SNPping away at the genetic basis of adolescent idiopathic scoliosis

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Abstract: Adolescent idiopathic scoliosis (AIS) is a genetically complex disorder of spine development, defined by a lateral curvature of the spine of 10° or greater which affects children during their pubertal growth spurt. Prior linkage and candidate gene approaches to elucidating the genetic basis of AIS have been of limited use for identification of candidate genes for this condition. Genome wide association studies (GWAS) have recently identified single nucleotide polymorphisms (SNPs) in *LBX1* and G protein-coupled receptor 126 (*GPR126*) that contribute to AIS occurrence. These discoveries support prior etiologic hypotheses regarding altered somatosensory function and skeletal growth in AIS. However, these loci account for a small percentage of the phenotypic variance associated with AIS, indicating the vast majority of the genetic causes of AIS remain to be delineated. A major translational application regarding understanding the genetic contributions to AIS relates to bracing efficacy.

Keywords: Adolescent idiopathic scoliosis (AIS); bracing; genome wide association study; single nucleotide polymorphism (SNP); skeletal growth; somatosensory function

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Adolescent idiopathic scoliosis (AIS) is defined as a lateral curvature of the spine of 10° or greater which affects children during their pubertal growth spurt for which no cause can be determined (www.srs.org). Although there is evidence that genetic and environmental factors are likely to play a role in its occurrence the mechanisms responsible for AIS remain uncertain at the present time. Rotational deformity is measured by an inclinometer in the forward bending position, and the scoliometer as an angle of trunk rotation (ATR). Confirmation of scoliosis is obtained by obtaining a lateral spine film and measuring the degree of curvature or Cobb angle.

The incidence of idiopathic scoliosis (IS), encompassing all age groups in the general population ranges from 2-3%, varying with the definition of the magnitude of the curve. Older IS subclassification is based on the age of presentation categorized as: (I) infantile (birth to age 3 years); (II) juvenile (age 3 to 11 years); and (III) adolescent (11 years and older). The incidence of AIS has ranged from 0.47% to 5.2% (1).

Population studies indicate that 11.1% of 1st degree relatives are affected with significant spinal curvature, in contrast to 2.4% of 2nd degree, and 1.1% of 3rd degree relatives (2). By the age of 16 years, 0.6% of affected people will have required intervention through active treatment with a full-time thoraco-lumbar-sacral orthosis (TLSO) or surgical correction with instrumentation (3,4).

These late consequences associated with IS are not surprising in light of the pathological consequences associated with the disorder. Significant health problems reported in association with IS, include chronic back and neck pain, disc herniations flatback syndrome, osteoporosis, kyphosis, cosmetic dissatisfaction, disability and psychologic distress (5). Patients with curves >70° (severe scoliosis) are 3 times more likely to die from cardiopulmonary disease than unaffected individuals (6).

Hypotheses put forth to explain pathogenesis of IS include abnormalities in the composition of the connective tissue matrix, alteration in body axis, calmodulin, melatonin, neuromuscular imbalance and altered vestibular function.

The mode of inheritance of IS has not been solidly established and is under debate (2,5,7-11). Inheritance patterns reported include autosomal dominant with variable penetrance, autosomal recessive, multifactorial and X-linked dominant modes. Previous studies reviewed in (12) demonstrated genetic heterogeneity for IS. Family based linkage studies have been used to identify genomic regions associated with IS (13,14). While these studies are of some use, they have a disadvantage because of their low power to detect genes associated with more complex disorders and identify relative large chromosome segments with a large number of candidate genes for a particular condition (15).

