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Genetic biomarkers associated with pain flare and dexamethasone response following palliative radiotherapy in patients with painful bone metastases

Anthony Furfari[1], Bo Angela Wan[1], Keyue Ding[2], Andrew Wong[3], Liting Zhu[2], Andrea Bezjak[4], Rebecca Wong[4], Carolyn F. Wilson[2], Carlo DeAngelis[5,6], Azar Azad[7], Edward Chow[1], George S. Charames[3,7,8]

[1]Department of Radiation Oncology, Odette Cancer Centre, Sunnybrook Health Sciences Centre, University of Toronto, Toronto, Ontario, Canada; [2]Canadian Clinical Trials Group, Cancer Research Institute, Queen's University, Kingston, Ontario, Canada; [3]Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario, Canada; [4]Princess Margaret Cancer Centre, Radiation Medicine Program, Ontario Cancer Institute, University of Toronto, Toronto, Ontario, Canada; [5]Department of Pharmacy, Odette Cancer Centre, Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada; [6]Leslie Dan Faculty of Pharmacy, [7]Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada; [8]Lunenfeld-Tanenbaum Research Institute, Sinai Health System, Toronto, Ontario, Canada

Contributions: (I) Conception and design: R Wong, CF Wilson, C DeAngelis, A Azad, E Chow, GS Charames; (II) Administrative support: A Furfari, BA Wan, A Wong, CF Wilson; (III) Provision of study materials or patients: A Bezjak, R Wong, E Chow; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Dr. George S. Charames, PhD, FACMG. Pathology and Laboratory Medicine, Mount Sinai Hospital, Suite 6-500, 600 University Ave, Toronto, Ontario, M5G 1X5, Canada. Email: George.Charames@sinaihealthsystem.ca.

Background: In patients who receive palliative radiation therapy (RT) for painful bone metastases, 40% experience a transient increase in pain known as a pain flare. Prophylactic dexamethasone has been shown to reduce pain flare incidence to 25%. We aimed to identify DNA biomarkers associated with pain flare and dexamethasone response.

Methods: Daily pain levels were recorded by 81 patients who received a single 8 Gy RT for painful bone metastases, of which 50 also received prophylactic dexamethasone. To identify single-nucleotide variants (SNVs), patient saliva samples obtained at day of RT were sequenced for 4,813 disease-associated genes, then filtered for genes associated with inflammation, radiation or immune response, and DNA damage. Significant SNVs (P<0.005) identified by the Cochran-Armitage trend test underwent the Penalized LASSO method with minimum Bayesian Information Criterion to select a multi-SNV model that jointly predicted pain flare, and pain flare despite prophylactic dexamethasone (dexamethasone response). The corresponding estimated effects of the multi-SNVs were used to drive the prognostic score of developing pain flare for each patient, who were divided into three risk groups of roughly equal sizes.

Results: Risk groups were significantly predictive of pain flare (P<0.0001) and dexamethasone response (P<0.0001). The high-risk patient groups had a 78% chance of developing pain flare, and pain flare despite dexamethasone [OR =24.6, 95% confidence interval (CI): 1.8–342.7, P=0.02]. The multivariable model for pain flare included 15 variants, with effect sizes ranging from –4.97 (*NBPF1* rs3872309 C>T) to 5.54 (*DNM2* 10940838 A>C). The multivariable model for dexamethasone response included 6 variants, with effect sizes ranging from –1.03 (*NBPF1* rs3872309 C>T) to 0.85 (*TSEN54* rs62088470 C>G).

Conclusions: Significant SNVs associated with pain flare were found in genes with functions in biosynthesis (*DHODH*, *PECR*), lipid excretion and metabolism (*UGT2A1/2*, *VLDLR*), and intracellular signalling (*DNM2*, *SEC23A*). Significant SNVs associated with dexamethasone response were from genes involved in extracellular matrix (*HAS1*, *ADAMTS16*) and cytoskeleton regulation (*GAS2L2*). Identification of SNVs predictive of pain flare and dexamethasone response enables targeted prophylactic therapy according to a patient's predisposed response.

