analysis was performed using minor allele frequency in 1,000-genome project and in-house exome database, protein prediction tools (SIFT and PolyPhen-2) and genetic evolution data crossing several animal species (PhastCons). Nucleotide variants obtained by NGS were classified their predicted pathogenicity based on American College of Medical Genetics and Genomics: known pathogenic, likely pathogenic, variant of unknown certain significance (VUS), likely benign and benign. The findings from NGS were subsequently confirmed by capillary sequencing.

Results: Nine HCM patients were enrolled for this study and two of them (22.22%) showed the pathogenic variants in *MYBPC3*, which was the common causative gene for this condition. One case (11.11%) revealed VUS in *TPM1*, which presented the possibility to be pathogenic by protein prediction analysis and evolution data, whereas the rest (66.67%) was unable to uncover the variants. For DCM, 17 patients were also enrolled. Three of them (17.65%) were identified for known pathogenic variants in *SCN5A*, a common gene for sudden cardiac death in Thai population. One of them (5.88%) showed a likely pathogenic variant in *TTN*, the common gene for DCM in global population. No variants were found in three cases (17.65%), while the others (58.82%) were characterized with VUS in various genes.

Conclusions: This is a preliminary study to demonstrate the molecular characterization of HCM and DCM in Thailand. NGS is proposed as an effective tool to detect pathogenic variants to facilitate risk stratification in patients and family members.

Keywords: Cardiomyopathy; hypertrophic; dilated; next-generation sequencing (NGS)

Cite this abstract as: Trachoo O, Panthan B, Jittorntam P, Phusanti S, Mukdadilok A, Srisukh S, Sae-Chew P, Charoenyingwattana A, Pasomsub E, Vathesatogkit P, Chantratita W, Tangcharoen T. Next-generation sequencing as a tool for molecular diagnosis of hypertrophic and dilated cardiomyopathies in Thai patients. Ann Transl Med 2015;3(S2):AB138. doi: 10.3978/j.issn.2305-5839.2015.AB138

AB139. The role of CD44 in the osteoblastic differentiation from mesenchymal stem cells

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Background and objective: Osteoblastic differentiation from mesenchymal stem cells (MSCs) is regulated

by many hormonal and autocrine/paracrine factors. Recently, immunocytochemistry revealed that CD44, a transmembrane glycoprotein, is more expressed in osteoblasts than in MSCs during osteoblast differentiation (Kim *et al*., 2008). We examined whether CD44 might be involved in osteoblast differentiation.

Methods: CD44 transcript, CD44 protein and calcium deposit levels were measured by RT PCR, real time PCR, Western blot and Alizarin Red S staining during osteogenic differentiation of mouse multipotent stem cells (D1 cell line) in various time points.

Results: Our data have shown that CD44 expressed in both MSCs and osteoblasts. CD44 transcriptional levels changed during osteoblastic differentiation, but that alkaline phosphatase (ALP) transcript levels gradually increased. In addition, the ectopic expression of CD44 protein in MSCs did not affect osteoblastogenesis. Calcium deposit was not different between D1 cells and CD44-over expressed D1 cells. When CD44 protein was blocked with CD44 antibody, ALP mRNA, osteocalcin protein and calcium deposit levels in inhibited cells were similar to in uninhibited D1 cells.

Conclusions: Our data suggest that CD44 does not directly effect on the osteoblastic differentiation of MSCs, but that it can be used as a marker protein for detecting osteoblasts produced by the osteogenic differentiation of MSCs.

Keywords: Osteoblastic differentiation; mesenchymal stem cells (MSCs); CD44

Cite this abstract as: Phuong DT, Yoon TR, Kim HK, Lee ES. The role of CD44 in the osteoblastic differentiation from mesenchymal stem cells. Ann Transl Med 2015;3(S2):AB139. doi: 10.3978/j.issn.2305-5839.2015.AB139

AB140. Ten years experiences of diagnosis spinal muscular atrophy using molecular techniques

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Background and objective: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder caused by mutations in the survival motor neuron gene (*SMN*). This article aims to identify the deletion exon 7 of *SMN*1/*SMN*2 genes in postnatal diagnosis and prenatal diagnosis with spinal muscular atrophy.

Methods: A total of 1,111 patients suspected SMA and 66 pregnant women who had affected children were collected from 2005 to 2015. The deletion of exon 7 *SMN*1/*SMN*2 genes was identified by PCR-restriction fragment length polymorphism (RFLP) techniques.

Results: We identified a homozygous deletion exon 7 of *SMN*1 in 353/1,111 (31.77%); 55/1,111 (4.95%) patients had deletion exon 7 of *SMN*-2 gene. In 66 pregnant women, there is 14/67 (20.9%) fetuses had deletion exon 7 of *SMN*-1 gene; 2/67 (2.98%) fetuses had deletion exon 7 of *SMN*-2 gene and 51/67 (76.12%) fetuses had no deletion.

Conclusions: Using molecular techniques to detect the deletion exon 7 of *SMN*1 gene is useful for postnatal and prenatal diagnosis with SMA.

Keywords: Spinal muscular atrophy (SMA); MLPA; PCR-restriction fragment length polymorphism (PCR-RFLP); prenatal diagnosis

Cite this abstract as: Nguyen TP, NguyenTM, Ngo MT, Ly TT, Ngo TT, An TL, Vu DQ, Nguyen XH, Ngo DN, Bui PT, Nguyen NK, Vu CD, Tran DC. Ten years experiences of diagnosis spinal muscular atrophy using molecular techniques. Ann Transl Med 2015;3(S2):AB140. doi: 10.3978/j.issn.2305-5839.2015.AB140

AB141. Multiplex PCR-based procedure establishment for simultaneous detection of two mutations occurring most frequently in FMS-like tyrosine kinase-3

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Abstract: Acute myeloid leukemia (AML) is a hematological disorder strongly correlated to several gene mutations which have been used in diagnosis and prognosis of disease. FMS-like tyrosine kinase-3 (*FLT3*) is a receptor tyrosine kinase, involving in cell differentiation and proliferation. In AML, abnormal haematopoietic cells carry most commonly two *FLT3* mutations, namely internal tandem duplication (ITD) and tyrosine kinase domain (TKD) found in approximately 25% and 5% among patients, respectively. Previously, two separate PCR based procedures was widely used to detect two mutations. In this study, we established a detection procedure for both mutations consisted of two major steps: multiplex PCR for simultaneous amplification of a 329 base-pair amplicon for ITD containing region and a 146 bp amplicon for TKD prone region, and digestion of the PCR products with EcoRV followed by analysis on 8% polyarylamide gel. The procedure was performed with previously confirmed ITDwt/TKDwt, ITDmt/TKDwt, and ITDwt/TKDmt samples and showed consistency with earlier result obtained by using separate procedures for each mutations. Moreover, the procedure is rapid, consuming 6 hours compared to 10 hours and more effective, using less reagents for PCR and polyacrylamide electrophoresis in detection of two mutants. In conclusion, we successfully established more productive multiplex PCR-based method for detection of two common AML-related mutations in *FLT3* gene.

Keywords: Multiplex-PCR; FMS-like tyrosine kinase-3-internal tandem duplication (*FLT3*-ITD); acute myeloid leukemia (AML)

Cite this abstract as: Tran HT, Pham Y, Trinh PL. Multiplex PCR-based procedure establishment for simultaneous detection of two mutations occurring most frequently in FMS-like tyrosine kinase-3. Ann Transl Med 2015;3(S2):AB141. doi: 10.3978/j.issn.2305-5839.2015.AB141

AB142. Clinical, biochemical and growth hormone receptor polymorphism profile of children with short stature presenting to a tertiary care centre

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Background and objective: To evaluate the clinical and biochemical profile and growth hormone receptor polymorphism of children presenting with short stature to a tertiary care centre. This would thus be directing resources to patients with GH deficiency who would respond best to it.
Materials and methods: This was an observational study on short stature children presenting to a tertiary care hospital over a period of 3 years. All children enrolled underwent extensive baseline work up to investigate for causes of short stature like endocrine causes, malnutrition, chronic diseases, celiac disease, syndromic association, skeletal dysplasia, familial short stature, constitutional short stature and idiopathic short stature. In children with pathological short, serum growth hormone (GH), IGF-1 and IGFBP-3 levels were estimated using two different pharmacological stimuli. GH value of less than 10 mg/L was considered to be GH deficient. Children with GH deficiency were subjected to analysis of the GHD3 exon deletion status. For the genotyping of GHR exon 3 locus, the frequency of GHR transcript variants with retention (GHRfl) or exclusion (GHRd3) of exon 3 was tested by the multiplex PCR assay. This was performed with primers G1, G2, and G3 with a well-defined protocol.
Results: A total of 473 children with a median age of 3.65 years (range, 2-18 years) were enrolled. Twenty three percent of the children each were diagnosed as growth hormone deficient and idiopathic short stature. Celiac disease also contributed significantly in 18% of cases. The other causes seen were skeletal dysplasia (7%), syndromic (12%) and malnutrition (2%). Amongst children with endocrine disorders, 40% children had hypothyroidism, panhypopituitarism was seen in 10% children and 50% had Laron's syndrome. In children with chronic disorders, 72% were diagnosed with thalassemia, 21% with chronic kidney disease and one child had renal tubular acidosis. Constitutional and familial short statures were seen in 6%

and 2% children respectively. Amongst patients with GHD, 60.7% had wild type (GHRfl/fl), 19.2% were heterozygous (GHRfl/GHRd3) and 20.1% were homozygous (GHRd3/d3), whereas for idiopathic short stature they were 67.5%, 14.5% and 18% respectively.

Conclusions: The diagnosis could be attained in 85% of cases. Growth hormone deficiency and celiac disease contribute significantly even though majority is normal variants. Also, genotyping done would help in prediction of response to recombinant GH therapy in a resource constraint resulting in appropriation of finances which could be utilized for a higher priority area.

