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Males with Familial Idiopathic Scoliosis: A Distinct Phenotypic Subgroup

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Abstract

Study Design—Statistical analysis of genomic screening and fine mapping data.

Objective—The goals of this study were to analyze a region on chromosome 17 and to identify specific genetic determinants within this region linked to familial idiopathic scoliosis (FIS) in a subgroup of families in which affected males have undergone surgery.

Summary of Background Data—The high prevalence and variability of FIS is indicative of genetic heterogeneity. To localize genes related to scoliosis, identification of groups of families with common clinical characteristics is a strategy that reduces genetic heterogeneity. Two independent studies have implicated a region on chromosome 17 as related to FIS.

Methods—With approval of the Institutional Review Board, the initial study population consisted of 202 families (1198 individuals), each of which had 2 or more affected individuals; 17 of those families had an affected male who had surgery. Individuals underwent genomic screening and subsequent fine mapping. Results were obtained using model-independent linkage analysis, with scoliosis set as a qualitative and as a quantitative trait, as implemented in SIBPAL (S.A.G.E., v4.5). The level of significance was set at $P \leq 0.05$.

Results—The initial study population had significant results at markers d17s975 and d17s2196. Analyses of a subgroup of families with males having undergone surgery using a customized single nucleotide polymorphism panel resulted in increased significance of this region.

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Key Points:

- Males with idiopathic scoliosis have phenotypic differences from females that are affected with this disorder.
- An area on chromosome 17 has been identified through two independent investigations as potentially significant in the etiology of idiopathic scoliosis in some families.
- Males with severe idiopathic scoliosis who have undergone surgery may represent a distinct genetic group.
- An area on chromosome 17 adjacent to the neurofibromatosis Type I locus is significant in relation to a specific group of families with idiopathic scoliosis.

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Conclusion—The data confirm a previously reported genetic locus on chromosome 17 as statistically significant in the etiology of FIS within a subgroup of families in which an affected male had spinal surgery.

Keywords

chromosome 17; genomic screen; linkage analysis; idiopathic scoliosis

Introduction

Idiopathic scoliosis, a fixed structural lateral curve of the spine of ≥10°, affects 2% to 3% of children in the western hemisphere.[1] Although the female to male ratio is approximately 1:1 for minor curves, the proportion of females increases dramatically as the curve magnitude increases; the reason is unknown.[1] Because of the predominance of females that demand active treatment, the disorder is classically addressed in relation to females, not males. Nevertheless, scoliosis in males can be progressive and can result in bracing and/or surgery. Therefore, in an effort to identify specific parameters that may be unique to their therapeutic management, this population has become a focus of multiple studies.[2-8]

Evidence obtained via observation and experimental data provide a compelling argument for a genetic basis for familial idiopathic scoliosis (FIS).[9-15] It is well known that there is an increased incidence of scoliosis among families of patients with idiopathic scoliosis compared with that of the general population.11›12 A meta-analysis of all reports involving monozygotic and dizygotic twins showed a marked increase in prevalence among monozygotic twins, indicating a statistically significant genetic component to this condition.[16] Current genetic statistical techniques have shown that, within specific populations, FIS segregates as a single gene disorder that follows Mendelian genetic patterns with varying degrees of penetrance and heterogeneity.[9,17,18] Despite this finding, the mode of inheritance has been controversial. The lack of disease transmission from father to son, noted by Cowell *et al*[10] in 1970, led to the conclusion that the disorder is X-linked-dominant in nature. Multiple subsequent reports[19-21] have refuted this theory and have reported an autosomal-dominant or multifactorial mode of inheritance.

The advent of genome-wide scanning has resulted in specific areas on the human genome being identified as potentially significant in the etiology of FIS. Wise *et al*[22] reported on loci on chromosomes 6p, 10q, and 18q in one single multiplex family. In a population of 7 Chinese families, Chan *et al*[23] reported on loci on chromosomes 19p and 2q. In a study of a 3-generation Italian family, Salehi *et al*[24] reported linkage to an area on chromosome 17p. In 2005, Miller *et al*[25] analyzed a large study population (202 families; 1198 individuals) and reported linkage to loci on 6p, 6q, 9q, 16q, 17p, and 17q. The area on 17p correlated with that reported in the previous study by Salehi *et al.*[24] Before any statistical analyses were performed, the initial study population was stratified based on clinical characteristics and most likely mode of inheritance.25 This stratification resulted in the formation of a group of families that had severely affected males who underwent surgery. Linkage analysis of this group highlighted regions on chromosomes 5p, 6q, 13q, 17p, and 17q, inclusive of the critical region on chromosome 17 noted previously. The independent experimental observations of distinct study populations linked to this critical loci area affords us reasoning to pursue this area as potentially related to the disease phenotype of idiopathic scoliosis.

