Genetic Pathway Analysis of Adolescent Idiopathic Scoliosis

By
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May 2017



Allegheny College
Department of Biology

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A Senior Comprehensive Project in Partial Fulfillment of the Requirements for a Bachelor of Science Degree from Allegheny College

I hereby recognize and pledge to fulfill my responsibilities as defined in the Honor Code and to maintain the integrity of both myself and the College community as a whole.

_____
Pledge

Approval by the Senior Thesis Committee:

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Dr. Rebecca Dawson

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Dr. Tricia Humphreys

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To Millie,

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ABSTRACT

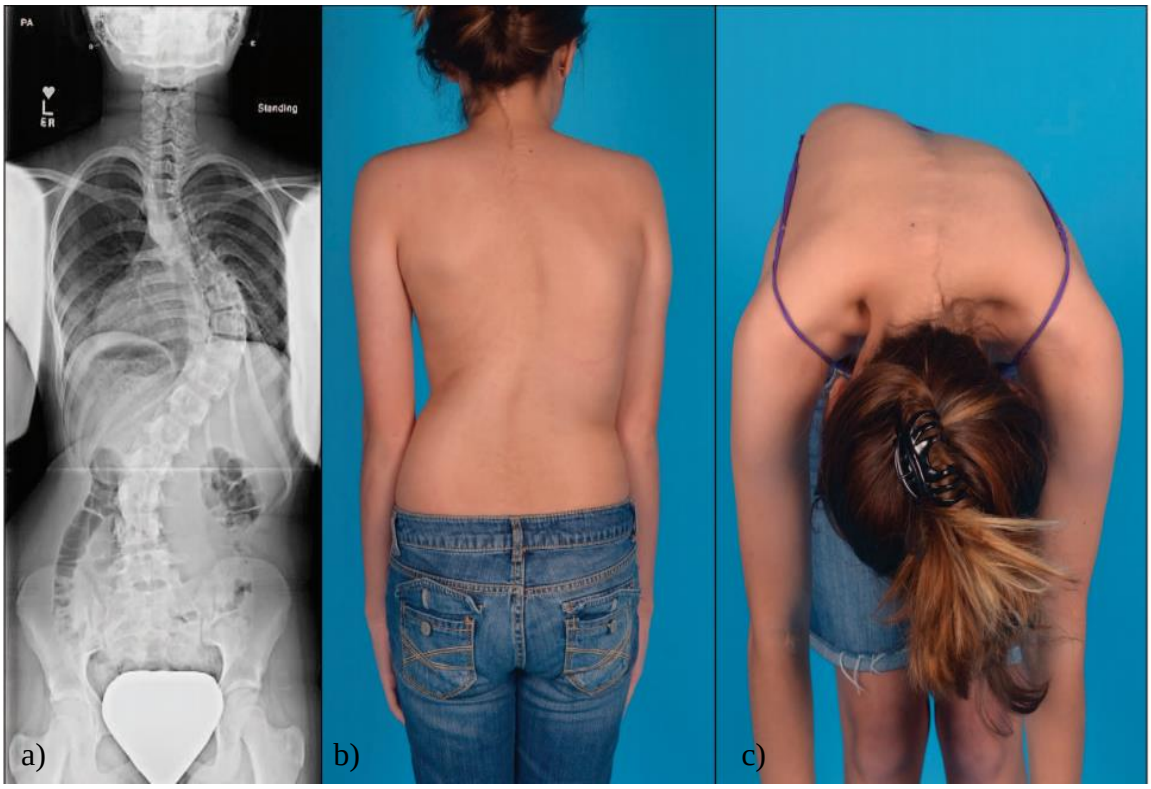
Adolescent idiopathic scoliosis (AIS) is a musculoskeletal disorder that causes a sideways curve of the spine. The etiology of AIS is currently unknown, but there are numerous genes and single nucleotide polymorphisms (SNPs) that have been associated with AIS in recent genome-wide association studies (GWAS) and gene association studies. The purpose of this project is to investigate the potential that gene participants of a biomolecular pathway are overrepresented within the list of statistically significant genes and that variations to genes in this pathway are the underlying cause of AIS. The traditional method of pathway analysis where a systematic review of publications presenting statistically significant SNPs that are associated with genes was conducted. In addition, a novel method named "intra-GWAS pathway analysis", which uses a list of statistically significant SNPs associated with genes from within a particular publication, was utilized. This study found multiple overrepresented pathways in the gene list generated by the traditional method of pathway analysis, but failed to find overrepresented pathways using the intra-GWAS pathway analysis. Pathways pertaining to endocrine resistance, pancreatic cancer, endochondral ossification, senescence and autophagy in cancer, stem cell differentiation, spinal cord injury response, cardiac hypertrophy, and lung fibrosis were found to be overrepresented in the population of statistically significant genes. This project was successful in identifying many candidate pathways for further investigation.