Keywords: Short stature; growth hormone receptor

Cite this abstract as: Singh P, Pasrija D, Polipalli S, Khalil S, Kapoor S. Clinical, biochemical and growth hormone receptor polymorphism profile of children with short stature presenting to a tertiary care centre. Ann Transl Med 2015;3(S2):AB142. doi: 10.3978/j.issn.2305-5839.2015.AB142

AB143. Berardinelli-Seip congenital lipodystrophy and its diagnostic implications

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Abstract: Berardinelli-Seip congenital lipodystrophy (BSCLD) (OMIM#269700) is a rare autosomal recessive disorder characterized by marked paucity of adipose tissue, a muscular habitus, insulin resistance, hypertriglyceridemia and hepatic steatosis. It is an anabolic syndrome due to the deficiency of leptin, a lipid modulator, leading to the inappropriate mobilization of fat. Four types of BSCLD have been described based on the site of genetic mutation. Type 2 accounts for an earlier onset, more severe complications and intellectual disability. This is due to the lower leptin levels and the loss of functional seipin in type 2 BSCLD. We report the evolving phenotype of type 2 BSCLD in a boy clinically diagnosed at age 5 months and confirmed by molecular genetic study which revealed a pathogenic mutation in the *BSCL2* gene: c.782dupG, p.lle262Hisfs*12homozygous. He is the firstborn of non-consanguineous parents with no significant family history. He has a muscular habitus with a distinct facies, hypertrichosis, absence of subcutaneous fat and hepatomegaly. Investigations revealed hypertriglyceridemia and insulin resistance. A conventional approach to management with dietary fat restriction successfully controlled his biochemical parameters, thus preventing catastrophic complications such as systemic arterial hypertension, heart failure and pancreatitis. Genetic counseling was conferred and prenatal testing recommended for future pregnancies. On follow-up, he had a muscular habitus with hepatomegaly and global developmental delay. Despite early correction of his biochemical parameters, an abdominal ultrasound revealed early onset fatty liver disease and an echocardiogram showed a thickened interventricular septum suggestive of early hypertrophic obstructive cardiomyopathy (HOCM). A fasting serum leptin done was low at 0.8 ng/mL (normal range, 2.0-5.6 ng/mL) confirming leptin deficiency. A leptin analog is now available as an adjunct to mollify the metabolic complications of BSCLD. However, it is yet to be available in developing nations and whether it is able to assuage progressive hepatic cholestasis and HOCM remains a field to venture. This report highlights the importance of early recognition and confirmation by molecular diagnosis to direct appropriate surveillance of complications. This also enables early genetic counseling and prenatal testing for future pregnancies. We describe the evolving phenotype, pertinent investigations and the conventional approach to the management of BSCLD.

Keywords: Autosomal recessive; generalized lipodystrophy; hypertriglyceridemia; insulin resistance; leptin

Cite this abstract as: Muthukumarasamy P, Thong MK. Berardinelli-Seip congenital lipodystrophy and its diagnostic implications. Ann Transl Med 2015;3(S2):AB143. doi: 10.3978/j.issn.2305-5839.2015.AB143

AB144. Study on the relationship between *MYCN* status and other prognostic factors in 41 patients with neuroblastoma

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Background and objective: Neuroblastoma (NBL) is the most common extracranial solid cancer of childhood and is characterized by a remarkable biological heterogeneity, resulting in favorable or unfavorable outcomes. There are many prognostic factors like age, stage, histopathology, plasma and urinary markers and the molecular characteristics of tumor cells. Amplification of the *MYCN* gene, observed in 20-25% of NBL, is established as the most powerful prognostic factor, and so as the first tumor genetic marker which has been used for risk stratification in all neuroblastoma clinical trials. This article aims to investigate, in a series of 41 NBL patients ascertained in 2014, the relationship between amplification of *MYCN* and some other prognostic factors: patient's age, histopathology, VMA/HVA ratio and LDH level.

Methods: Amplification of *MYCN* was identified by FISH. The *MYCN* status was compared with the other clinic-biological factors.

Results: Amplification of *MYCN* is found on 9/41 NBL patient. The proportion of amplified *MYCN* according to the age group is 11% (<12 months patients), 22% (12-18 months) and 67% (>18 months). The favorable and unfavorable histology cases show a different frequency of *MYCN* amplification, 25% and 75%, respectively. All patients with amplified *MYCN* have a VMA/HVA ratio below 1, and 63% of them have LDH level above 2,000 IU/mL (both associated with worse prognosis).

Conclusions: The amplification of *MYCN* is strongly associated with age at diagnosis >18 months, unfavourable histology, VMA/HVA ratio below 1 and LDH level above 2,000 IU/mL. The VMA/HVA ratio and LDH level could be valuable markers for diagnosis and monitoring of disease status, however, the *MYCN* status determined by FISH is one of the most important tools for treatment stratification.

Keywords: Neuroblastoma; MYCN; age of diagnosis; biochemistry parameters; histopathology

Cite this abstract as: Vu DQ, Nguyen TH, Phung TL, Tran NS, Le DC, Hoang NT, Tran TT, Nguyen XH, Ngo DN. Study on the relationship between *MYCN* status and other prognostic factors in 41 patients with neuroblastoma. Ann Transl Med 2015;3(S2):AB144. doi: 10.3978/j.issn.2305-5839.2015.AB144

AB145. Comparative metabolomic analyses in term and preterm Malaysian infants

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Background: Metabolomics, which involves profiling and comprehensive analysis of cellular metabolites, is a promising new tool for clinical diagnostic in neonatology. Urine is considered to be the most predictive of phenotypic outcome in neonatal conditions. Management of sick neonates could be improved with the availability of information on perinatal/neonatal maturational processes and their metabolic background. This study was carried out to compare metabolites identified in the urine sample obtained from term and preterm infants from the postnatal and neonatal intensive care unit (NICU) of University of Malaya Medical Centre.

Methods: Experiments were carried out to compare the metabolomic profiles between (I) collection of urine using urine bag and cotton ball from preterm infants, (II) urine collection at different time-points from preterm infants, (III) preterm and term infants, (IV) different birth weights of preterm infants and (V) between preterms with and without respiratory distress syndrome (RDS). Urine samples were

stored at -40 °C freezer until analysis. Metabolites were extracted using cold methanol extraction. The extracted samples were analyzed on an Agilent 6540 Accurate-Mass LC/QTOF mass spectrometer. Qualitative analysis was done using MassHunter Professional Profiler.

Results: In relation to principle component analysis (PCA) plot, there were no observable differences between collection of urine using urine bag and cotton ball. Thus, urine samples were collected using cotton ball for all subsequent experiments. There were also no significant differences between the metabolomic profiles of week 1 and week 2 preterm infants. It was found that 47 metabolites and two biological pathways were found to differ significantly between preterm and term infants (P value <0.01). On the other hand, metabolomic profiles between preterm infants <1 kg and those >1 kg differed in 17 metabolites (P value <0.01). Importantly, 110 metabolites and 39 biological pathways differed significantly (P value <0.01) between metabolomic profiles of preterm infants with and those without RDS.

Conclusions: Urine metabolomic profile is stable with time of collection over a period of 2 weeks but varies with birth weight and pathological conditions in preterm infants. It is of interest to note that there are significant differences in the urine metabolomics profile between preterm infants with and without RDS. Thus, urine metabolomics has the potential to be applied to the investigation of other pathological conditions in neonates.

Keywords: Urine; metabolomics; LC/MS; neonatal

Cite this abstract as: Muthukanoo RD, Loke MF, Choo YM, Kamar AA, Ishak MT, Vadivelu J, Thong MK. Comparative metabolomic analyses in term and preterm Malaysian infants. Ann Transl Med 2015;3(S2):AB145. doi: 10.3978/j.issn.2305-5839.2015.AB145

AB146. *CD38* gene expression and social phenotypes: blood genomics as a proxy for CNS function

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Abstract: Given the moderate heritability of most complex traits, in which environment plays an important role, the sole pursuit of genetic markers alone may fail to reveal the fullness of phenotypic variance. A complimentary approach is to measure biomarkers in accessible tissues such as blood. Gene expression, which reflects both hereditable and environmental influence, is a particularly attractive focus of investigation Measurement of mRNA levels is likely to capture more of the phenotypic variance, both genomic and epigenetic than a unitary gene based approach. Most importantly, expression levels of many genes show good correspondence between peripheral blood and brain. These considerations have catalyzed an increasing number of investigations demonstrating a relationship between peripheral transcription of both specific candidate genes as well as whole genome expression and many behavioral syndromes. Indeed, so-called 'blood genomics' is becoming an important tool in dissecting complex behaviors. However, no studies to our knowledge have yet leveraged blood genomics towards understanding social decision-making. In a large ongoing study of risk and social decision-making in Singapore and China the B2ESS group has genotyped (GWAS) more than 3,000 Han Chinese subjects. In a subsample of subjects gene expression was measured in peripheral blood. We find an intriguing association between *CD38* gene expression in blood and a number of social

phenotypes including scores on Baron-Cohen's autism quotient questionnaire (N=192, P=0.002, coef =3.933), TCI novelty seeking (N=187, P=0.004, coef =–7.186) and scores on the Hope questionnaire (N=228, P=0.048, coef =0.905). *CD38* gene activity is also correlated with proposer's offer in ultimatum game, but only in males (N=120, P=0.018, coef =2.797). These findings suggest that blood genomics can be a complementary approach to understanding the role of *CD38* in human social behavior.

Keywords: *CD38*; gene expression; social phenotypes; autism; vitamin A

Cite this abstract as: Ebstein RP, Monakhov M, Chew SH, Lai PS. *CD38* gene expression and social phenotypes: blood genomics as a proxy for CNS function. Ann Transl Med 2015;3(S2):AB146. doi: 10.3978/j.issn.2305-5839.2015.AB146

AB147. Treatment-focused genetic testing (TFGT)—is it too soon for Malaysia?

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Background and objective: The knowledge of a woman's BRCA mutation status around the time of breast cancer diagnosis can be used to guide surgical treatment and preventative options for women. This concept, known as treatment-focused genetic testing (TFGT), has been previously reported to be acceptable in a population of Australian women. However, little is known of the acceptability of TFGT to women with breast cancer in Malaysia. The fact that Malaysia is a multi-cultural and religious country poses challenges for the implementation of BRCA genetic testing into clinical practice. Despite this, the benefits in terms of risk management options and the potential reduction in incidence and mortality of breast cancer that may follow the introduction of TFGT in Malaysia are considerable.