The goal of this study was to analyze this region on chromosome 17p in order to identify specific genetic loci related to scoliosis in a subgroup of families in which affected males have undergone surgery.

Materials and Methods

Population and Initial Genome-Wide Screening

The initial study population consisted of 202 families (1198 individuals), each of which had 2 or more affected individuals.[25] Criteria for a diagnosis of scoliosis were history and physical examination consistent with a spinal curvature in the sagittal plane and standing anteroposterior radiographs exhibiting a minimum of 10° of lateral curve by the Cobb method26 with pedicle rotation.27-29 Individuals with congenital deformity and neuromuscular causes were excluded. The sample population used in the study was consistent with that of our previous epidemiologic study with regard to gender, curve type, and curve size as measured by the Cobb angle.[20] If there was any historical evidence within the family of other genetic conditions, regardless of whether that member did or did not have scoliosis, the family was excluded from the study sample.

Blood samples were obtained from all eligible individuals, and DNA was extracted with a Stratagene DNA extraction kit (Stratagene, La Jolla, CA) via standard protocols. The initial study population of 202 families underwent a genomic screen under Institutional Review Board approval by the Center for Inherited Disease Research (Baltimore, MD) with a modified CHLC v.9 marker set consisting of 391 short tandem repeat markers. Markers were primarily tri- and tetra-nucleotide repeats. The average heterozygosity of the markers was 76%, and the average distance between the markers was approximately 9 centiMorgans (cM). Genotyping was performed on an ABI 377 (Applied Biosystems Inc. Foster City, CA) platform. To assess error rates, 4 blind duplicate samples were run on each 96-well plate. Based on 20,157 paired genotypes, the blind duplicate error rate was 0.13%.

The initial genome-wide screen25 and subsequent fine mapping of the initial study population were analyzed by model-independent linkage analysis via SIBPAL (Statistical Analysis for Genetic Epidemiology [S.A.G.E.], version 4.5, Case Western Reserve University, Cleveland, OH). Strategies of family stratification have been published.[25] Recognizing clinically that males with severe curvatures may represent a distinct phenotypic subgroup, a criterion selected as a basis for a family subset was one in which an affected male had undergone surgery for progressive, severe scoliosis. From the initial study population, 17 families (120 individuals) met this criterion and comprised the subset that is the focus of the current study. This subpopulation was analyzed statistically with the initial genomic screening markers, and, subsequently, through fine mapping markers.

Single Nucleotide Polymorphisms (SNPs)

Candidate genes were identified within the critical region on chromosome 17 (18.3Mb – 31.47Mb, Table 1). Fifty SNPs with a reported minor allele frequency in dbSNP of ≥0.2 were chosen within the 13.17 Mb area at an approximate density of 1 SNP every 250 kilobases (kb), with attention to SNPs intragenic to the identified candidate genes. SNP genotyping or allelic discrimination was performed with the Taqman method (Assays-on-Demand, Applied Biosystems Inc., Foster City, CA) to determine the zygosity of the individuals at each SNP site. A 5-ul reaction mixture [20 ng of genomic deoxyribonucleic acid, 2.5 μl of Taqman Universal polymerase chain reaction (PCR) master mix (Applied Biosystems Inc.), and 0.25 μl of 20× Assays-on-Demand SNP mix (Applied Biosystems Inc.)] was prepared according to the manufacturer's specifications. PCR conditions were 95 C for 10 minutes, followed by 40 cycles of 92 C for 15 seconds and 60 C for 1 minute. The PCR product then was analyzed for fluorescence with the PRISM 7900 Sequence Detection System (Applied Biosystems Inc.).

A second battery of 24 SNPs was selected over a 2.31-Mb area (24.50 – 26.81 Mb) within the area detailed from the first fine mapping analysis on chromosome 17. SNPs were intragenic

when possible, with a reported minor allele frequency in dbSNP of ≥0.2. SNP polymorphism genotyping or allelic discrimination was performed via the Taqman method as described above.