Keywords: Adolescent Idiopathic Scoliosis, pathway analysis, intra-GWAS pathway analysis, genome-wide association study, genetics

INTRODUCTION

Adolescent Idiopathic Scoliosis (AIS)

Scoliosis is a musculoskeletal disorder characterized by a sideways curve of the spine. Scoliosis is defined as a spinal curve of at least a 10° Cobb angle (Lazar, Martins, and Wajchenberg, 2015). Moderate curves include 25°-35° Cobb angles, while severe curves include Cobb angles greater than 45° (Akoume et al., 2013). Cobb angles are measured using standing spinal radiographs (Paria and Wise, 2016).



a) Lateral radiograph of a right thoracic spinal curve b) Patient with a right thoracic spinal curve c) Patient with a rib hump evident in a forward bent position

Scoliosis may be caused by a number of diseases and disorders including but not limited to cerebral palsy, muscular dystrophy, and poliomyelitis. However, adolescent

idiopathic scoliosis (AIS) occurs from an unknown cause in otherwise healthy pre-adolescents, especially females (Vital, 2015). It is typically manifested during developmental stages characterized by rapid growth and development, such as infancy or the onset of adolescence (Gao et al., 2008). Many elementary schools now screen for AIS in order to detect the disorder early. Early detection can open many preventative treatment opportunities such as the use of a scoliosis brace (Gao et al., 2008). AIS affects approximately 2-3% of the pediatric population worldwide (Paria and Wise, 2016).

AIS has the potential to cause many health issues including lung and heart damage, back pain, and spinal osteoarthritis (Mayo Clinic Staff, 2016; Gao et al., 2008). While less severe spinal curves generally stop progressing after the full development of the child's skeletal structure, more severe curves can continue to progress in adults, further escalating the patient's symptoms (Mayo Clinic Staff, 2016).

Treatments and Risk Factors

One out of every six children diagnosed with AIS will require treatment. The total estimated hospital cost for treating AIS in the United States was $2.7 billion in 2010. This expense is attributed to a variety of treatments that have been largely unchanged over the past few decades (Akoume et al., 2013). Modern treatments for AIS include the use of scoliosis braces, which prevent further progression, and surgery, which is used in more extreme cases to both reduce severity and prevent progression. Alternative available treatment methods include chiropractic manipulation and muscle electrical stimulation (Mayo Clinic Staff, 2016).

The most common and predictive risk factors of AIS are gender and age. While both young males and young females have comparable rates of AIS, females are more likely to present a spinal curve that progresses in severity. All ethnic populations are affected by AIS, although population percentages vary due to population stratification (Gao et al., 2008; Konieczy, Krauspe, and Senyurt, 2013).

The lack of understanding of the etiology of AIS is partially due to the lack of an animal model that can accurately simulate a developing human spinal column. Multi-genic influence on AIS development also complicates etiology. However, recent progress in molecular biology and genetics has opened new avenues into studying the genetic influences on AIS (Paria and Wise, 2016).

AIS is a Complex Disorder

Heritability of AIS is estimated to be 38%, which strongly indicates that genetics play an important role in both its development and progression (Riseborough and Wynne-Davies, 1973; Gerdhem, Grauers, and Rahman, 2011). AIS tends to be inherited, but it is a complex disorder and is unable to be accounted for by Mendelian inheritance (Riseborough and Wynne-Davies, 1973; Gao et al., 2008; Brandon et al., 2011; Bangping et al., 2014; Kesling and Reinker, 1997). Twin studies and genetic testing comparing AIS severity between monozygotic and dizygotic twins were historically popular in determining the underlying genetic, environmental, and epigenetic factors influencing AIS presence and severity (Lazar, Martins, and Wajchenberg, 2015). The field of genetic epidemiology is evolving to include conducting pathway analyses of genome-wide association studies (GWAS) in order to better understand the complex etiology of AIS.