Methods: A qualitative study was performed with 20 Malaysian women who attended the Breast Clinic at University Malaya Medical Centre (UMMC). A modified semi-structured interview was used to explore their hypothetical views on TFGT (as if they were being offered the testing if it was available in Malaysia). All interviews were audio-recorded, transcribed and analysed for concordance by three independent coders. Thematic analysis was facilitated by NVivo 10.0 software (QSR International).

Results: Major challenges to implementing TFGT in Malaysia were identified including limited knowledge of health and the genetics of breast cancer, cultural aspects, attitudes towards risk-reducing surgery and the cost of genetic testing. Most participants had difficulty understanding the concept of TFGT and misunderstood the role of genetic testing in breast cancer treatment. Risk reducing mastectomy was perceived to be too extreme, with significant concerns raised about body image. Social stigmatization about a breast cancer diagnosis and being a BRCA mutation carrier, along with the high cost of genetic testing were other identified barriers/challenges to TFGT implementation in Malaysia. Participants preferred face-to-face discussion with their treating doctor rather than written materials, and information regarding TFGT soon after a breast cancer diagnosis was felt to be too much for most participants to receive at that time.

Conclusions: The lack of understanding of genetic testing and poor health literacy are barriers to the introduction of TFGT in Malaysia. Further education in the role of genetics in breast health is essential but there is also a need to consider cultural influences before TFGT implementation in Malaysia. This study provides important insights into the challenges to breast healthcare and cancer genetic counseling in Malaysia.

Keywords: Attitudes; BRCA genetic testing; breast cancer; Malaysia; treatment-focused genetic testing

Cite this abstract as: Mazlan RA, Barlow-Stewart K, Gleeson M, Hwang TS, Yee YS, Hooi TG, Keong TM, Taib NA. Treatment-focused genetic testing (TFGT)—is it too soon for Malaysia? Ann Transl Med 2015;3(S2):AB147. doi: 10.3978/j.issn.2305-5839.2015.AB147

AB148. The introduction of a contingent model of first trimester screening using non-invasive prenatal testing

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Background and objective: The availability of non-invasive prenatal testing (NIPT) has resulted in a paradigm shift in prenatal screening for Down syndrome and other common aneuploidies. NIPT has been validated in both high and low risk populations. The test has now reached a price-point (under AU$500) where consumer demand is high and services need to devise a model under which NIPT is made available. This article aims to describe our experience from 2012 as the first centre in Sydney to offer NIPT, and the progression towards the adoption of a contingent model of aneuploid screening in the first trimester.

Methods: An audit of the first 118 NIPT patients was undertaken by retrospective file review. Following this, a prospective study was undertaken by questionnaire and structured interview in an additional 84 NIPT patients. Maternal anxiety at the time of NIPT and 1 week following results delivery was measured using the Spielberger State-Trait Anxiety Inventory (STAI).

Results: (I) Contingent screening is a model that will increase the detection rate of Down syndrome beyond that of combined first trimester screening as well as reducing the number of miscarriages related to invasive testing; (II) receiving a low risk NIPT result leads to a significant decrease in maternal anxiety ($P<0.01$) and alters decision making regarding invasive testing; (III) we have identified women's motivations for NIPT beyond that of their first trimester screening result.

Conclusions: Following review of the first cohort of patients to undergo NIPT, we established a contingent model of aneuploidy screening in the first trimester. Under this model, the offer of NIPT is contingent on the patient's result from combined First trimester screening. NIPT is offered to patients with an aneuploidy risk higher than 1:1,000, though the potential benefit of invasive testing for those with a risk higher than 1:50 is also discussed. Our results demonstrate that NIPT is considered a valuable addition to prenatal care by women with either high risk or low risk combined first trimester screening results. This is important data that represents that attitudes and preferences of women regarding aneuploid screening.

Keywords: Non-invasive prenatal testing (NIPT); maternal anxiety; contingent screening

Cite this abstract as: Richmond Z, Fleischer R, Chopra M, Pinner J, D'Souza M, Fridgant Y, Hyett J. The introduction of a contingent model of first trimester screening using non-invasive prenatal testing. Ann Transl Med 2015;3(S2):AB148. doi: 10.3978/j.issn.2305-5839.2015.AB148

AB149. Long-range PCR sequencing as a novel approach in genetic—analysis of *MYH3*: a preliminary result

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Background and objective: Genetic diagnosis of large genes are usually complicated by large transcript size, complexity of the gene region and the high level of gene variations. Long-range PCR is a flexible, fast, efficient and cost-effective choice for sequencing large candidate genomic regions, especially when combined with next-generation sequencing (NGS) platforms. This article aims to develop and optimise novel mutation screening assay for *MYH3* gene which is 40 kb in size by directly sequencing four LR-PCR products using Illumina MiSeq sequencing platform.

Methods: Genomic DNA was isolated from 1 mL of peripheral blood was extracted using GeneAll DNA extraction kit (GeneAll, Korea), following the manufacturer's protocol. Four sets of primers that span the entire gene were synthesised. MaxTaq DNA Polymerase (Vivantis) was used to amplify each amplicons with cycling conditions; 94 ℃ for 2 min, 94 ℃ 12 s, annealing temperature for 30 s, 68 ℃ for 10 min, 68 ℃ for 7 min for a total of 35 cycles. The amplified amplicons were pooled together and library was prepared using Nextera XT DNA Sample Preparation Kit (Illumina Inc., CA, USA). Pooled library was generated by mixing all libraries equally for high-throughput sequencing on MiSeq™ Benchtop Sequencer (Illumina Inc., CA, USA). The sequence was aligned to the hg19 assembly of the genome with BWA enrichment analysis tool (Illumina Inc., CA, USA) and variants were called using Variant Studio (Illumina Inc., CA, USA). Only variants with at least 6-fold read coverage and a phred scale SNP ≥30 were included for further analysis.
Results: The four 10 kb amplicons were successfully amplified with annealing temperatures; 63.1, 62.4, 64.7 and 64.7 ℃ respectively, employing standard PCR cycling method within a short period of time. Targeted sequencing using Illumina MiSeq paired-end sequence resulted in 11,947,785 reads (98.05%) of which 99.8% could be aligned with the human genome. This provided 99.8% coverage of the sequence at a minimum sequencing depth of 6×. With this method, a total of 2,280 known variants and 848 unknown variants were detected, which will be subjected to further downstream analysis.
Conclusions: Current method enables amplification of the entire gene with four reactions, each generating product sizes of 10 kb circumventing the need for specific PCR amplification of individual exons. LR-PCR allows direct sequencing and screening methods for detecting genetic variations, achieving high sensitivity and improved intronic coverage with a faster turnaround time and lower costs, and providing a reliable tool for complex genetic analyses.

Keywords: Large gene; long-range PCR; next-generation targeted sequencing

Cite this abstract as: Maran S, Ee R, Faten SA, Taib WR, Ibrahim WP, Wong AR, Ankathil R, Zain MR, Chan KG, Tan HL. Long-range PCR sequencing as a novel approach in genetic—analysis of *MYH3*: a preliminary result. Ann Transl Med 2015;3(S2):AB149. doi: 10.3978/j.issn.2305-5839.2015.AB149

AB150. Mutation spectrum of the *fibrillin-1* (*FBN1*) gene in Taiwanese patients with Marfan syndrome

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Background and objective: Marfan syndrome (MFS) is an autosomal dominant genetic disorder that involves in multisystem connective tissues. The various phenotypic manifestations of MFS are skeleton, ocular and cardiovascular system. MFS is mainly caused by mutations in the *fibrillin-1* gene (*FBN1* gene) on chromosome 15 which contains 66 exons consist of 11,695 bp mRNA and coding 2,872 amino acids. To date, there are more than 1,000 mutations in *FBN1* have been registered in Human Gene Mutation Database (HGMD). Mutation scanning of the *FBN1* gene with DNA direct sequencing is time-consuming and expensive because of its large size. The aim of this study was to establish a national database of mutations in the *fibrillin-1* (*FBN1*) gene that cause MFS in the Taiwanese population. And we present an alternative method for high-throughput *FBN1* gene variant scanning by melting curve analysis with the Roche LightCycler 480 (LC480) system.
Methods: We screened 390 patients from 231 families for the presence of *FBN1* mutations using polymerase chain reaction/high-resolution melting analysis (PCR/HRM). All the temperature-shifted melting curves will be analysis by Sanger Sequencing.
Results: We identified 76 mutations in 96 of the 231 (41.5%) families including 69 single-base substitutions (51 missense mutations, 7 nonsense mutations, and 11 splicing sites), one small insertion, four small deletions, one small indel (insertion and deletion), and one exonic deletion (exon 36). When family history and Ghent criteria for MFS were taken into consideration, the mutation detection rate rose to 97% (62/64). That finding implies that family history and the Ghent criteria play a more important role than clinical manifestations in establishing a clinical diagnosis of MFS. Among the 76 mutations found in this study, 13 (17%) have not been registered in the HGMD or in the Universal Mutation Database (UMD).
Conclusions: This is the largest study of the mutation

spectrum of MFS in a cohort of patients in Taiwan. The database is expected to considerably improve genetic counselling for and medical care of MFS families. And our results support the use of this technology as an alternative method for the diagnosis of Marfan syndrome as well as its suitability for high-throughput mutation scanning of other large genes.