Statistical Analyses

Allele frequencies were obtained with FREQ (S.A.G.E., v4.5). Familial relationships were verified with RELCHECK30, which infers the most likely relationship between pairs of relatives by using identity-by-descent sharing estimates. Mendelian inconsistencies were determined with PEDCHECK.31 The genetic and physical map distances for the short tandem repeat polymorphisms (STRPs) were obtained from the University of California-Santa Cruz Human Genome browser (March 2004 release). Physical map distances for the SNPs were obtained from the National Center for Biotechnology Information (dbSNP) database (human build 35).

Model-independent sib-pair linkage analysis was used to screen for linkage between the trait and each marker (S.A.G.E., v4.5). Scoliosis was analyzed as a qualitative trait, as defined by a curve threshold of ≥10°. This threshold was used to dichotomize the degree of lateral curvature as affected or unaffected. The level of significance for all statistical analyses was set at $P \leq 0.05$.

Before multipoint linkage analyses, SNPs were assessed for linkage disequilibrium using Haploview (version 3.232). SNPs in high linkage disequilibrium were defined as those having a D′=1.0 and an $r^2 \geq 0.4$. The SNP with the highest information content from each cluster of SNPs in linkage disequilibrium was retained for multipoint linkage analysis.

Results

In the initial sample population (202 families), when the threshold for disease curvature was ≥10°, qualitative linkage analyses revealed significant results at 2 successive markers: D17s2196 ($P = 0.0387$) and D17s975 ($P = 0.0276$).[25] When the families were segregated based on most likely mode of inheritance, a subset of families representing an autosomal-dominant pattern (101 families) had heightened significance within the same region at 3 markers: D17s1303 ($P = 0.0370$), D17s799 ($P = 0.0084$), and D17s2196 ($P = 0.0086$). However, intervening fine-mapping markers proved not to be significant (Table 2). Two additional markers, D17s1293 and D17s1298, proximal and distal to those above, were also significant ($P = 0.0026$ and $P = 0.0433$, respectively). It is noteworthy that Salehi *et al*[24] also reported marker D17s799 as significant within a multigenerational Italian family (logarithm of odds = 3.20; $\theta = 0$).

A subset of families identified through a male individual having undergone surgery for progressive scoliosis consisted of 17 families (120 individuals). With a disease threshold of 10° Cobb angle, there were 79 affected individuals (34 males and 45 females) and 41 unaffected individuals (22 males and 19 females). Within these families, 66% of individuals, 61% of the males, and 70% of the females were affected with scoliosis. These numbers represent a ratio of 1.3 affected females to 1.0 affected male, a ratio lower than that of the initial family population (202 families; 3.9 affected females per affected male). The average Cobb angle of the affected males within this subset of 17 families was 38° (range, 10°--107°); the average Cobb angle of the affected females was 31° (range, 10°--70°). Of the affected males and females who had surgical intervention, the average preoperative Cobb angle was 62° and 69°, respectively. The average Cobb angle of the affected males that did not have surgical intervention was 17° (range, 10°--40°); the angle was between 10° and 15° in 10 individuals, between 16° and 20° in 3 individuals, and between 22° and 40° in 4 individuals.

Linkage analyses of this subset of families resulted in regions suggestive of linkage, defined as 2 adjacent markers with a *P* value ≤ 0.05, on 5p, 6q, 13q, 17p, and 17q (all data not shown). In the context of this work related to chromosome 17 (Table 2), qualitative single-point analyses (disease curvature threshold of 10°) resulted in significance at markers D17s1298 (*P* = 0.0372), D17s969 (*P* = 0.0101), D17s975 (*P* = 0.0003), D17s1293 (*P* = 0.0475), D17s784 (*P* = 0.0217), and D17s928 (*P* = 0.0099). Multipoint analysis results within this region were significant at markers D17s1298 (*P* = 0.0266), D17s784 (*P* = 0.0028) and D17s928 (*P* = 0.0032). Of note, marker d17s799, which had been noted to be significant in a previous subset and by Salehi *et al*[24], is within this area; however, that particular marker did not prove to be significant within this specific subpopulation. To confirm and define this region further, this subset of families underwent additional analyses with flanking markers (D17s969, D17s918, and D17s689); however, results were not significant (Table 2).

