RESEARCH ARTICLE

Functional genomic variant patterns in Caucasian patients diagnosed with idiopathic scoliosis: a controlled, observational study.

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Abstract

Previous studies have identified various genetic variants associated with idiopathic scoliosis. However, only individual single nucleotide polymorphisms (SNPs) were described, making application to clinical practice difficult. The current study examined functional SNP groups, where individual SNPs were collectively categorized based upon a common metabolic function or pathway. The number and types of functional SNP groups were identified and compared between a group of Caucasian idiopathic scoliosis patients, and a group of Caucasian, non-scoliosis patients. Intergroup comparison showed a significant difference in 19 functional SNP groups out of 131 reported. Intragroup comparison of the scoliosis group found a statistically significantly higher average number of the 19 significant SNP groups in the scoliosis patients with curves above surgery threshold compared to those below. Within the scoliosis patient group, those patients above the recommended surgery threshold of 50° were found to have a higher average number of positive SNP categories when compared to patients below the recommended surgery threshold. It remains to be determined if these functional SNP group differences may explain some of the metabolic abnormalities reported in patients with idiopathic scoliosis. Further research should explore means of treating or supporting these SNP group impacts on metabolic function.

Keywords: Genomics; Idiopathic; Scoliosis

Introduction

The etiopathogenesis of idiopathic scoliosis has been the focus of many recent genetic studies. Many of these studies only focus on one singular genetic variation. Although the incidence of scoliosis in adolescence is 2-3%,[1] its occurrence in relatives who have idiopathic scoliosis may range from 9% in males to 29% in females, suggesting a distinct genetic influence.[2] For example, studies have examined the LBX1 locus,[3] which is responsible for spinal cord differentiation and somatosensory signaling.[4] Genomic variants causing abnormal spinal ossification (GPR126),[5] craniofacial osteocartilaginous development (BNC2),[6] vitamin D receptor polymorphism (VDR),[7] and leptin receptor polymorphism (LEPR)[8] have also been confirmed. However, methods or treatments to minimize the impact of these genetic variants have not been developed. Zaydman et al[9] identified genomic variants relating to delayed chondrocyte activation on the concave side of vertebral growth plates in scoliosis, leading to asymmetrical growth. Despite this information, proposed treatments or solutions for these genomic variants is lacking.

When metabolic or hormonal signalling is examined in idiopathic scoliosis, many authors have previously observed distinct variations in such functions when compared with non-scoliotics. Estradiol, for instance, has been shown to inhibit chrondrocytes in vertebral growth plates.[10] In addition to these direct effects on chondrogenesis, estrogen receptors seem to be related to circulating levels of leptin, which in turn up-regulates certain estrogen receptors, and down-regulates collagen production.[11] Abnormal leptin bioavailability,[12] and central leptin resistance,[13] have also been implicated in the onset of scoliosis.

While many genetic variants such as those mentioned above have been identified, little is presently known about how to create medical interventions that reduce or eliminate their impact on the development or progression of idiopathic scoliosis. Advances in genomic testing have provided unique insights into many diseases, and its increased accessibility to clinicians has allowed them to apply this information to their management strategies. Ultimately, the ability to apply genomic information into daily clinical practice seems entirely dependent upon the genomic variants examined. For example, Morningstar et al[14] found that nearly 50% of idiopathic scoliosis patients were either A1298C homozygous or C677T/A1298C heterozygous positive for the methylenetetrahydrofolate receptor (MTHFR) variant, compared to about 20% of non-scoliotics. The novelty of identifying this type of genomic variant in idiopathic scoliosis patients is that there have been functional medicine strategies developed in efforts to minimize the impact of this variant on downstream physiology.[15]

In the present study, we evaluated the genomic test results of a cohort of idiopathic scoliosis patients and compared them to the same results in a cohort of non-scoliotics. This genomic testing looked at a series of variants for which functional medicine treatments have previously been created and published.[16]

Materials and Methods

All subjects whose provided their raw data had previously provided online informed consent for the evaluation of their data. This study was granted IRB exemption through IntegReview IRB. Subjects' data were collected according to the following inclusion criteria: 1) Subject had a