Keywords: Genetic biomarker; single nucleotide variant; palliative radiotherapy; pain flare; dexamethasone

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Introduction

Radiation therapy (RT) is commonly used in palliation of painful bone metastases. However, a transient increase in pain within 10 days since treatment known as a "pain flare" is experienced by around 40% of patients who receive palliative radiotherapy (1). As radiation is inherently cytotoxic, it may cause pain flare by evoking an endogenous inflammatory response. Analysis of levels of urinary inflammatory proteins showed an overall increase in levels of cytokines and chemokines following radiotherapy. Patients who experienced pain flare also had different urinary protein profiles compared to patients who did not experience pain flare. These patients had significantly lower levels of pro-inflammatory chemokines interleukin-8 (IL-8), IL-10, and macrophage derived chemokine (MDC) (2).

It has been postulated that the imbalance of pro-inflammatory and anti-inflammatory cytokines contributes to the development of pain flare. Dexamethasone is prophylactically prescribed for managing side effects from radiation of brain metastases. A phase 3 trial conducted across 23 Canadian cancer centers found that dexamethasone is also effective in reducing the incidence of pain flare from 35% to 26% after palliative radiation to bone metastases (3). It has been proposed that differences in dexamethasone response are due to variations in metabolism among individuals, which may also be observed at a genetic level.

Genetic biomarkers have been used to identify individual propensities in treatment response. For example, 58–78% of the individual differences in sensitivity to radiotherapy can be attributed to heritable genetic factors (4). Many of these identified genetic differences are single nucleotide variations (SNVs) of genes involved in DNA damage response, inflammation, and growth factor signalling pathways. For example, the SNV rs12757998 from the gene RNASEL that encodes for a pro-inflammatory ribonuclease has been found to be associated with outcome in prostate cancer patients who received RT (5). Our study aimed to identify genetic biomarkers associated with pain flare and dexamethasone response. This would enable individualised treatment plans for more effective management of pain in patients with bone metastases.

Methods

Patient population

This study was approved by the Ontario Cancer Research Ethics Board (OCREB) (No. 10-094). Written informed consent was obtained for cancer patients at the Odette Cancer Centre receiving a single 8 Gy dose of palliative RT for painful bone metastases before enrollment into the multi-centre NCIC Clinical Trials Group (NCIC CTG) Symptom Control 23 (SC.23) study (3). Patients were randomized into one of two arms: prophylactic dexamethasone (two 4 mg tablets) taken at least 1 hour before RT and for 4 days after RT, or placebo pills for control.

Data collection

To assess pain flare and dexamethasone response, patients filled out the brief pain inventory (BPI) and recorded their analgesic intake on day of RT, every day for 10 days post-RT, and at day 42 post-RT. The BPI ranked pain on a scale of 0 to 10, with 0 being no pain, and 10 being pain as bad as you can imagine. Analgesic intake was converted into daily oral morphine equivalent. Pain flare was defined as at least a two-point increase in the worst pain score without reduction in analgesic intake, or at least a 25% increase in analgesic intake without worst pain score reduction. To be categorized as pain flare rather than pain progression, pain score and analgesic intake must have returned to baseline during the 10 days after RT. Dexamethasone response was determined by whether patients had pain flare despite having taken prophylactic dexamethasone.

Genomic analysis

Saliva samples were taken at day of RT, and underwent DNA sequencing using the Illumina TruSight™ One Panel. This identified SNVs in 4,813 genes harbouring

disease-causing variants. Burrows-Wheeler Aligner's Smith-Waterman alignment (BWA) was used to map the raw sequencing data from Illumina's MiSeq platform hg19 to a reference genome (6). As outlined by Genome Analysis Tool Kit (GATK) Best Practice, we performed base quality score recalibration, indel realignment, duplicate removal, and variant calling (7).

Variant selection

Genes that were associated with inflammation, radiation response, immune response, or DNA damage were selected for variant analysis. The Cochran-Armitage trend test was used to assess associations between variants and pain flare or pain flare treated with dexamethasone.