Secondary fine mapping within this same region with a customized SNP panel (the first panel extending from 18.30 to 31.47 Mb, and the second panel extending from 24.50 to 26.81 Mb), was performed. Two SNPs were dropped because they were not in Hardy-Weinberg equilibrium (*P* < 0.0001). Seventy-two SNPs were retained. Once adjusted for linkage disequilibrium, 46 SNPs were retained and were merged with the STRPs. Single-point analyses resulted in significance at multiple SNPs. Two regions that had at least 2 contiguous SNPs of significance (*P* <0.05) were delineated and are reported in Table 3. The point of highest significance was at SNP rs4325622 (*P* = 0.00028). Multipoint analyses resulted in significance at marker rs7342921 (*P* = 0.00945), which is 0.09 Mb downstream of rs4325622 and 0.52 Mb downstream of D17S975. Of interest is the proximity of this region to the deletional area responsible for the spectrum of phenotypic variation related to neurofibromatosis type I (NF1).[33]

Discussion

FIS is a complex genetic disorder with multiple genes and genetic interactions responsible for its ultimate expression. The theory that adolescent males with severe idiopathic scoliosis represent a unique group harboring specific characteristics is supported by clinical studies.[2-4,34,35] Our current study focused on a unique subgroup of families, each of which has an adolescent male with severe scoliosis, in which genetic loci on chromosomes 5, 6, 13, and 17 have been identified as significant. The data on chromosome 17p corroborate previously published results from an independent investigation[24] in which the same region was found to be significant in a 3-generation Italian family. With multiple genetic factors potentially influencing the expression of this complex disorder, the replication of a specific genetic locus from 2 independent investigations is highly significant. This finding emphasizes the potential role of this region in the etiology of scoliosis within a family subgroup and was the focus of the current study.

The identification of this particular subgroup was based on studies related to clinical presentation and clinical responsiveness to therapeutic strategies.[2-4,34,35] Natural history studies have shown significant differences between males and females with idiopathic scoliosis. In 1988, Suh and MacEwen[34] studied 50 males with idiopathic scoliosis of at least 20°. Male curves were shown to progress into adolescence until a Risser 5 level of maturity, a maturity level later than that seen in female populations. A second study by Karol *et al*[3] of 210 boys with idiopathic scoliosis showed an increased risk of curve progression in boys with an earlier Risser sign, those who were younger at diagnosis, and those who had a larger curve at time of presentation. The authors further concluded that males generally had a later onset and prolonged progression of scoliotic curvature than did females.

In relation to therapeutic algorithms and outcomes, a study by Karol,[2] which focused on nonoperative bracing treatment in males, found that bracing was poorly effective for curves >30°. Several other studies have reported on operative outcomes from functional and quantitative perspectives with differing results. White *et al*[35] evaluated 121 patients undergoing operative intervention for idiopathic scoliosis and found that females reported better outcomes in a functional and self-image domain after surgical intervention than did males. In contrast, Merola *et al*[7] found no statistically significant differences between males and females. In matched male and female populations, Helenius *et al*[4] showed greater major curve correction in females than in males. However, there were no significant differences in the final correction of isolated thoracic curves and outcome measurements between the 2 genders. In a matched retrospective study, Sucato *et al*[36] found males had larger curves at the time of surgery, longer surgical times, more intraoperative blood loss, and less curve correction than did females. Recently, Marks *et al*[6] retrospectively reviewed data from 547 patients (98 males) and found that male curves were more rigid preoperatively but had similar degrees of postoperative scoliosis correction compared with female curves.

Based on the above-listed studies, our current investigation distinguished a subgroup of families with idiopathic scoliosis in which males underwent surgery for severe scoliotic curvature. FIS is a complex genetic disorder believed to be the result of multiple genetic and biologic factors. When evaluating a large sample population, the identification of subgroups based on detailed clinical data is one method of reducing heterogeneity and garnering a more homogenous sample. Clinical studies support the hypothesis that severe scoliosis in males *versus* females presents a unique clinical situation and a distinct phenotype.[2,3,6,7,36]

In the current study, genomic screening results of families with male patients having undergone surgery for idiopathic scoliosis resulted in areas on chromosome 17 of potential significance, one of which has been previously reported by an independent investigation.[24] Further definition of the initial large region, through customized SNP panels, resulted in narrowing the focus of the region to an area of approximately 2.5 Mb that is adjacent and contiguous with the microdeletional area associated with NF1. Within this region, 5 SNPs are significant ($P < 0.05$), giving weight to the potential relationship of this area to the pathology of the scoliotic deformity (see Table 3). Intermediate or adjacent SNPs that are not significant may be explained through lower marker heterozygosity resulting in a decreased power to detect linkage, a sample population that is not completely homogeneous, or a type-II error.