Keywords: Marfan syndrome (MFS); *FBN1* gene, high-resolution melting (HRM); Taiwan

Cite this abstract as: Hsieh SC, Lu YH, Chen YC, Pai JS, Hsieh YP, Niu DM. Mutation spectrum of the *fibrillin-1* (*FBN1*) gene in Taiwanese patients with Marfan syndrome. Ann Transl Med 2015;3(S2):AB150. doi: 10.3978/j.issn.2305-5839.2015.AB150

AB151. *CHD7* variants identified by next-generation sequencing

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Background: CHARGE syndrome is a genetic disorder with clinical features including ocular coloboma, heart defects, choanal atresia, retardation or developmental delay, genital hypoplasia, ear anomalies and deafness. Mutations in chromodomain helicase DNA binding protein 7 (*CHD7*) regulatory gene have been associated with this syndrome. *CHD7* gene mutations accounted for more than half of patients with CHARGE syndrome. Advancement of next-generation sequencing technologies like the introduction of bench-top next-generation sequencers has enabled cost-effective and accurate detection of mutations in large genes or disorders which are difficult to diagnose clinically. Molecular diagnosis based solely on Sanger sequencing is time consuming and less efficient. The aim of this study was to test the ability of a targeted gene panel and bench-top sequencing to molecularly diagnose patients with CHARGE syndrome.

Methods: Patients with some clinical characteristics of CHARGE syndrome were sequenced using the Haloplex ICCG targeted panel (containing 180 genes including *CHD7*) on the Ion Torrent PGM. Sequences were mapped to the human reference genome (hg19). Variants were called using the Torrent Variant Caller and annotated using the web-based GeneTalk software (GmbH, Berlin, Germany). Candidate variants that are potentially pathogenic with population frequencies less than 1% were filtered and prioritized for confirmation by Sanger sequencing.

Results: Sanger sequencing confirmed two truncating and three missense heterozygous CDH7 variants in five children. The R2631X and Q201X pathogenic variants have been previously reported in other patients with CHARGE syndrome. Of the missense variants, the G1504E and T1910 variants are novel but the T894A variant has a population frequency of 0.004%. Parental testing and correlation with clinical phenotypes will be helpful in assessing the pathogenicity of these missense *CHD7* variants.

Conclusions: Our results indicate that the use of targeted gene panels like the HaloPlex ICCG panel combined with a bench-top sequencer is useful as a cost-effective and rapid screening tool for molecular diagnosis of clinically heterogeneous disorders like CHARGE syndrome.

Keywords: CHARGE syndrome; *CHD7*; targeted gene sequencing; Haloplex ICCG targeted panel; Ion Torrent PGM

Cite this abstract as: Lee SP, Brett M, Lim E, Lai A, Tan ES, Lim JY, Cham B, Tan EC. *CHD7* variants identified by next-generation sequencing. Ann Transl Med 2015;3(S2):AB151. doi: 10.3978/j.issn.2305-5839.2015.AB151

AB152. Inborn errors of metabolism spectrum in symptomatic children of north India: 5-year prospective data from tertiary care centre

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Background: Children with high suspicion of IEM is a more effective screening strategy in a resource limited country like India. We present a prospective analysis of symptomatic children with red flag signs suggestive of IEM referred for analysis by LCMSMS. This study investigated the spectrum of IEM in symptomatic children over a period of 5 years (1st June 2010 to May 31st 2015).

Methods: A total of 3,250 symptomatic children for IEM were screened. Dried blood spots were collected and processed by MS/MS (API-2000 & 3200 Qtrap), using a non derivatized kit, analysed by R-4 Stork algorithm.

Results: A total of 3,250 children, 1,803 boys (56.34%), 1,397 girls (43.66%) with a median age of 20.8 months (range, 0.04-148.2 months) were screened. The 125 were diagnosed with an inborn error of metabolism, with a detection rate of 3.90%. Of these, 78 (62.40%) were males and 47 (38.60%) were females with a median age of 6.55 months. Clinical variation among the patients were unexplained encephalopathy, seizures, convulsions, delayed milestones with global developmental delay, persistent metabolic acidosis with increase anion GAP. The commonest group was amino acid disorders affecting 61 (48.8%) with phenylketonuria (n=5), hyperphenylalaninemia (n=4), maple syrup urine disease (n=8), hypermethioninemia (n=3), hyperglycemia (n=14), tyrosinemia (n=5), classic neonatal onset citrullinemia (n=4), 3 with hyperornithinemia, 10 with rasied alanine (as a secondary indicator), 3 with argininemia and 2 with remethylation defect. Organic acidemias 37 (29.60%) were methylmalonic academia (n=15), malonic aciduria (n=3), propionic aciduria (n=5), glutaric academia type I (n=5) and with 3-Methyl crotonyl-CoA carboxylase deficiency (n=9). Fatty acids disorders were seen in 27 (21.60%) children with medium-chain acyl-CoA dehydrogenase deficiency being the commonest (n=5), and very-long chain acyl-CoA dehydrogenase deficiency (n=2), carnitine palmitoyltransferase Ia deficiency (n=12), carnitine palmitoyltransferase II deficiency (n=8).

Conclusions: Mental retardation, delayed motor and language milestones are difficult to reverse as most of the patients belongs to higher age. Availability of restrained diets are rate limiting. Prospective symptomatic screening in large population with high rates of consanguinity & interbreeding will likely to decreased neonatal and infant mortality rate.

Keywords: IEM-inborn errors of metabolism; msms-mass spectroscopy

Cite this abstract as: Kumar S, Lomash A, Varughese B, Bidhan S, Khalil S, Polipalli SK, Kapoor S. Inborn errors of metabolism spectrum in symptomatic children of north India: 5-year prospective data from tertiary care centre. Ann Transl Med 2015;3(S2):AB152. doi: 10.3978/j.issn.2305-5839.2015.AB152

AB153. Incidence of IEM from perspective of new born screening at a tertiary health care center in India

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Background: Inborn errors of metabolism form a large class of genetic diseases involving congenital disorders of metabolism leading to significant morbidity and mortality in the population. In most of the disorders, signs and symptoms arise due to accumulation of metabolites which are toxic or interfere with normal function or the effects of reduced ability to make essential compounds. The aim of this study was to determine the incidence of IEM in the healthy newborns in the population at a tertiary health care center in India.

Methods: Dried blood spot samples of 32,333 newborns babies were taken after 24 h of birth, on a Whatmann 903 S

card, by heel prick method for a period of 6 months. Level of various metabolite was checked by LCMSMS (3200 MD QTrap), analysed by using R-4 Stork Algorithm.

Results: Five out of 32,333 samples were positive for IEM. Further confirmation with GCMS has found them to be cases of 2 maple syrup urine disease, 1 carnitine palmitoyl transferase-1 deficiency, and 1 case of cirullinemia and 1 case of tyrosinemia type 1.

Conclusions: We identified the incidence of IEM in the general population to be 1 in every 6,466 live births. Even this is higher than the normal incidence rate for the IEM, this can be explained by epigenetic factors, high degree of consanguinity. India is a large country with 110 million population, with 20.22 births occurring per 1,000 people in a year, so the incidence of IEM amounts to a very large population. Timely detection of IEM, along with clinical intervention might avert a large amount of morbidity and mortality. But the implementation of such a screening program with skilled workforce and clinicians will require a lot of planning & infrastructure and the cost effectiveness of such a program for a large population is yet to be determined.

Keywords: IEM-inborn errors of metabolism; LCMSMS-liquid chromatography mass spectroscopy mass spectroscopy; GCMS-gas chromatography mass spectroscopy

Cite this abstract as: Bidhan S, Kumar S, Lomash A, Varughese B, Thelma BK, Kapoor S. Incidence of IEM from perspective of new born screening at a tertiary health care center in India. Ann Transl Med 2015;3(S2):AB153. doi: 10.3978/j.issn.2305-5839.2015.AB153

AB154. Molecular characterization of Filipino patients with variant galactosemia

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Background: Classical galactosemia is a disorder of carbohydrate metabolism caused by low to absent activity of the *GALT* (galactose-1-phosphate uridyltransferase) enzyme. The clinical manifestations occur early and are severe if untreated. In contrast, patients with variant galactosemia (VG) are apparently healthy and do not manifest elevated galactose levels in spite of low *GALT* activity. The question of whether to restrict dietary galactose in variant VG remains unanswered. The Philippine newborn screening program does not impose any dietary restriction on patients with VG but monitors their total blood galactose for 5 years. The objective of the study was to determine the molecular basis of VG in 13 clinically diagnosed patients.

Methods: The coding sequence of the *GALT* gene of 13 Filipino patients clinically diagnosed to have VG was examined. *GALT* exons were PCR-amplified using genomic DNA as template and subsequently sequenced in both forward and reverse directions.

Results: None of the patients had the D/G galactosemia variant genotype. Five patients were heterozygous for classic galactosemia allele/potential G allele (G/- genotype), while three patients were heterozygous for the Duarte allele, p.N314D in cis configuration with c.-119_-116delGTCA, (D/- genotype). Five patients did not have detectable mutations in the coding region of the *GALT* gene. Two mutations, p.R80Q and p.Y89C, are novel, but cursory *in silico* analysis predicts that these are deleterious mutations. Three of the five patients without detectable mutations by sequence analysis were each assessed to have one of the following: learning disability, attention deficit disorder and global delay. Another two patients were assessed to have an error of refraction.

Conclusions: Mutations not evident by direct sequence analysis may be present in the *GALT* gene of the five patients without detectable mutations. Therefore, additional molecular testing aside from direct sequence analysis (e.g., whole gene deletion detection) is needed to fully analyze the *GALT* gene in VG patients.

Keywords: Variant galactosemia (VG); molecular diagnosis; *GALT* gene

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AB155. Clinical presentation and its relationship with chromosomal abnormalities in Turner syndrome

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Background: Turner syndrome is a relatively common chromosomal disorder. The disease affects only females, causing hypogonadism and short stature. Early treatment can improve short stature and hypogonadism. The study aims to describe chromosomal abnormalities, clinical characteristics and its relationship with chromosomal abnormalities in patients with Turner syndrome.

Methods: A total of 213 patients with Turner syndrome diagnosed in National Hospital of Pediatrics, Hanoi. A cross section study was used.