For the multivariable model predictive of pain flare or pain flare treated with dexamethasone, statistically significant variants with P<0.005 underwent backwards elimination. The *hpgenselect* procedure on SAS was applied using the LASSO method of variable selection with the minimum Bayesian Information Criterion. The prognostic scores for pain flare were produced from the sum of the estimate of effect in the *hpgenselect* model of each of SNVs in the multivariable model, multiplied by the corresponding SNV value. The prognostic scores were calculated for each patient and used to divide patients into risk groups of low (<1/3 quartiles), medium (≥1/3 quartiles but <2/3 quartiles) and high (≥2/3 quartiles). Univariate analysis of the risk groups was conducted using the Cochran-Armitage trend test. For multivariable analysis of pain flare, a logistic regression model was generated, using pain flare as outcome (low risk of pain flare as the referent group), and baseline factors (gender and primary cancer type)-adjusted risk group model of pain flare as the independent factor.

Univariable analysis of pain flare after treated with dexamethasone was conducted in patients who received dexamethasone. Variants were then selected from the significant variants for the multivariable model and prognostic score, which was used to calculate risk scores of patients in both arms of dexamethasone and placebo. Patients from each arm were then divided into three risk groups. Data from both arms were combined to produce a logistic regression model with pain flare as the outcome variable, fit with risk groups, treatment arm, and interaction terms, adjusted for gender and primary cancer site.

The identified variants in the prognostic model underwent pathway analysis and literature search to identify potential biological mechanisms associated with pain flare or dexamethasone response.

Results

A total of 81 patients were included in biomarkers analysis, which consisted of 50 patients who received prophylactic dexamethasone and 31 patients who did not receive prophylactic dexamethasone. The median patient age was 72 (range, 33–95) years old, and 56% of patients were male (*Table 1*). The most common primary cancer sites were prostate (32%), followed by breast (24%) and lung (24%). The majority of patients had a Karnofsky performance status between 70–80 (62%), and a worst pain score at baseline of 7–10 (56%). Radiation to the pelvis, hips, or lower limbs was most common (35%). Twenty-two (27%) patients experienced pain flare. In 50 patients who took prophylactic dexamethasone, 11 (22%) had pain flare.

Variants associated with pain flare

In the univariate analysis, we identified 50 variants associated pain flare (*Table S1*). The most significant variants associated with pain flare were the A>C variant of *DNM2* at position 10940838 (P=0.0002), and the G>A variant of *UGT2A1-UGT2A2* at position 70505162 (P=0.0002).

Fifteen variants were selected in the multivariable model (*Table 2*). Individuals with high prognostic scores belonged to higher risk groups, which were associated with the development of pain flare. In univariate analysis, the risk groups were significantly predictive of pain flare (P<0.0001), with patients in the high-risk group patients having a 78% chance of developing pain flare (*Table 3*).

In the multivariable model, the variant with the largest positive effect size was also one of the most significant variants identified in the univariate analysis, namely the A>C variant at position 10940838 of *DNM2* (effect size =5.54). This variant produced an amino acid change from histidine to leucine at position 772. One variant had a negative effect size. This was rs3872309 of *NBPF1*, which had a C>T variant at position 16891333 that produced an amino acid change from glycine to arginine at position 1049 (effect size =–4.97, P=0.00016).

The X-chromosome gene *OFD1* had four variants in the multivariable model. These were the G>C variant at position 13785256 and 13785266, the C>A variant at position 13785272, and the G>A variant at position 13785269. All four variants had an effect size of 1.35, and P=0.0013 in univariate statistical analysis.

Table 1 Baseline characteristics

Characteristic	N (%)
Median age [years (range)]	72 [33–95]
Sex	
Male	45 (55.6)
Female	36 (44.4)
Primary cancer site	
Prostate	26 (32.1)
Breast	19 (23.5)
Lung	19 (23.5)
Other or unknown	16 (19.8)
Karnofsky performance status	
40–60	26 (32.1)
70–80	50 (61.7)
90	5 (6.2)
Worst pain score at baseline	
1–4	15 (18.5)
5–6	21 (25.9)
7–10	45 (55.6)
Index site of radiated bone lesion	
Pelvis, hips, or lower limbs	28 (34.6)
Ribs, clavicle or sternum	20 (24.7)
Lumbo-sacral spine	19 (23.5)
Cervical-thoracic spine	12 (14.8)
Humerus	2 (2.5)
Pain flare (PF)	
No pain flare (NPF)	59 (72.8)
PF	22 (27.2)
PF with dexamethasone	
Responders (no PF)	39 (78.0)
Non-responders (PF)	11 (22.0)
Total (N)	72

Variants associated with pain flare despite dexamethasone

In univariate analysis, we identified 25 variants with P<0.005 that were associated with pain flare with dexamethasone (*Table S2*). Among these variants, the most significant were the G>A variant of *CREBBP* at position 3795363 (P=0.0007), and the G>A variant of *HAS1* at position 52220351 (P=0.0007).