NF1 is an inherited disorder whose diagnosis depends on the presence of at least two of seven clinical features.[37] Those individuals with a diagnosis of NF1 have a mutation within the NF1 gene. Scoliosis, the most common osseous manifestation of NF1, is seen in 10% to 30% of patients.[38] In-depth screening of the patients and families in the current study clearly negated NF1 within any individual. Molecular studies have linked NFl to a deletional area on chromosome 17 that includes 14 genes, one of which is the NF1 gene.[39,40] Recent evidence has shown that epigenetic mechanisms of gene regulation exist throughout this region and are related to the phenotype variation of NF1 itself and to the numerous clinical conditions that overlap with the NF1 phenotype.[41] The critical region shown by the SNP data in the current study maps adjacent to the centromeric breakpoint of the NF1 microdeletional region.[41] The question is whether potential common genetic variations causally related to scoliosis reside in this region.

The most significant SNPs (rs4325622 and rs2066713 with *P* values of 0.00028 and 0.00241, respectively) are located in the serotonin transporter gene, SLC6A4. The SLC6A4 gene is of interest for its ability to modulate serotonin (5-HT) activity.[42] Serotonin transport inhibition is known to result in altered architecture and decreased bone mineral content, bone mass, and mechanical bone strength.42 Idiopathic scoliosis has been associated with decreased bone

mineral density in some ethnic groups.43 Patients with NF1 have decreased bone mineral density, a finding most pronounced in NF1 patients who exhibit scoliosis that requires surgical treatment.43,44 Pathophysiologically, decreased bone mineral density has a direct impact on the structural biomechanical stability of the spine and can lead to collapse through osseous deformation. Hypothetically, genetic factors influencing bone mineral density could be common elements leading to spinal deformity within these 2 disorders.

Additional significant SNPs within the area are associated with the genes SSH2 (slingshot 2), BLMH (bleomycin hydrolase), and Rab11-FIP4 (Rab11 family interacting protein 4). The BLMH gene encodes for an enzyme that inactivates bleomycin and has been related to treatment strategies in patients with testicular germ-cell cancer.[45] Rab11-FIP4 is one of a family of Rab11-interacting proteins that function in the control of membrane traffic in cytokinesis or cell division.46 Although neither of these genes have any obvious relationship to scoliosis, SSH2 was initially highlighted in this region as a candidate gene because of its role in the phosphorylation/inactivation of cofilin, a molecule integral to cytoskeleton organization. Aberrations in cofilin activation lead to specific defects in extracellular matrix assembly that may have distinct ramifications in growth patterns at the cellular level. This process could be proposed as a novel causal mechanism for the development of scoliosis, a disorder that primarily is expressed during periods of rapid growth.

Conclusion

In summary, FIS is a complex genetic disorder in which multiple genetic and biologic factors lead to its expression. The identification of a subset of families with males who underwent surgery has isolated a locus on chromosome 17, which was previously identified in the literature as potentially significant. Further work has delineated the region to an area contiguous with the microdeletional area associated with NF1. The primary region is inclusive of multiple genes of interest. Identifying genetic mutations within these genes via sequencing, and showing segregation among affected family members, would be necessary to establish true candidacy. Our future goals include the expansion of this population and the identification of the significance of this genomic area in the etiology of FIS.

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Table 1
Candidate Genes Identified on Chromosome 17 within the Areas of Interest

Candidate Gene	Approximate Map Position (Mb)*	Function
Lethal giant larvae homolog 1 (LLGL1)	18	Cytoskeletal protein, cell cycle control
Microfibular-associated protein 4 (MFAP4)	19.2	Protein metabolism and modification
Nitric oxide synthase 2A (NOS2A)	23	Synthase, electron transport
Slingshot 2 (SSH2)	25	Phosphatase, cell structure/motility
Solute carrier family 6 (SLC6A4)	25.5	Serotonin transporter, signal transduction, bone accrual regulation
Carboxypeptidase D (CPD)	25.7	Intracellular trafficking
Neurofibromin 1 (NF1)	26.4	Neuronal signaling molecule