Results: Mean age on diagnosis was 12.2±4.9 years. Monosomy 45,XO occupied 54,31%; 45,X/46,XX was seen in 14.66%; 27.59% had structural disorders of chromosome X. Short stature was found in all patients aged more than 15 years. Severity of short stature and percentage of patients with short stature went up with age. There was no difference in term of height between karyotype groups. In group aged ≥12 years, 95.2% of cases had hypogonadism. Other symptoms frequently seen were nail hypoplasia (77.4%), cubitus valgus (74.7%), broad chest (69.2%)/abnormalities in face and neck were epicanthic fold (55.6%), low posterior line (51.3%), excessive skin in the back of the neck/webbed neck (42.5%). In a group aged <1 year, lymphoedema of hands/feet, epicanthic fold, broad chest, cubitus valgus were found in 100%. Majority of symptoms, congenital defects of heart/kidney were seen more frequently in 45,X group.

Conclusions: Lymphoedema of hands/feet in infants, low growth velocity, delayed puberty, abnormalities in face and neck, and other symptoms should be checked to early diagnose and treat Turner syndrome. Patients with 45,X had more severe presentation compared to patients with 45,X/46,XX and structural abnormalities of X chromosome.

Keywords: Turner syndrome

Cite this abstract as: Thao BP, Dung VC, Khanh NN, Ngoc CT, Hoan NT, Phuong NT. Clinical presentation and its relationship with chromosomal abnormalities in Turner syndrome. Ann Transl Med 2015;3(S2):AB155. doi: 10.3978/j.issn.2305-5839.2015.AB155

AB156. Clinical and laboratory characteristics of Prader-Willi syndrome

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Background: Prader-Willi syndrome (PWS) is a complex multisystem genetic disorder due the lack of expression of paternally inherited imprinted genes on chromosome 15q11-13. Clinical presentation includes hypotonia, hyperphagia, obesity, hypogonadism, learning difficulty. The article aims to study clinical and laboratory of patient diagnosed and treated in National Hospital of Pediatrics, Hanoi (NHP).

Methods: A total of 80 patients diagnosed of PWS by FISH in NHP from 2007 to 2015 were recruited in the descriptive study.

Results: Male/female ratio was 6:1. Patients diagnosed before 5 years occupied 53.5%. The 85.7% of patients were found to have hypotonia at age of 4.9±2.0 months. A total of 86.4% of patients had hyperphagia at age of 20.7±11.1 months. In patients aged of >2 years, weight SDS was +8.7±4.7 SD compared to gender and age. The figure of BMI was +10.3±6.3 SD. Four in seven of patients aged ≥6 years had micropenis. A total of 91.7% of patients had cryptorchidism. 4/24 of patients (14.3%) had type 2 diabetes mellitus.

Conclusions: Based on clinical presentation, more PWS patients could be diagnosed and treated early.

Keywords: Prader-Willi syndrome (PWS); clinical

presentation; laboratory characteristics

Cite this abstract as: Thao BP, Dung VC, Khanh NN, Ngoc CT, Ngoc ND, Nhung DT, Lan AT, Mai NT, Dat NP, Hoan NT. Clinical and laboratory characteristics of Prader-Willi syndrome. Ann Transl Med 2015;3(S2):AB156. doi: 10.3978/j.issn.2305-5839.2015.AB156

AB157. Evaluation of thalassemia screening program by using red blood count in pregnant women at Hung Vuong Hospital, Ho Chi Minh City, Vietnam

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Background: To evaluate thalassemia carrier screening program using red blood count indices (RBC) at Hung Vuong hospital, Ho Chi Minh City, Viet Nam, from June 2010 to March 2013.

Methods: All pregnant women visiting Hung Vuong hospital were screened thalassemia carrier by using RBC. Serum ferritin and hemoglobin electrophoresis were performed among microcytic hypochromic anemia women [mean cellular volume (MCV) <80 fL or mean cellular hemoglobin (MCH) <28 pg]. Their partners were also asked to be screened by these tests. The anemia couples were consulted to identify thalassemia mutation. The couples who were at high risk of having thalassemia major fetus were then advised to undergo invasive procedure such as amniocentesis or cordocentesis. The couples having confirmed thalassemia major fetus were offered pregnancy termination.

Results: Among 2,982 microcytic hypochromic anemia pregnant women, 21% of them (633/2,982) have their partners detected to have the same condition. Among those anemia couples, 51% (324/633) were both alpha thalassemia carriers, 10% (62/633) were both beta thalassemia carriers and 39% (247/633) were two different types of thalassemia carriers. Among alpha thalassemia mutations, --SEA mutation has the highest proportion (67%, 337/502). The mean of MCV and MCH of --SEA mutation carriers were 67.9±4.7 and 21.9±1.5 respectively. Among 1,249 beta thalassemia cases diagnosed through RBC and hemoglobin electrophoresis, 54% (678/1,249) had HbE peak with 75.7±5.1 MCV and 25.2±1.9 MCH and the other 46% (571/1,249) had 67.7±6.7 MCV and 22±2.3 MCH. Among the couples who were both microcytic hypochromic anemia, only 33% (209/633) of them agreed to undergo amniocentesis or cordocentesis to identify the affected fetuses. We found 40 hemoglobin Bart hydrops fetalis syndrome cases and 4β thalassemia major cases. All the thalassemia major fetuses were terminated, except 1β thalassemia major fetus carrying Cd 26 (HbE disease) and Cd 41/42 mutations.

Conclusions: Thalassemia is a common inherited condition in Viet Nam. The number of α thalassemia carriers is twice as many as beta thalassemia carriers. --SEA and CD 26 (HbE disease) mutations are the most common mutations of α thalassemia and beta thalassemia, respectively. In order to reduce the number of major thalassemia fetuses, thalassemia screening strategy by RBC in preconceptional couples or in first-visit pregnant women is effective and applicable in Viet Nam.

Keywords: Thalassemia; red blood count indices (RBC); mean cellular volume (MCV); mean cellular hemoglobin (MCH)

Cite this abstract as: Nguyen TV, Van Nguyen Le K, Pham VH, Nguyen TT, Le LK, Le KK, Nguyen TV. Evaluation of thalassemia screening program by using red blood count in pregnant women at Hung Vuong Hospital, Ho Chi Minh City, Vietnam. Ann Transl Med 2015;3(S2):AB157. doi: 10.3978/j.issn.2305-5839.2015.AB157

AB158. Report of a Gardner's syndrome case with an *APC* gene mutation

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Background: Gardner's syndrome is an autosomal dominant disorder with complete penetrance, caused by mutations in the adenomatous polyposis coli gene (*APC* gene). Gardner's syndrome is characterized by intestinal polyposis, osteomas and dental abnormalities. *APC* mutations are mostly point mutations, causing a truncated and dysfunctional *APC* protein. Detection of mutations in *APC* gene from a patient with Gardner's syndrome.

Methods: A 19-year-old female patient with typical symptoms of Gardner's syndrome was sent from the Hospital of Odonto-Stomatology, Ho Chi Minh City for detection of *APC* gene mutations. We performed *APC* gene sequencing and used bioinformatics tools to detect *APC* gene mutations and predict the effects of mutations.

Results: We detected the p.Gln1517ArgfsX6 mutation in *APC* gene and analyzed the effects of this mutation on functions of the *APC* protein.

Conclusions: We reported a p.Gln1517ArgfsX6 mutation in *APC* gene from a patient with Gardner's syndrome.

Keywords: Gardner's syndrome; adenomatous polyposis coli (*APC*); sequencing; p.Gln1517ArgfsX6

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AB159. Endocrine disrupting chemicals: toxicological risk assessment *in vivo* and *in vitro* models

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Abstract: In several studies, scientists asserted that many of endocrine disruptors (EDs), which have been involved in developmental, reproductive, neural, immunological, and other problems in wildlife and laboratory animals. Some environmental EDs, such as di-(2-ethylhexyl) phthalate (DEHP), flutamide (Flu), parabens, are used in many products in life and environment. However, the adverse effects caused by EDs can be temporary or permanent and the mechanism(s) through which these chemicals elicit their effects on biological systems of human and animal health is not clearly understood. The specific aim of this study is to evaluate endocrine disrupting chemicals-induced impact on the male or female reproductive system. An attempt is also made to elucidate the impact of these EDs in an *in vitro* model, i.e., GH3 rat pituitary cell line. A great deal of work has been carried out on the toxicity of phthalate, Flu, parabens *in vivo* and *in vitro* models. In brief, studies have been indicated that long-term and short-term exposure to various endocrine disrupting compounds (i.e., DEHP, Flu, parabens) during development stage (i.e., gestation, neonatal, immature, peripubertal) were done to find alternative dysfunctions later in animal life. The development and function of male or female reproductive tract showed many abnormalities, e.g., menstrual cycle irregularities; impaired fertility, endometriosis, and polycystic ovarian syndrome in female or morphological and functional gonadal dysfunction, e.g., infertility and decreased libido, congenital malformations (altered embryonic and fetal intrauterine development) and testicular dysgenesis syndrome in male. In addition, the differential gene expression patterns by microarray analysis following EDs exposure were found, particularly in steroid hormone synthesis, androgen and/or estrogen synthesis, and sex determination-related gene. On the other hand, studies revealed that parabens, a weak estrogenic chemical, exerted their actions on the stimulation of *CaBP-9k* gene, an estrogenic biomarker, via binding to estrogen

receptor and/or progesterone receptor in immature female rat and GH3 cell line. An increasing number of chemical compounds in the environment have been identified as endocrine disruptor *in vivo* and *in vitro* bioassay. A future challenge is required to confirm a theoretical toxicology and risk assessment of EDs for human and animal health.

Keywords: Endocrine disruptors (EDs); theoretical toxicology; risk assessment; reproductive system

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AB160. Fuminal hepatic failure in Wilson disease

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Background: Wilson disease (WD) is an autosomal recessively inherited disorder of copper metabolism. Mutation of the *ATP7B* gene on chromosome 13 leads to accumulation of copper in the liver, brain, kidney and cornea. Clinical presentation is particularly in liver and central nervous system. Fuminal hepatic failure in WD is rarely and mortality rate is very high.
Methods: Retrospective description. Describe clinical, preclinical characteristics of patients acute liver failure due to WD.
Results: A total of 6 patients with acute liver failure due to WD (Leipzig 2001) at Hepatology Department in NHP from January 2014 to June 2015. Common symptoms: jaundice, edema, ascites, hepatic encephalopathy, hyperbilirubinemia, hemolytic anemia, hypoalbuminemia, severe coagulation disorder, ceruloplasmin <0.2 g/L, urinary copper/24 h >100 mcg/dL. Five patients improved after treatment, one patient died due to fulminant liver failure.
Conclusions: Fulminant hepatic failure which cause by WD is very rare in children. The disease can be rescued if it is diagnosed and treated promptly. WD should be suspected in fuminal hepatic failure unknown the origin.