Association studies such as the finding of over transmission of the *CHD*7 associated polymorphism, rs4738824 in patients with IS have helped to elucidate genetic contributions to IS. Substitution of the A allele of this polymorphism with the G allele is predicted to disrupt a possible binding site for caudal-type (cdx) homeodomain-containing transcription factors. Mutations in *CHD*7, a chromeodomain helicase DNA binding protein are associated with CHARGE syndrome (coloboma of the eye, heart defects, atresia of the choanae, retardation of growth and/or development, genital and/or urinary abnormalities, and ear abnormalities and deafness) (16). A hypothesis for the development of IS is *CHD*7 may act postnatally to alter spinal growth during the adolescent growth spurt. *CHD*7 in zebrafish is expressed in somites, brain, eye and otic vesicle. *CHD*7 enables proper symmetric expression of critically important somitogenesis associated genes located downstream from Wnt including *her*7, *cdx1a*, *dlc*, *mespa* and *ripply*. Zebrafish morpholinos in which *CHD*7 was knocked down were noted to have tail kinks and a progressively shortened axis, thus providing supporting evidence for an important role for *CHD*7 in body axis formation (17).

Genome wide association studies (GWAS) have circumvented challenges associated with linkage and candidate gene approaches because they measure association between common variation across the entire human genome in the form of single nucleotide polymorphisms (SNPs) and identify genetic associations with observable traits (15). Two recent GWAS studies performed by the same group of investigators highlight their discovery potential (18,19). In the first study a GWAS was performed using the Illumina Human610 Genotyping BeadChip and the Illumina HumanHap550v3 Genotyping BeadChip for 1,050 Japanese females with AIS and 1,474 control subjects respectively. Three SNPs on chromosome 10q24.31, located within the same linkage disequilibrium block, which is defined by the association of inheritance between two closely linked alleles, reached genome-wide significance of $P<1.0\times10^{-7}$ with rs11190870 demonstrating the strongest association in both the initial GWAS and replication study ($P=1.27\times10^{-10}$). Through the use of imputation or statistical inference using MACH (Markov Chain framework for genotype imputation and haplotyping) the imputed SNPs and the three original SNPs on 10q24.3 were all localized to the 3' flanking region of *LBX1*.

In GWAS studies it is necessary to control for population stratification. This refers to the presence of genetic differences between cases and controls that represent discongruity in sampling from the respective populations and are not related to the underlying disease process. Nearly all subjects fell into the main cluster of the Japanese population. Additionally, using logistic regression, the principal components were not associated with disease status, arguing against population stratification.

LBX1 is a homeobox gene with homology to the drosophila ladybird late (*lbl*) gene (20). It is expressed in the dorsal part of the spinal cord, hindbrain, a subpopulation of cardiac neural crest cells and muscle precursor cells. *LBX1* in mice is an important determinant of dorsal spinal neurons and hindbrain somatosensory neurons (21). Somatosensory information from the periphery to higher brain centers is mediated by the dorsal spinal cord. The observation of somatosensory dysfunction in some individuals with AIS, in addition to *lbl* expression data support a hypothesis for *LBX1* having some type of somatosensory role (22). The exact genetic mechanism of *LBX1* action is not precisely understood.

In a second GWAS study performed by the same research group (18) 10,641 X-chromosome SNPs were added to the initial study sample, with six SNPs suggestive of AIS association ($P<1\times10^{-5}$). One SNP, rs6570507 on chromosome 6q24.1 reached was associated with AIS ($P=3.02\times10^{-5}$). This finding was significant when Bonferroni-corrected (adjustment for error due to false negative test results; $P<1.67\times10^{-2}$) and when replicated in an independent set of Japanese patients consisting of 786 cases and 24,466 controls with a combined P value of 2.25×10^{-10} [OR 1.28; 95% confidence interval (CI), 1.18-1.38]. Further association of rs6570507 was observed in a replicate set derived from a Chinese Han population of 743 cases and 1,209 controls and a population of European ancestry which consisted of 447 cases and 737 controls resulting in a combined P value of 1.27×10^{-14}. Using genome wide imputation, suggestive association was observed in 12 loci ($P<1\times10^{-5}$) with rs11190870 located on chromosome

10q24.31 reaching genome wide significance ($P<5\times10^{-8}$). Nineteen additional SNPs yielded evidence for association and were strongly correlated ($r^2>0.9$) with rs6570507, all localized in intronic regions of G protein-coupled receptor 126 (*GPR126*). Two SNPs, (rs7774095 and rs9403380) were evaluated by using Encyclopedia of DNA Elements (ENCODE) (23) annotated elements and hypothesized to have a regulatory role of transcriptional activation of *GPR126*. Zebrafish knockdown morpholino experiments blocking translation and messenger RNA splicing, demonstrated *GPR126* morphants had a significantly less mean body length and delayed ossification of vertebrae.