history of idiopathic scoliosis, 2) Subject had previously completed a salivary DNA genomic test, and 3) a copy of the subject's raw DNA file was forwarded to the authors for analysis. All subjects had been diagnosed with idiopathic scoliosis by current accepted radiographic standards.[17] An additional set of DNA files were compiled from subjects who did not have a history of idiopathic scoliosis. Many of these DNA files were provided by non-scoliosis family members of the scoliosis group, while the remainder were non-scoliosis patients of the authors (MNS, MWM). These subjects served as the control group. All of the patients in the study were Caucasian, and resided in the United States Midwest and East Coast regions, comprising a total of 6 states. The average of the scoliosis group was 31 years, while the non-scoliosis group was an average of 27 years. Patient samples were collected beginning in 2018 through early 2019. All DNA files were analyzed by an online genomic website, the NutriGenetics Research Institute (**http://www.nutrigeneticresearch.org**). The software evaluated up to 7000 genes, and produced a subject single nucleotide polymorphism (SNP) report on specific genomic variants associated with specific metabolic pathways, such as methylation, transsulfuration, detoxification, and mitochondrial activity. The DNA samples were analysed using the Axiom myDesign Genotyping Array (ThermoFisher Scientific). These resultant SNPs were divided into specific functional genomic groups based on common metabolic function. Patterns of positive homozygous or heterozygous genomic variants were identified and recorded according to their function and loci. These were recorded for both the scoliosis group as well as the control group. Once the analysis was completed, and the report generated, various genetic polymorphisms within specific functional genomic groupings were identified. As an example, rather than to look at just the MTHFR C677T or A1298C SNPs, 3 additional SNPs were included in the same functional grouping. All 5 of these SNPs collectively code for different functional aspects of the folate methylation pathway. A total of 131 such genomic groups were analyzed for both patient groups. Figure 1 illustrates an example of two different SNP groupings, where two are considered positive and the other negative.

Gene Name	Variants	Metrics
BH4 Factors		
CBS C699T (rs234706)	2	AA 11%
MTHFR A1298C (rs1801131)		TT 47.6%
SHMT2 (rs34095989)	1	GA 47%

Gene Name	Variants	Metrics
BH4 Factors		
CBS C699T (rs234706)		GG 45.9%
MTHFR A1298C (rs1801131)	1	TG 42.7%
SHMT2 (rs34095989)	1	GA 47%

Gene Name	Variants	Metrics
BH4 Factors		
CBS C699T (rs234706)		GG 45.9%
MTHFR A1298C (rs1801131)		TT 47.6%
SHMT2 (rs34095989)	1	GA 47%

Figure 1: These are examples of a functional genomic grouping that are positive (top, middle) and negative (bottom).

Results

From the inclusion criteria listed, a total of 93 scoliosis subject DNA files were selected. The non-scoliosis control group was comprised of 115 DNA files. The scoliosis group was comprised of 12 males and 81 females. The control group had 29 males and 86 females.

The average number of individual genes in the 131 functional genomic groups was 7. Since at least 50% of the genes in a given functional genomic group needed to have a positive variant to consider the genomic group positive as a whole, we conducted a power analysis to determine if a sample size of 93 was enough to establish a statistically significant effect size of 14% difference. This difference required a

minimum sample size of 51 patients in each group. Among the 131 total genomic groups evaluated, 19 total groups were significantly more likely to be found in the idiopathic scoliosis group than the control group. All genomic groupings were analyzed in Microsoft Excel using independent t-tests with unequal variance. With a 95% confidence interval, each of the 19 groups attained a P value of <.05. Table 1 shows the 19 genomic groups, along with their respective P value.

Notable SNP differences on Intergroup comparison

SNP Group	Scoliosis (n=198)	Non-Scoliosis (n=208)	P Value
FUT2	132	164	0.002331
HNMT	223	40	0.017452
PANK1	197	84	9.83094E-07
PANK2	134	68	0.000333
PANK3	202	38	0.047161
PANK4	37	12	3.91E-05
FADS1	117	95	0.003992
FADS2	111	96	0.033005
FADS3	159	150	0.032982
SOD1	85	60	0.001746
SOD2	142	131	0.033396
SOD3	166	162	0.030746
PON1	103	73	0.0003344
MTHFR	143	132	0.035155
B12F	153	133	0.002058
MAOA	161	149	0.013888
COMT	144	166	0.039539
BH4	144	82	3.189E-12
VDR	133	116	0.009082

Table 1: The SNP Groups that reached a statistically significant difference at P<.05.

Once the 19 statistically significant functional genomic groups were identified, the scoliosis group was further broken down to see if there was a difference between those with scoliosis below 50°, compared to those with scoliosis between 10-49 degrees. Among the 93 scoliosis patients, 40 had curves below 50°, while the remaining 53 had curves above 50°. Of those above 50°, 29 had a positive past surgical history of spinal fusion for their idiopathic scoliosis. Figure 2 shows an intragroup comparison of the scoliosis group and their respective number of positive functional genomic groups when categorized by curve severity.