Keywords: Wilson disease (WD); fuminal hepatic failure

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AB161. High resolution melting analysis of buccal DNA revealed a significant association between *UGT1A1* c.211G>A and neonatal hyperbilirubinemia development in Malay population

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Background: Severe neonatal hyperbilirubinemia or neonatal jaundice (NNJ) characterised by an elevated total serum bilirubin (TSB) level may result in kernicterus or even death. Uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) is the key enzyme which conjugates bilirubin with glucuronic acid for the subsequent bilirubin excretion. Conversely, constitutive androstane receptor (CAR), encoded by nuclear receptor subfamily 1, group I, member 3 (*NR1I3*) gene, regulates bilirubin excretion by activating the components of the bilirubin clearance pathway. Thus, genetic variants in *UGT1A1* and *NR1I3* genes may modulate bilirubin excretion and lead to NNJ. This study aimed to determine the association between *UGT1A1* and

NR1I3 genetic variants and NNJ development in Malay population by genotyping the DNA isolated from buccal swabs. The accuracy and reliability of the genotyping results produced by buccal DNA was also compared with that of the whole blood DNA.

Methods: Buccal swabs were collected from 232 hyperbilirubinemia and 232 non-hyperbilirubinemia newborns admitted to and/or born in Hospital Universiti Sains Malaysia (HUSM). Hyperbilirubinemia subjects were those with TSB levels ≥250 μmol/L within the first week after birth while non-hyperbilirubinemia subjects were newborns without significant hyperbilirubinemia. The *UGT1A1* (c.211G>A) and *NR1I3* [MPJ6_1I3008 (G>A), IVS8+116T>G and 540A>G] variants were genotyped by using high resolution melting (HRM) analysis. Binary logistic regression was used to assess the association between variant genotypes and risk of NNJ. Whole blood samples were also collected from 60 subjects and genotyped to compare the HRM genotyping results with that of the buccal swabs.

Results: When compared with wild-type genotype, both heterozygous and homozygous variant genotypes of MPJ6_1I3008 (G>A), IVS8+116T>G and 540A>G were not significantly associated with NNJ. However, the heterozygous genotype (GA) of c.211G>A was found to increase the risk of NNJ (OR: 1.96, 95% CI, 1.13-3.39, P=0.014). Besides, all buccal DNA samples demonstrated 100% genotype call rates and achieved complete genotype concordance with blood DNA samples.

Conclusions: The heterozygous genotype of c.211G>A could be a genetic risk factor of NNJ in Malay population. Since buccal DNA produced complete genotype call and concordance rates, the non-invasive buccal swabs collection can be used as an alternative to blood sampling especially in genetic studies involving paediatric population.

Keywords: Neonatal hyperbilirubinemia; *UGT1A1*; *NR1I3*; buccal cells; Malay

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AB162. Genes variation in three families of Vietnamese dioxin victim

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Abstract: Dioxins are a class of chemical contaminants that are formed during combustion processes such as herbicide manufacturing, waste incineration, forest fires, and backyard trash burning. The most toxic chemical in the class is 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD). Approximately 18 million gallons of Agent Orange were sprayed by US Airforce on southern of Vietnam from 1962 to 1971. About 0.3% of Agent Orange consisted of TCDD. Dioxins have been considered highly toxic and able to cause cancer, reproductive and developmental problems, damage the immune system, and interfere with hormones. In this paper we studied gene variation in some families of dioxin victims of Vietnamese army veterans who have been exposed directly under sprays or carried out missions for at least 2 years in the heavily sprayed regions. Of the first family, we found 21 nucleotide variants in *TP53* gene, 13 nucleotide variants in *AhR* gene. All of theme leading to amino acid change. We also found R554K in ThB4.VT16 and ThB4.VT17. This mutation changes activity for CYP1A1 induction in lymphocytes. In the second family, we identified 29 nucleotide variants in *TP53* gene. Although we could not found any variant associated with phenotype of the family members but previous studies have found P295L associated with gastric carcinoma, L299P associated with pancreatic cancer, G279E associated with colorectal carcinoma and cancer of male sex cells. In the third family, we found 22 nucleotide variants in *TP53* gene and 9 variants in *CYP1B1* gene. For understanding of whole genome sequence variation, whole genome of 3 member of each family has been sequenced by Illumina HiSeq 2000/2500 platform. The whole genome sequence data have started analysing.

Keywords: AhR; CYP1A1; dioxin; TP53; whole genome

sequence

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AB163. Microsatellite markers for preimplantation genetic diagnosis in Vietnamese *DMD* and hemophilia: a female carriers

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Abstract: Microsatellite polymorphic markers were powerful tool to perform single cell diagnosis for preimplantation genetic diagnosis (PGD) in X-linked recessive disorders. This type of analysis requires haplotypes information of carrier mothers and affected sons. We present 12 Vietnamese families with duchenne muscular dystrophin (*DMD*) or Hemophilia A affected sons, six with each disorder. We established haplotypes based on linked microsatellite polymorphic markers in these families and performed diagnosis enabling embryo transfer from the PGD cycle. We also perform haplotypes analysis in five more families for each disease to identify more informative markers among other, in order to construct better strategy for future diagnosis. Microsatellite polymorphic markers flanking the *F8* and *DMD* gene were used to identify haplotypes. Polar bodies (PB) were biopsied and analyzed to determine allelic association between the mutation and markers in multiplex PCR reaction. The results showed that 13 out of 28 embryos were found to be not affected by *F8* or *DMD* gene inherited mutations and were available for transfer. Marker DXS9907, DSTR44, DSTR49 for *DMD* gene and marker FXS1073, DXS9897, DXS1073 for *F8* gene were identified as the most frequent markers shown heterozygous alleles in mother carriers. PB analysis by microsatellite markers were proved to be useful technique for PGD of *DMD* and Hemophilia A families. Better strategy for PB diagnosis was built.

Keywords: Preimplantation genetic diagnosis (PGD); *F8* gene; *DMD* gene; polar bodies; microsatellite markers

Cite this abstract as: Tuan-Pham LA, Tran HH, Tran DQ, Minh NT, Huong NL, Tien NV, Ta VT, Bui TH, Tran VK. Microsatellite markers for preimplantation genetic diagnosis in Vietnamese *DMD* and hemophilia: a female carriers. Ann Transl Med 2015;3(S2):AB163. doi: 10.3978/j.issn.2305-5839.2015.AB163

AB164. Methylmalonic acidemia/propionic acidemia in Taiwan

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Background: Methylmalonic acidemia (MMA) comprises a heterogeneous group of disorders, which are characterized by accumulation of methylmalonate in the body due to deficiency of methylmalonyl/proprionic coenzyme A mutase or defects in the uptake, transport or synthesis of 5'-deoxyadenosylcobalamin. Propionic acidemia (PA) refers to propionyl-CoA carboxylase deficiency. Most these patients might have

very-early onset signs and symptoms that occur even before the results of newborn screening (NBS) are available, and die immediately or survive with significant neurodevelopmental disability. The associative outcome of MMA/PA was scarcely reported in Asia.

Methods: From January 2000 to December 2014, elevated C3 and elevated C3/C2 ratio cases were collected from Taipei Institute of Pathology (TIP), Chinese Foundation of Health (CFH) and National Taiwan University Hospital (NTUH). Demographic data, initial presentation, whether or not undergoing liver transplantation (LT) and postoperative prognosis including survival rate, DQ/IQ performance, admission length, tube feeding time and were analyzed.

Results: During this period, 2,735,122 newborns were screened. Overall incidence rate of MMA was 1/109,405; PA, 1/683,781. Referral time was 323 days before the era of NBS *vs.* 8.8/7.5 days after the introduction for MMA/PA (P<0.05). MMA mutase type generally possesses higher AST, ALT, NH3 value and lower pH value compared to cobalamin type (P<0.05). Seventeen out of 25 MMA mutase type patients received LT; two out of 4 PA patients did LT. Mean admission length shortened from 90.6 days/year (pre-LT) to 9.1 days/year (post-LT) for MMA patients (P<0.0005). Tube feeding ratio decreased from 74.56% to 0.56% (P<0.00005). The anxiety level from the caregiver also down-escalated from 33.4 to 27.2 (P=0.001). DQ/IQ performance ameliorated after LT as well from 50 to 60.1, even though not statistically significant.

Conclusions: There is still room for improved regarding to management of MMA/PA. LT patients do survive with less admission length, less tube feeding, less anxiety from caregiver; nevertheless, their DQ/IQ performance still necessitates further monitor.

Keywords: Methylmalonic acidemia (MMA); propionic acidemia (PA); liver transplantation (LT); newborns

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AB165. Extended follow-up of Taiwanese Chinese patients treated early for 6-pyruvoyl-tetrahydropterin synthase deficiency

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Background: The 6-pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is the most important type of BH4 deficiency related to hyperphenylalaninemia. It is also accompanied by various neurological signs and symptoms due to impaired synthesis of catecholamines and serotonin. In this report, we aimed to report the long-term results of early initiation of treatment PTPS.

Methods: Between 1988 and 2014, 23 newborns with PTPS deficiency who underwent early treatment at our hospital were identified. All patients received tetrahydrobiopterin replacement in a daily dosage between approximately 2 and 4 mg/kg. The dosages of levodopa replacement were 10 to 15 mg/kg/d, which is considerably higher than the typically recommended dosages of less than 7 mg/kg/d for patients aged younger than 2 years and 8 to 10 mg/kg/d for patients aged 2 years or older. Replacement with 5-hydroxytryptophan varied widely among patients. We examined the IQ score of the patients of age greater than 3 years.