The multivariate model included six variants, with their effect size and statistical significance from the univariate model shown on *Table 4*. High prognostic scores are predictive of developing pain flare for patients on dexamethasone (P<0.0001, *Table 5*). Patients who were on dexamethasone were also significantly less likely to develop pain flare (P=0.023). After adjustment for the preselected baseline factors, the high-risk group remained significantly predictive of pain flare after radiotherapy in patients treated with dexamethasone [OR =24.6; 95% confidence interval (CI) 1.8–342.7, P=0.02].

The SNV with the largest effect size was rs62088470 of *TSEN54*, a C>G variant at position 73520359 that produced an amino acid change from proline to alanine at position 483 (effect size =0.85, P=0.0010). One SNV had a negative effect size. This was the rs3872309 variant of NBPF1, corresponding to a C>T variant at position 16891333 that produces an amino acid change from glycine to arginine at position 1049 (effect size =–1.03, P=0.0013).

Variants with pre-existing clinical investigations from literature review

Our literature review identified none of the genetic variants in the model predicting pain flare. However, two SNVs in the model predicting dexamethasone response have been published. This includes rs1053878 of the gene *ABO* that corresponds a G>A variant at position 136131651 which produces an amino acid change from proline to leucine at position 156. Through a retrospective cohort study, Cozzi *et al.* identified that ovarian cancer patients who are minor allele carriers of rs1053878 had better overall survival (OS) (8). This corresponds to improved survival in patients with blood type A, when compared to those with blood types B or O.

This also includes rs11084111, a synonymous variant of a G>A change at position 52220351 of *HASI*, a gene that produces the extracellular matrix compound hyaluronic acid. Bulatova *et al.* studied whether polymorphisms of rs11084111 affected disease progression in patients with chronic hepatitis C (9). However, the authors found no significant difference between rs11084111 alleles among healthy patients compared to patients with chronic hepatitis C.

Discussion

We identified SNVs that belong to several significant pathways, including those of biosynthesis, metabolism,

Table 2 Genetic variants associated with PF

Gene	Chr	Position	dbSNP ID	R>A	Protein change	PF SNV: 0, 1, 2	NPF SNV: 0, 1, 2	ExAC	Function	P value	Effect size
DNM2	chr19	10940838	Unavailable	A>C	p.His772Leu	17, 5, 0	59, 0, 0	Unavailable	Intracellular signalling, neural development, apoptosis, cytokinesis, bone resorption	0.0002	5.54
UGT2A1/ UGT2A2	chr4	70505162	rs28404221	G>A	p.Ala267Val	17, 5, 0	59, 0, 0	0.03653	Excretion of lipophilic compounds	0.0002	5.42
DNAH11	chr7	21639572	rs17144747	A>G	Synonymous	17, 5, 0	58, 1, 0	0.0504	Respiratory cilia movement	0.0013	4.68
SEC23A	chr14	39545199	rs17108797	A>G	p.Pro309Pro	18, 4, 0	59, 0, 0	0.04867	Intracellular trafficking	0.0008	4.65
TSEN54	chr17	73520359	rs62088470	C>G	p.Pro483Ala	15, 7, 0	57, 2, 0	0.05846	tRNA splicing	0.0003	4.5
UTRN	chr6	144809978	Unavailable	C>T	Unavailable	17, 5, 0	58, 1, 0	Unavailable	Neural membrane maintenance	0.0013	4.15
DHODH	chr16	72057421	rs61733129	C>T	p.Ala341Val	16, 6, 0	57, 2, 0	0.027	Pyrimidine biosynthesis	0.0014	1.49
OFD1	chrX	13785256	Unavailable	G>C	Unavailable	17, 5, 0	58, 1, 0	Unavailable	Centrioles, signalling, autophagy	0.0013	1.35
OFD1	chrX	13785272	Unavailable	C>A	Unavailable	17, 5, 0	58, 1, 0	Unavailable	Centrioles, signalling, autophagy	0.0013	1.35
OFD1	chrX	13785266	Unavailable	G>C	Unavailable	17, 5, 0	58, 1, 0	Unavailable	Centrioles, signalling, autophagy	0.0013	1.35
OFD1	chrX	13785269	Unavailable	G>A	Unavailable	17, 5, 0	58, 1, 0	Unavailable	Centrioles, signalling, autophagy	0.0013	1.35
MYO18B	chr22	26422980	rs2236005	A>G	p.Gln1501Arg	10, 12, 0	50, 8, 1	0.257	Muscle-associated gene	0.0014	0.42
PECR	chr2	216923679	rs1429148	C>T	p.Glu3Lys	14, 6, 2	53, 6, 0	0.1103	Fatty acid biosynthesis	0.0019	0.14
VLDLR	chr9	2648773	rs6148	A>G	Synonymous	12, 8, 2	49, 10, 0	0.145	Metabolism of lipids	0.0025	0.08
NBPF1	chr1	16891333	rs3872309	C>T	p.Gly1049Arg	18, 4, 0	25, 34, 0	0.2778	Unknown function, but implicated in developmental disorders	0.0016	–4.97