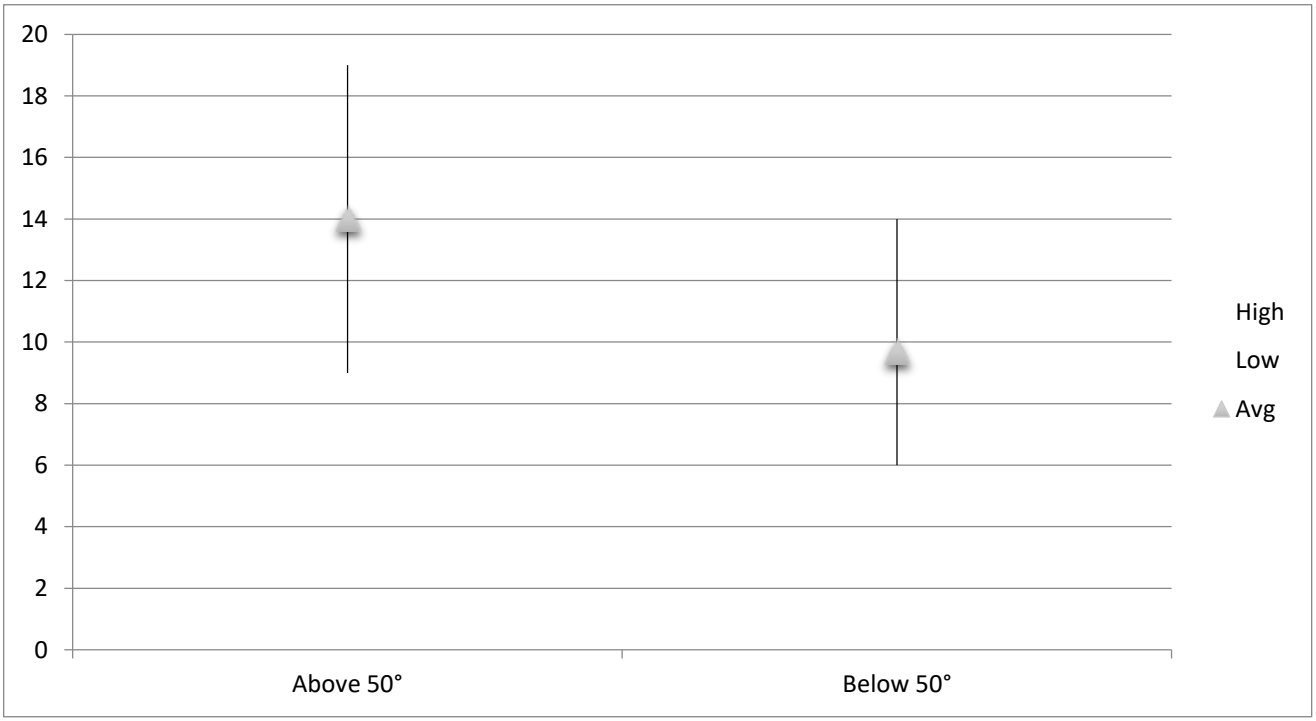

Figure 2: Scoliosis intragroup comparison based on curve magnitude. Difference was statistically different with a P value at 1.558 e^{-12}

Patients with curves below 50° had an average of 9.7 positive functional genomic groups out of the total 19 (range 6-14). Patients whose curves exceeded 50° had an average of 14 positive groups, ranging from 9-19. This intragroup difference was statistically different at P<.001.

Discussion

Although previous studies have identified several genetic variants in scoliosis, it is not clear how these genetic variants directly influence the spinal curvature.[17] Although newer studies have begun investigating the epigenetic influences of certain environmental factors on the DNA methylation of some of these genetic variants,[18,19] it has not yet resulted in a translation to clinical scoliosis management.

The main difference between the present study and previously published studies on scoliosis genetics is that researchers have attempted to create associations or causal relationships to single gene variants. This has led to the discovery of several isolated gene variants in the literature that were singularly linked to scoliosis.

Some of these studies showed different singular genes based on different subject ethnicities.[20]
This is the first study in which specific functional groups of genetic SNPs have been investigated in scoliosis patients. As such, it is unknown if or how the present findings can be compared to previously published scoliosis genetic variant data. The present data is approached from a functional basis. The goal of future studies will be to evaluate the impact on scoliosis occurrence and/or progression in those patients where these functional genomic groups are identified, and subsequent treatment interventions are utilized in the hopes of minimizing the impact of the functional genomic variants on some of the downstream metabolic functions that may or may not govern scoliosis etiopathogenesis.

Conclusions

Caucasian patients with a history of idiopathic scoliosis, when compared with a non-scoliosis Caucasian patient group, displayed a significant number of functional genomic groups with a majority of their respective genes having a single nucleotide polymorphism (SNP). When examining and subcategorizing the scoliosis patient group based upon their curve severity, those with curves below surgery threshold had a lower average number of functional genomic SNPs compared to those above surgery threshold. This research may have implications for predictive genomic testing and/or early metabolic interventions for the identified functional genomic groups.

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