Results: The overall incidence rate of PTPS according to the newborn screen result showed 1/115,000. The mean (SD) IQ score of our PTPS-deficient patients was 96.2 (range, 90-119), which is considerably higher than previous reports of other populations of PTPS-deficient patients (IQ: 76; range, 56-98). All patients reached a normal IQ on high daily dosages of levodopa replacement, without developing apparent long-term levodopa-induced adverse effects. We also observed a correlation between long-term IQ score and genotype, birth weight, and age at initiation of treatment.

Conclusions: An effective newborn screening referral program and early initiation of appropriate therapy preserved the IQ scores of PTPS-deficient patients.

Keywords: Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency; IQ score; newborn screening

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AB166. Replicative genetic association analysis reveals genetic markers of schizophrenia and Alzheimer's disease in Russians and Kazakhs and demonstrates overlapping associations pattern between two diseases

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Abstract: Replicative analysis of genetic markers, previously identified in genome-wide association studies (GWAS) for neuropsychiatric diseases, in population of various origins may contribute to better understanding of genetic composition of the diseases. In this study 50 SNPs found in GWAS for schizophrenia (SZ), Alzheimer's diseases (AD) and cognitive endophenotypes were genotyped in patients and control samples from Russian and Kazakh populations. Eight polymorphic markers (SNPs in regions of genes for *APOE*, *APOJ*, *CSMD1*, *CCDC60*, *SNX29*, *PICALM*, *NOTCH4* and *NRIP1*) were associated with AD in Russian population. Associations of 10 genetic markers with SZ in Russian populations (markers in regions of genes for *NRGN*, *KCNB2*, *NRIP1*, *CCDC60*, *LSM1*, *LOC100129100/LOC100509857*, *CSMD1*, *CPVL*, *POM121L2* and *NDE1P1/PRMT6*) and 8 genetic markers in Kazakh population (*VRK2*, *KCNB2*, *CPVL*, *BRD1*, *PVRL2*, *ARHGAP1*, *CD33 u GPR89P/TRV-AAC1-5*) were replicated. Multidimensional reduction methods revealed epistatic interaction of several genetic loci in formation of susceptibility to SZ in populations of various ethnic origins. Overlapping fields of genetic associations for AD and SZ, demonstrating some similarity in inherited background for both diseases, were found. This interaction may be implemented through common unknown levels in pathogenesis of AD and SZ, mediated by cognitive endophenotypes. Bioinformatic analysis of biological functions of associated genes revealed that among primary biological processes enriched by genes under study are the processes of lipid metabolism, cellular interactions, various regulatory and transport processes in neural cells, processes of immune response regulation. Substantial part of investigated genes forms an interacting network, which consisted of distinct clusters corresponding to major biological processes, were genes under study are involved, and to basic molecular functions of their products. In general, data obtained in the project, extend the understanding of genetic component in neuro-psychiatric diseases, as well as the biological processes and functions, implemented in the manifestation of genetic susceptibility to AD and SZ.

Keywords: Genetic associations; schizophrenia; Alzheimer's disease (AD); Russian population; Kazakh population

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AB167. Congenital adrenal hyperplasia (CAH) caused by mutations in the *CYP21A2* and *CYP11B1* gene of Vietnamese children patients

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Abstract: 21-hydroxylase (*CYP21A2*) and 11β-hydroxylase (*CYP11B1*) are two important enzymes catalyzing conversion of adrenal and steroid hormone biosynthesis. While *CYP11B1* only participates in cortisol synthesis pathway, *CYP21A2* catalyzes conversion of both cortisol and aldosterone. Mutations in these two genes lead to congenital adrenal hyperplasia (CAH) which is a genetic disease resulting from autosomal recessive traits. The typical manifestations of this disease are virilization, salting loss, dehydration, hypertension and even gonad deformation in severe female inborn patients. Mutations in the *CYP21A2* gene which occupy about 90% cases are the main cause contributing in CAH meanwhile *CYP11B1* gene mutants accounting for just 5-8% cases are the second main cause of this disease. In our study, entire *CYP21A2* and *CYP11B1* gene were amplified by PCR and directly sequenced to detect mutations. In further research, the effect of mutations was predicted and evaluated by protein 3D modelling analysis and enzyme assay in COS-1 cell line. As the results, three novel mutations (IVS6+5G>T, R51K and Y395X) in the *CYP11B1* gene were detected in Vietnamese children diagnosed suffering from CAH. In terms of *CYP21A2* gene, three mutations including 30 kb deletion, I2 splicing and E246 frameshift were found and also described previously. In conclusion, the results of our study have considerable significance in early diagnosis through understanding the relationship between genotype and phenotype of patients. Furthermore, mutagenesis detection and analysis could assist doctors bring out genetic consultants for patients as well as their parents.

Keywords: Congenital adrenal hyperplasia (CAH); *CYP11B1*; *CYP21A2*; genetic disease; mutations

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AB168. Novel *DYM* compound heterozygous mutations in a Malaysian boy with Dyggve-Melchior-Clausen syndrome

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Background: Dyggve-Melchior-Clausen (DMC) syndrome and Smith-McCort Dysplasia (SMC) are rare, progressive, autosomal recessive skeletal dysplasias caused by mutations in the *Dymeclin* (*DYM*) gene, mapped to chromosome 18q21.1. These are allelic disorders and share many features including short stature, a barrel-shaped chest, platyspondyly, abnormalities of the epiphyses and metaphyses, and a distinctive lacy appearance of the iliac crest. The distinguishing feature is that individuals with DMC have intellectual disabilities whereas SMC is associated with normal intelligence.

Case presentation: We present a 6-year-old Malaysian boy, the elder of two children born to a non-consanguineous Chinese couple. He was a term baby but was small and short for gestational age at birth. He initially presented to the paediatric endocrinologist for concerns of short stature and was subsequently referred prior to the age of three for suspicion of mucopolysaccharidosis (MPS) from his vertebral radiological findings. Clinical evaluation revealed that he had short stature, microcephaly and prominent pectus carinatum. He had normal early

developmental milestones but on follow-up, it became obvious he had learning difficulties with expressive speech delay. His skeletal radiographs showed platyspondyly with a double hump and anterior beaking, broad ribs, widened metacarpals, abnormally shaped femoral heads and lacy crests of the iliac wings. Molecular testing of the *DYM* gene identified novel compound heterozygous mutations—a deletion c.242_249del8 in exon 4 was inherited from his father and a single nucleotide duplication c.1917dupT in exon 17 was inherited from his mother. Both these mutations cause a frameshift and result in aberrant mRNA processing. The parents are therefore heterozygous carriers. Our patient was initially thought to have Smith-McCort dysplasia SMC but his diagnosis had since been revised to DMC when it became evident he had speech delay and was faltering with his learning from the age of four. This diagnosis would also seem to be more in keeping with his genotypic change. A formal psychometric assessment was planned to evaluate his intelligence more objectively.

Conclusions: We wish to highlight the phenotypic and radiological features of this rare entity and the role of molecular testing. It is important to remember that certain other disorders especially Morquio A (MPS type IVA) may mimic this condition on vertebral radiological changes. Diagnosis confirmation with finding the *DYM* mutations not only allows for accurate genetic counselling and prenatal testing, but also directs one to be vigilant for potential life-threatening complications in this disorder like atlanto-axial instability.

Keywords: *DYM*eclin (*DYM*); skeletal dysplasia; 18q21.1; short stature

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AB169. Engaging the genetic counsellor in the implementation of precision oncology in Singapore

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Abstract: Cancer is a genetic condition driven by a series of both inherited (germline) and tumour specific (somatic) mutations. The analysis of tumour to identify somatic alterations and generate a genetic profile has given rise to a personalised approach for treating oncology patients, guiding optimal therapy. Next generation sequencing (NGS) technology is now routinely utilised in oncology to screen for both genes known to be associated with improving treatment response, as well as, genes of uncertain significance to contribute to new findings in cancer treatment. In the development of genome wide mutation analysis of tumour tissue, germline DNA has also been routinely collected for comparative analysis in order to identity tumour specific mutations. As the identification of germline mutations is secondary to the main purpose of tumour testing for targeted treatment, the management of germline findings has been extensively debated, in particular, around if and when to communicate these findings to patients. The role of the genetic counsellor in this process has been pertinent to these discussions. Clinical practice, however, is shifting towards a preference of sequencing tumour tissue alone to characterise its molecular profile for reasons such as reducing cost and facilitating logistics of sample collection. This method raises the question of how the testing of tumour alone contributes to the identification of germline mutations. The laboratory at POLARIS, Singapore, has generated genomic data of 130 colon tumours using a panel of 84 genes, including 29 germline susceptibility genes associated with

known inherited syndromes. The analysis of this data has given an insight into the proportion of mutations that are potentially germline, which in turn will impact how patients are consented for tumour profiling and the delivery of the results. In generating these findings, however, the analysis process has demonstrated a number of the complexities in interpreting the pathogenicity of the variants, as well as, determining the somatic or germline origin of the variant. The challenges in interpreting germline mutations from tumour next generation data, the importance of conveying a germline finding to the patient and the integration of the genetic counsellor in this process will be discussed.

Keywords: Genetic counsellor; precision oncology; next generation sequencing (NGS); tumour profiling

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AB170. Loss of heterozygosity in child with multiple congenital anomaly

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Background: Multiple congenital anomaly (MCA) has become an increasing problem in worldwide, since it significantly contributes to infant mortality rate (IMR) and caused many morbidities during neonatal until childhood period. Prevention has to be done to decrease the incidence through prenatal diagnosis, hence a proper postnatal definitive diagnosis should be established as a reference. Microaray system is one of the leading technique in order to detect copy number variations (CNV's) and loss of heterozygosities (LOH's) which may responsible to phenotype. This report is aimed to demonstrate a case of MCA with normal G-banding result, no pathologic CNV's, with wide area of LOH's contain several genes which may responsible to the phenotype.