Chr, Chromosome of variant; position, chromosomal location of variant; dbSNPID, SNV identification; R>A, reference allele and alternative allele; protein change, change of amino acid; NPF SNV and PF SNV, number of individuals with 0, 1, or 2 copies of the alternative allele; ExAC, population frequency of alternative allele from Exome Aggregation Consortium; gene, name of gene harbouring SNV; function, biological function of gene; P value, significance found in univariate analysis, as determined by the Cochran-Armitage trend test; effect size, estimate of effect on predicting response to radiotherapy.

Table 3 Prognostic risk group by status for PF

PF status/ prognostic groups	Low	Middle	High	Total
NPF	26 (100.0%)	27 (96.43%)	6 (22.2%)	59 (72.8%)
PF	0 (0.0%)	1 (3.6%)	21 (77.8%)	22 (27.2%)
Total	26	28	27	81

excretion, and intracellular signalling. These pathways may be important in the mechanisms underlying variation among patients in experiencing pain flare and in response to dexamethasone.

Pain flare has been proposed to be due to an inflammatory response from certain individuals in response to cell death induced by radiotherapy (10). One of the genes implicated in pain flare in our model is *DNM2*, which produces the protein dynamin. Dynamin is an intracellular signalling protein that is member of the GTPase family, and is involved in a variety of cellular processes including apoptosis (11). Therefore, variation in this gene may lead to differences in the cellular response to radiation and subsequent inflammatory response.

Metabolic genes and genes involved in excretion had been identified as significantly associated with pain flare. For instance, rs28404221 was identified in the genes *UGT2A1-UGT2A2* of the UDP glucuronosyltransferase 2 family, which produce proteins that catalyze the conjugation of lipophilic substrates with glucuronic acid to increase water solubility and enhance excretion (12). UGT2A1 is known as a detoxification enzyme, and some of its polymorphisms have been identified as playing potential roles in tobacco-associated carcinogenesis (12).

Another gene involved in metabolism is *VLDLR*, which produces a transmembrane receptor for very-low-density lipoproteins (VLDLs). Our study identified that one of its synonymous variants, rs6148, is in a model that may be predictive of pain flare. VLDLR is important in cholesterol homeostasis, and therefore may be contribute to the physiological response to dexamethasone, which, like cholesterol, is a member of the steroid family (13). Moreover, it has been suggested that VLDLR is involved in inducing adipose tissue inflammation (14). Therefore, variation in *VLDLR* may also contribute to the development of pain flare.