The observed *GPR126* results validate a prior hypothesis which postulates that scoliosis can be mediated by abnormal skeletal growth (24). Several other experimental lines of evidence are consistent with this hypothesis. Limb posture abnormalities and growth failure are observed in *GPR126*-null mice in addition to a hypomyelinating neuropathy (25). Zebrafish *GPR126* morphants display a slower escape time (triggered by impulsive hydrodynamic or visual stimulus) which may be related to myelination defect. Prior GWAS studies have demonstrated association between common SNPs in *GPR126* and stature in children and adults (26).

The irony of these thorough and meticulous analyses is that *LBX1* and *GPR126* account for only a modest 1% of the total phenotypic variance associated with AIS. This indicates multiple layers of complexity associated with AIS that can be hypothetically attributed to multiple gene and other environmentally mediated factors. In a recent comprehensive review, this complexity is underscored by the citation of 50 genetic studies related to IS in which genes associated with connective tissue metabolism, bone growth and metabolism, melatonin signaling and pubertal growth were identified by various researchers (27).

The translational value of identifying genes contributing to the development of AIS relates to possible benefit in the utilization of genetics to help predict which scoliosis curves will fail bracing. While the results of the Bracing in Adolescent Idiopathic Scoliosis Trial (BrAIST) indicate that bracing significantly increases the likelihood of reaching skeletal maturity with a curve of less than 50 degrees as compared to observation alone, bracing treatment fails in approximately 15% of patients with AIS (28).

Attempts to use modeling derived from Cobb angle, Lenke classification of curve type, Risser score, menarchal status and 53 SNPs involved with calcium metabolism, neurodevelopment and signal transduction resulted in a negative predictive value nearing 100% for low risk scores less than 41. While 99% of high risk scores greater than 190 or greater resulted in severe curve progression it was not possible to predict outcome for scores in between 41 and 190 (29). These patients with intermediate risk scores would require close follow up for curve progression by an orthopedic surgeon.

The promoter polymorphism (rs11063714) in the neurotrophin 3 (*NTF3*) gene is associated with curve severity for IS in the Chinese Han population. Individuals affected with IS having an AA genotype had lower mean maximum Cobb angle as compared to patients with AG and GG genotypes (30). Patients who were skeletally immature with an AA genotype had greater success for treatment with bracing as compared to patients with GG genotype. In the corresponding mouse model, Egr $3^{-/-}$, affected mice fail to express *NTF3* and have proprioceptive dysfunction due to muscle spindle agenesis, apoptosis of proprioceptive neurons, proprioceptive neuron apoptosis and disruption of synaptic connectivity between muscle sensory and motor neurons. Moreover, a reduction in the number of muscle spindles and malfunction has been demonstrated in spinal muscle obtained from patients with IS, examined histologically and histochemically, suggesting that abnormalities in proprioception may contribute to IS pathogenesis (31).

In summary two recent GWAS studies highlight association between AIS and SNPs in *LBX1* and *GPR126*. The hypothetical effect that SNPs in these genes can have on spine development in utero and after birth is consistent with prior hypotheses related to altered somatosensory function and skeletal growth. There is much more work that needs to be done before genetic analyses can be used to predict clinical course in scoliosis. Further research to elucidate the complexity of genetic and possibly epigenetic contributions will likely require the collaboration of larger numbers of patient centers and functional analysis of genetic variants.

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