* From the www.genome.ucsc.edu website

Table 2

Model-Independent Linkage Analyses Results for Candidate Region on Chromosome 17

Marker	Approximate Map Position (Mb)*	Initial Population (*P* value)	Autosomal-dominant Subgroup (*P* value)	Families with Males with Idiopathic Scoliosis Treated with Surgery	
				Single-point (*P* value)	Multipoint (*P* value)
D17s1298	3.61	0.1513	**0.0433**†	**0.0372**	**0.0266**
D17s974		0.6514	0.0911	0.4547	0.2103
D17s1303	10.8	0.6973	**0.037**	0.0855	0.1575
D17s969§	11.79	N/A¶	0.3981	**0.0101**	0.0976
D17s799§‡	13.11	0.4442	**0.0084**	0.1082	0.1634
D17s918§	15.1	N/A	0.0589	0.4122	0.2185
D17s2196	17.21	**0.0387**	**0.0086**	0.2076	0.2661
D17s689§	20.9	N/A	0.1018	0.7156	0.1904
D17s975	25.12	**0.0276**	0.0864	**0.0003**	0.1937
D17s1293	29.58	0.0841	**0.0026**	**0.0475**	0.1478
D17s784	75.41	0.4397	0.8057	**0.0217**	**0.0028**
D17s928	77.84	0.4111	0.4429	**0.0099**	**0.0032**

*From the www.genome.ucsc.edu website

†Boldface indicates statistically significant values ($P \leq 0.05$)

§Fine-mapping marker

¶N/A, not applicable.

‡Logarithm of odds score was 3.20[24]

Spine (Phila Pa 1976). Author manuscript; available in PMC 2011 January 15.

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Table 3

Model-Independent Linkage Results for SNPs on 17q11.2

Marker	Position* (Mb)	Associated Gene	Familial Idiopathic Scoliosis Families with Males Treated with Surgery		Location
			Single-point (*P* value)	Multi-point (*P* value)	
rs1472081	24.42	--†	**0.01827**§	0.19811	Unknown
rs8067041	24.5	MYO18A	0.31121	0.19735	Intron
rs6505114	24.52	MYO18A	0.63171	0.19713	Intron
rs894606	24.93	GIT1	0.65590	0.19514	Intron
rs3110492	24.95	--	**0.02810**	0.19506	Unknown
d17s975	25.12	SSH2	**0.00037**	0.19371	Intron
rs2467334	25.16	SSH2	0.18985	0.19349	Intron
rs4436830	25.41	EFCAB5	0.17881	0.18809	Intron
rs4325622	25.55	SLC6A4	**0.00028**	0.10264	Intron
rs2066713	25.58	SLC6A4	**0.00241**	0.09264	Intron
rs3816828	25.62	BLMH	0.46811	0.07718	Intron
rs7342921	25.64	BLMH	**0.00409**	**0.00945**	Exon
rs3794794	25.74	CPD	0.09817	0.07765	Intron
rs2191351	25.78	CPD	0.05990	0.07954	Intron
rs170053	25.92	--	0.07734	0.08812	Unknown
rs2905787	26.51	NF1	0.36028	0.16494	Intron
rs2057769	26.72	NF1	0.42398	0.20561	Intron
rs2107359	26.72	NF1	0.68507	0.20578	Intron
rs2074153	26.88	Rab11-FIP4	**0.00331**	0.20277	Intron
rs739630	26.9	--	**0.03891**	0.20302	Unknown
rs886224	27.25	--	0.79680	0.21401	Intron
rs753750	27.64	RHBDL3	0.05750	0.23211	Intron

Genes: MYO18A = myosin 18A isoform b, GIT1 = G protein-coupled receptor kinase interactor 1, SSH2 = Protein phosphatase Slingshot homolog 2, EFCAB5 = EF-hand calcium binding domain 5, SLC6A4 = solute carrier family 6 member 4, BLMH = Bleomycin hydrolase, CPD = carboxypeptidase D precursor, NF1 = neurofibromin isoform 1, Rab11-FIP4 = Rab11 family interacting protein 4, RHBDL3 = rhomboid, veinlet-like 3

*From www.genome.ucsc.edu

† No gene association.

§ Boldface indicates statistically significant values ($P \leq 0.05$)