Table 4 Genetic variants associated with PF despite prophylactic dexamethasone (dexamethasone response)

Gene	Chr	Position	dbSNP ID	R>A	Protein change	PF SNV: 0, 1, 2	NPF SNV: 0, 1, 2	ExAC	Function	P value	Effect size
TSEN54	chr17	73520359	rs62088470	C>G	p.Pro483Ala	7, 4, 0	38, 1, 0	0.05846	tRNA splicing	0.001	0.85
HAS1	chr19	52220351	rs11084111*	G>A	Synonymous	4, 5, 2	34, 4, 1	0.1307	hyaluronic acid	0.0007	0.84
ABO	chr9	136131651	rs1053878*	G>A	Pro156Leu	6, 3, 2	36, 3, 0	0.0888	ABO blood group	0.0008	0.62
ADAMTS16	chr5	5240002	rs11742370	C>A	Synonymous	4, 6, 1	30, 9, 0	0.1663	Metalloprotease	0.0047	0.55
GAS2L2	chr17	34072898	rs12602590	C>T	p.Ala540Thr	7, 2, 2	36, 3, 0	0.0702	Cytoskeleton regulation	0.004	0.2
NBPF1	chr1	16891333	rs3872309	C>T	p.Gly1049Arg	10, 1, 0	14, 25, 0	0.2778	Unknown function	0.0013	−1.03

Chr, chromosome of variant; position, chromosomal location of variant; dbSNPID, SNV identification (* indicates variant with published clinical associations); R>A, reference allele and alternative allele; protein change, change of amino acid; NPF SNV and PF SNV, number of individuals with 0, 1, or 2 copies of the alternative allele; ExAC, population frequency of alternative allele from Exome Aggregation Consortium; gene, name of gene harbouring SNV; function, biological function of gene; P value, significance found in univariate analysis, as determined by the Cochran-Armitage trend test; effect size, estimate of effect on predicting response to radiotherapy.

Table 5 Prognostic risk group by status for PF despite prophylactic dexamethasone

PF status/ prognostic groups	Low	Middle	High	Total
NPF	15 (100.0%)	17 (94.4%)	7 (48.2%)	39 (78.0%)
PF	0 (0.0%)	1 (5.6%)	10 (58.8%)	11 (22.0%)
Total	15	18	17	50

Being a synthetic steroid, dexamethasone exerts its physiological effects through binding and activating glucocorticoid receptors located in the nucleus, leading to transcription of genes that produce an anti-inflammatory response (15,16). Our panel included four glucocorticoid receptor genes: *NR3C1*, *NR3C2*, *NR4A2*, and *NR5A1*, but none yielded significant variants. Since the downstream anti-inflammatory effects of dexamethasone are multifaceted, and include suppressing prostaglandin synthesis, and transcription of pro-inflammatory interleukins such as IL-1β, IL-6, and Il-8, it is possible that the variation among how individuals respond to dexamethasone may be due to differences in how these pathways are influenced by it. Our study, limited by small sample size, at the most can serve as hypotheses generating and would require validation in future larger research studies.

Finding a panel of variants that can predict pain flare and dexamethasone response is of great clinical utility. With the rapid advancement of genetic technology in terms of efficiency and speed, genetic methods will likely become more accessible and affordable in identifying patient responses to treatment options and enable more effective care. As the genomic data of a patient are stable across time, gene assessment may also be conducted well in advance of the delivery of palliative care. This can reduce patient burden by eliminating treatments that are not likely to be beneficial. Therefore, further study is required to independently validate our proposed predictive genetic models. This would enable the identification of individuals who are at risk of pain flare, and therefore allow intervention before the onset of increased pain after palliative radiotherapy. This can also aid in the identification of an appropriate prophylactic medication, based on whether an individual will respond to dexamethasone. If not, analysis and further study of the non-responders may enable identification of alternative measures to prevent pain and improve well-being.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: This study was approved by the Ontario Cancer Research Ethics Board (OCREB) (No. 10-094). Written informed consent was obtained for cancer patients at the Odette Cancer Centre.