Methods: Using Online Mendelian Inheritance in Man (OMIM) and Pictures of Standard Syndromes and Undiagnosed Malformation (POSSUM) databases, certain syndrome were tried to identify. Chromosome analysis were performed to detect large aberration. Microarray examination was done using Infinium CytoSNP-850K DNA analysis bead chip kit from Illumina. Chip was scanned using Hi-scan scanner from Illumina. Data were extracted using genome studio software. Data were analyzed using Nexus software.

Results: A 8-year-old girl was brought by parents to hospital with a chief complains dyspnea and looks cyanosis since 1 month prior to admission. She was born spontaneously, full term, no cyanosis, with distinctive face. Birth weight was 2,800 g. Her growth was retarded, but her development was normal. There is no history of seizure. Patient was a student in 3rd grade of elementary school, with an average level of intelligence. Physical examination reveals tachypnea and cyanosis. There was pansystolic murmur without gallop. Clubbing fingers were noticed. There are several dysmorphic feature such as frontal bossing, wide frontal, depressed nasal bridge, hypertelorism, down slanting palpebrae, asymetric face (hemi hypoplasia), midfacial hypoplasia, strabismus. No chromosomal aberration was found. Microarray examination showed benign duplication regions at chromosome 21, 22 and 7. Benign deletion regions were found at chromosome 21 and 6. There are two large regions of heterozygosity (ROH) more than 5 Mb in size, which are located at chromosome 1 and 3. Those regions consist of 29 genes, which might responsible to the phenotype.

Conclusions: Unrecognized MCA with normal chromosome results needs further evaluation using microarray system. Although CNV's calling give normal result, SNP calling might shows important finding. In this case microarray examinations showed 2 large regions of heterozygosity (ROH) consist of 29 genes which might responsible to the phenotype.

Keywords: Multiple congenital anomaly (MCA); definitive diagnosis; microarray; loss of heterozygosity

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AB171. RNA alternative splicing modulator can effectively increase lymphoblast enzyme activity in patients with cardiac fabry disease caused by IVS4+919G >A mutation

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Background: In Taiwan, DNA-based newborn screening showed a surprisingly high incidence (1/875 in males and 1/399 in females) of a cardiac fabry mutation (IVS4+919G >A). The common cardiac variant fabry mutation, IVS4+919G >A, affects the splicing of *GLA* RNA by introducing a 57-nucleotide insertion between exons 4 and 5 that contains a stop codon and leads to a truncated protein and inactive enzyme. And this mutation affected males have up to 10% residual enzyme activity and present clinically with late-onset hypertrophic cardiomyopathy. Due to the high cost of enzyme replacement therapy and the large number of patients with this mutation, the development of alternative therapies is essential. Several low-molecular-mass compounds, such as histone deacetylase inhibitors or kinase/phosphatase inhibitors, have been identified as modulators of alternative splicing. It may offer a potential alternative to enzyme replacement therapy. We expect to find out a more economic and effective drug by the detailed study of the mechanism of the small molecule modulators on the IVS4+919G >A mutation for the greater benefits of patients with this mutation.

Methods: In this study, we used to generate Epstein-Barr virus-transformed lymphoblast cell lines and incubated with different concentrations of three HDIs (sodium butyrate, valproic acid, and trichostatin A) and Amiloride hydrochloride (Amiloride HCl). To identify the respond of these compound, we were monitored the relative amounts of normal and aberrant splice forms by quantitative real-time polymerase chain reaction, the relative amounts of the normal and truncated α-Gal A protein products were analyzed by Western blotting and enzyme activities.

Results: Western blotting revealed those females heterozygous for the IVS4+919G >A mutation had approximately 50% of the normal level of α-Gal A protein, whereas hemizygous males had approximately 10% of the normal level. The three HDIs were all found to rescue the aberrant RNA splicing and to increase the amount of normal α-Gal A protein. And amiloride HCl increased the splicing ratio (2.5 fold) and enzyme activity (2.2 fold) of α-Gal A in the lymphoblasts with IVS4+919G >A mutation.

Conclusions: Our results provide proof-of-concept that aberrant RNA splicing caused by the cardiac variant fabry mutation, IVS4+919G >A, can be rescued by HDIs. The use of HDIs may become a viable therapeutic strategy for patients with this highly prevalent mutation in the Han population.

Keywords: Uniparental disomy; recessive congenital methemoglobinemia; argininosuccinic aciduria; microsatellite genotyping

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AB172. High-throughput and cost-effective newborn screening method for female with fabry disease by DNA mass spectrometry in Taiwan

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Background: Our team has implemented a newborn screening program for fabry disease in Taiwan since 2008. However, current enzyme-based newborn screening method is not reliable to detect heterozygous female patients. Estimatedly, more than 80% of female patients are missed by this enzyme-based screening method. The study

aims to develop a simple, fast, reliable, and cost-effective method to screen fabry disease in females.

Methods: Because the *GLA* mutations are limited to only 21 different pathogenic mutations according to the reports from 668,087 newborns via our newborn screening program, a customized mass screening method for fabry mutations by Agena iPLEX assay is established in Taiwan. Simultaneously, 21 mutations can be analyzed in one reaction for one person.

Results: There were 20,154 female infants participated in this program. A total of 54 IVS4+919G>A and one c.656T>C female infants were identified. The incidence of female infants with fabry mutations is as high as 1/366. Owing to the particularity of 21 mutations detected in a single multiplex reaction, the cost of this method can be less than US $10 per infant in the Fabry mutation screening.

Conclusions: In this study, we demonstrated that the Agena iPLEX assay is a powerful tool with high specificity and sensitivity for germline mutation screening. The detectivity of fabry disease in females can be elevated by this DNA mass-based genotyping method. Considering the low cost, rapidity and flexibility of the Agena iPLEX assay, it has a great potential to be incorporated into the newborn screening in Taiwan.

Keywords: Fabry disease; Agena's MassARRAY®; Agena iPLEX assay; *GLA* genotyping

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AB173. Heterozygous carriers of classical homocystinuria tend to have higher fasting serum homocysteine concentrations than non-carriers in a folate deficiency area where has inordinately high homocystinuria prevalence

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Background: The newborn screening of homocystinuria in Taiwan has started since 1984. Out of 5 million newborns screened, only 3 newborns (Han Taiwanese) suffering from homocystinuria were detected in this newborn screening program. The prevalence is less than 1 in 1 million. However we recently found 8 patients presenting with homocystinuria in an Austronesian Taiwanese Tao tribe. All the Tao patients are homozygous for a novel mutation (p.D47E, c.141T > A). Among the 428 adult islanders screened for the D47E mutation, approximately 1 in 7.78 is a carrier of the mutation, and an estimated 1 in 240 islanders suffered from homocystinuria. This is the highest known prevalence of homocystinuria worldwide. The expression study revealed that this p.D47E mutation interferes not only with the function but also with the stability of the CBS protein *in vivo*. We evaluated if the CBS carriers tend to have higher fasting serum tHcy concentrations than non-carriers in presence of folate deficiency.

Methods: The serum tHcy and folate levels before and after folate replacement was measured in 48 adult Tao carriers, 40 age-matched Tao non-carriers and 40 age-matched Han Taiwanese controls.

Results: We found that serum tHcy level of the Tao CBS carriers (17.9±3.8 μmol/L) was significantly higher than in Tao non-carriers (15.7±3.5 μmol/L; P<0.008) in the presence of folate deficiency. Of note, the difference in tHcy levels between the carriers and non-carriers was eliminated by folate supplementation (carriers: 13.65±2.13 μmol/L; non-carriers: 12.39±3.25 μmol/L, P=0.321). This finding implies that CBS carriers tend to have the risk of cardiovascular disease in presence of folate deficiency.

Conclusions: CBS carriers tend to have a higher tHcy

level in the presence of folate deficiency than non-carriers. Although many reports have indicated that CBS carriers are not associated with cardiovascular disease, the risk for CBS carriers with folate deficiency has not been well studied. Owing to a significantly elevated level of fasting tHcy without methionine loading, this finding implies that CBS carriers tend to have the risk of cardiovascular disease in presence of folate deficiency.

Keywords: Folate; homocysteine; homocystinuria; hyperhomocysteinemia; Vitamin B_{12}

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AB174. Genetic counseling in the couple with compound heterozygous carrier based on the result of mutation effect analysis on cystic fibrosis transmembrane conductance regulator (CFTR) protein

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Background: Cystic fibrosis (CF) is an autosomal recessive disorder due to mutation(s) in the *Cystic Fibrosis Transmembrane conductance Regulator* (*CFTR*) gene. In Caucasian population, CF is routinely screened in the newborns and also couple with CF family history due the high incidence and prevalence. The V322A missense mutation was detected from a man whose expecting spouse is carrier for F508del. F508del is known as the most common severe CF-causing mutation, while the effect of V322A mutation is still unknown. If V322A is deleterious, the F508del/V322A baby will suffer from CF, thus prenatal diagnosis is necessary. To evaluate the impact of V322A mutation to CFTR protein *in silico* and *in vitro* and to provide proper genetic counselling to the couple with compound heterozygous carrier.

Methods: *In silico* and *in vitro* studies of the impact of V322A mutation on CFTR protein were conducted. The mutation effect was predicted by two *in silico* studies: PolyPhen-2 and SWISS-MODEL Workspace. Following the *in silico* studies, biological studies were performed to analyze the impact of V322A mutation on protein maturation and localization. The risk of having affected offspring if she/he carries both mutations (*F508del-V322A*) in the gene was estimated by using Bayesian calculation.

Results: PolyPhen-2 revealed that V322A mutation is predicted to be possibly damaging, while SWISS-MODEL Workspace showed that the mutation does not have deleterious effect to the structure of protein. Western blot results showed that V322A protein has the same maturation degree with the wild type. Protein localization by immunofluorescence revealed that V322A does not alter the CFTR trafficking process. Bayesian calculation predicted that the couple has low risk to having a CF baby.

Conclusions: V322A substitution is likely a normal CFTR variant though *in silico* tools showed different predictions. Since the risk of having CF baby is low, therefore prenatal diagnosis is conducted only if the parents consent to it.

Keywords: Cystic fibrosis transmembrane conductance regulator (CFTR); cystic fibrosis; genetic counselling

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