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Supplementary

Table S1 Significant variants associated with PF identified in univariate analysis (P<0.005)

Gene	Chromosome	Location	R>A	PF SNV (0, 1, 2)	NPF SNV (0, 1, 2)	P value
*DNM2**	chr19	10940838	A>C	17, 5, 0	59, 0, 0	0.0002
*UGT2A1/UGT2A2**	chr4	70505162	G>A	17, 5, 0	59, 0, 0	0.0002
*TSEN54**	chr17	73520359	C>G	15, 7, 0	57, 2, 0	0.0003
*SEC23A**	chr14	39545199	A>G	18, 4, 0	59, 0, 0	0.0008
DNM1L	chr12	32854366	A>C	9, 12, 1	46, 13, 0	0.0008
YARS2	chr12	32908237	C>A	9, 12, 1	46, 13, 0	0.0008
YARS2	chr12	32908518	G>A	9, 12, 1	46, 13, 0	0.0008
*OFD1**	chrX	13785256	G>C	17, 5, 0	58, 1, 0	0.0013
*OFD1**	chrX	13785266	G>C	17, 5, 0	58, 1, 0	0.0013
*OFD1**	chrX	13785269	G>A	17, 5, 0	58, 1, 0	0.0013
*OFD1**	chrX	13785272	C>A	17, 5, 0	58, 1, 0	0.0013
*UTRN**	chr6	144809978	C>T	17, 5, 0	58, 1, 0	0.0013
*DNAH11**	chr7	21639572	A>G	17, 5, 0	58, 1, 0	0.0013
*DHODH**	chr16	72057421	C>T	16, 6, 0	57, 2, 0	0.0014
*MYO18B**	chr22	26422980	A>G	10, 12, 0	50, 8, 1	0.0014
*NBPF1**	chr1	16891333	C>T	18, 4, 0	25, 34, 0	0.0016
*PECR**	chr2	216923679	C>T	14, 6, 2	53, 6, 0	0.0019
*VLDLR**	chr9	2648773	A>G	12, 8, 2	49, 10, 0	0.0025
CHRM1	chr11	62677220	G>A	15, 7, 0	55, 4, 0	0.0034
CHRM1	chr11	62678306	G>T	15, 7, 0	55, 4, 0	0.0034
APP	chr21	27394182	GTG>–	19, 3, 0	59, 0, 0	0.0038
GRHL2	chr8	102631911	G>A	19, 3, 0	59, 0, 0	0.0038
FUT7	chr9	139925983	G>A	19, 3, 0	59, 0, 0	0.0038
PRSS1	chr7	142460429	CAA>–	19, 3, 0	59, 0, 0	0.0038
MYO15A	chr17	18064722	C>T	19, 3, 0	59, 0, 0	0.0038
MYO15A	chr17	18075051	G>A	19, 3, 0	59, 0, 0	0.0038
SRGAP2	chr1	206634526	T>A	19, 3, 0	59, 0, 0	0.0038
LOXL2	chr8	23190926	C>T	19, 3, 0	59, 0, 0	0.0038
LOXL2	chr8	23190995	T>C	19, 3, 0	59, 0, 0	0.0038
DPYSL2	chr8	26481697	G>A	19, 3, 0	59, 0, 0	0.0038
GAS2L2	chr17	34072386	A>G	19, 3, 0	59, 0, 0	0.0038
SCN5A	chr3	38601665	C>T	19, 3, 0	59, 0, 0	0.0038
KRT17	chr17	39775870	C>T	19, 3, 0	59, 0, 0	0.0038
CD3EAP	chr19	45912750	G>A	19, 3, 0	59, 0, 0	0.0038
CCDC8	chr19	46915431	A>C	19, 3, 0	59, 0, 0	0.0038
CEP135	chr4	56883987	C>G	19, 3, 0	59, 0, 0	0.0038
CEP135	chr4	56885581	T>C	19, 3, 0	59, 0, 0	0.0038
BMP2	chr20	6759706	C>T	19, 3, 0	59, 0, 0	0.0038
DYSF	chr2	71762413	G>A	19, 3, 0	59, 0, 0	0.0038
KDM6B	chr17	7752283	A>C	19, 3, 0	59, 0, 0	0.0038
CARD14	chr17	78182150	C>T	19, 3, 0	59, 0, 0	0.0038
ASPN	chr9	9523702	–>TCATCATCA	19, 3, 0	59, 0, 0	0.0038
PECR	chr2	216908679	C>T	21, 1, 0	36, 18, 5	0.0039
LTBP2	chr14	74992800	A>G	8, 12, 2	41, 17, 1	0.0041
VLDLR	chr9	2644954	C>T	12, 8, 2	48, 11, 0	0.0043
PPARGC1B	chr5	149212243	G>C	16, 6, 0	56, 3, 0	0.0047
PPARGC1B	chr5	149212471	G>A	16, 6, 0	56, 3, 0	0.0047
BNC2	chr9	16435714	T>C	16, 6, 0	56, 3, 0	0.0047
ZDHHC8	chr22	20131116	G>A	16, 6, 0	56, 3, 0	0.0047
NR1H2	chr19	5088182	–>AAC	2, 8, 12	14, 33, 12	0.0049

Gene, genetic symbol gene housing variant (variants selected in multi-SNV model are marked with *); Chr, chromosome of variant; position, chromosomal location of variant; R>A, reference allele and alternative allele; PF and NPF SNV, number of individuals with 0, 1, or 2 copies of the alternative allele; P value, significance found in univariate analysis, as determined by the Cochran-Armitage trend test.

Table S2 Significant variants associated with PF despite prophylactic dexamethasone identified in univariate analysis (P<0.005)

Gene	Chromosome	Location	R>A	PF SNV (0, 1, 2)	NPF SNV (0, 1, 2)	P value
CREBBP	chr16	3795363	G>A	6, 5, 0	37, 2, 0	0.0007
*ABO**	chr9	136131651	G>A	6, 3, 2	36, 3, 0	0.0008
CSMD1	chr8	2820043	G>T	8, 3, 0	39, 0, 0	0.0008
DNM2	chr19	10940838	A>C	8, 3, 0	39, 0, 0	0.0008
VCAN	chr5	82808072	C>T	8, 3, 0	39, 0, 0	0.0008
SPG11	chr15	44944341	G>A	8, 3, 0	39, 0, 0	0.0008
DCAF17	chr2	172337812	T>A	8, 3, 0	39, 0, 0	0.0008
UGT2A1-UGT2A2	chr4	70505162	G>A	8, 3, 0	39, 0, 0	0.0008
DNAH11	chr7	21639572	A>G	8, 3, 0	39, 0, 0	0.0008
EPHB6	chr7	142561922	G>A	8, 3, 0	39, 0, 0	0.0008
*NBPF1**	chr1	16891333	C>T	10, 1, 0	14, 25, 0	0.0013
*GAS2L2**	chr17	34072898	C>T	7, 2, 2	36, 3, 0	0.0040
CREBBP	chr16	3795292	G>T	7, 4, 0	38, 1, 0	0.0010
FZD1	chr7	90895971	G>C	7, 4, 0	38, 1, 0	0.0010
*HAS1**	chr19	52220351	G>A	4, 5, 2	34, 4, 1	0.0007
SIGLEC12	chr19	52004759	C>T	8, 2, 1	39, 0, 0	0.0016
PER3	chr1	7887579	C>G	6, 5, 0	36, 3, 0	0.0026
MTUS1	chr8	17503501	G>A	7, 3, 1	37, 2, 0	0.0032
*ADAMTS16**	chr5	5240002	C>A	4, 6, 1	30, 9, 0	0.0047
*TSEN54**	chr17	73520359	C>G	7, 4, 0	38, 1, 0	0.0010
POLG	chr15	89861826	T>C	7, 4, 0	37, 2, 0	0.0049
TRPV4	chr12	110238481	G>A	7, 4, 0	37, 2, 0	0.0049
DHODH	chr16	72057421	C>T	7, 4, 0	37, 2, 0	0.0049
ZNF213	chr16	3190685	T>C	7, 4, 0	37, 2, 0	0.0049
ZAN	chr7	100364679	C>T	7, 4, 0	37, 2, 0	0.0049

Gene, genetic symbol gene housing variant (variants selected in multi-SNV model are marked with *); Chr, chromosome of variant; position, chromosomal location of variant; R>A, reference allele and alternative allele; PF and NPF SNV, number of individuals with 0, 1, or 2 copies of the alternative allele; P value, significance found in univariate analysis, as determined by the Cochran-Armitage trend test.