There are many association studies, including GWAS, that have identified polymorphic genes with alleles that are statistically correlated with AIS (Appendix #2). These genes have the potential to be analyzed with pathway analysis in order to further understand their overall contribution to the development of AIS.

Genome-Wide Association Studies (GWAS)

GWAS are genetic epidemiological studies that aim to identify associations between the presence or absence of a disease or continuous trait and single nucleotide polymorphisms (SNPs), also known as "variants", within the genome. These associations are grounded in the Common Disease Common Variant (CDCV) hypothesis, which hypothesizes that complex diseases are caused by common variants. Common variants are more frequently present in the genome but are hypothetically less influential on the phenotype than rare variants. However, there is the potential that many common variants collectively have a large effect on the phenotype (Manolio, 2013).

From 2005 to 2013, GWAS have identified nearly 2,000 associations between genes and complex diseases. This technique is relatively new but is effective in identifying statistical consistencies in the genomes of those suffering from a variety of diseases (Manolio, 2013).

There has been an abundance of GWAS conducted on patients with AIS (Appendix #2). However, none of them have discovered the cause of AIS or produced reliably predictive genotypes of individuals at risk of developing AIS. It has been noted that further genetic research can create insights into AIS disease pathways and open

opportunities to develop pharmaceuticals that could function in early intervention and prevention of AIS (Paria and Wise, 2015).

Pathway Studies

GWAS are limited in many aspects, including the modestly low predictive nature of the individual SNPs that are associated with the disease in question (Manolio, 2013). They also do not take into account how genes function within biological networks. Pathway analyses are statistical analyses where gene lists involved in biological pathways are tested for significant relationships with a particular phenotype (Moore et al., 2012). Pathway analyses have the potential to aggregate statistically significant SNPs from multiple GWAS in order to identify specific biomolecular pathways that involve a statistically higher number of genes whose SNPs the GWAS find significant than one would expect by chance. A statistically significant pathway is considered enriched. This pathway gives insight into the underlying biochemical processes that affect the development of the trait in question. This creates a clearer connection between the genotype and the phenotype of an individual, while SNPs identified using GWAS are generally not influential enough to form this connection on their own (Moore et al., 2012).

Novel Method for Pathway Analysis: Intra-GWAS Pathway Analysis

After preliminary research of AIS was conducted, it was noted that a substantial number of publications identify the same significant SNPs despite the vast number of publications on the genetics of AIS. Many GWAS ignore the long lists of SNPs that are

statistically significant but do not meet their often strenuous threshold. Butte, Khatri, and Sirota (2012) support this assertion, describing the information loss when marginally less significant genes are not considered (Butte, Khatri, and Sirota, 2012). The high standard for statistical significance helps to identify lone SNPs in search for point sources of genetic influence on AIS development. The high standard helps to create a threshold that eliminates much of what many perceive as the variable 'static' of many, less significant SNPs in the genome. For instance, Cheng et al. (2015) used $p < 5.0 \times 10^{-8}$ as its cutoff for genome-wide significance and concluded that only four gene-representative SNPs are significant. However, they also identified 43 SNPs that surpassed $p < 1.0 \times 10^{-5}$, which was their threshold for suggestive whole-genome significance (Cheng et al., 2015).

It appears that researchers are only publishing the most significant SNPs and are ignoring the small effect alleles that are also present in the data. However, pathway analyses are designed to present an overrepresentation of a biomolecular pathway within a list of genes that are significant. Therefore, it would be wise to utilize a lower threshold of statistical significance, accumulate a larger number of SNPs that would otherwise not be published due to the genetic epidemiology community's relatively stringent precedent of statistical significance, and then perform a pathway analysis of data from within a single study. Another benefit of conducting pathway analysis in this fashion is that it eliminates the need to control many of the variables that could confound results when comparing different populations. Data from one study would not have to consider genetic or environmental differences between many populations since one study usually only acquires participants from a single population. Using SNPs from many different populations can cause issues in this respect.

This pathway analysis method is named the "intra-GWAS pathway analysis". This research project will be the first to conduct an intra-GWAS pathway analyses, according to the current genetic epidemiological literature.

Objective

The objective of this project is to analyze published GWAS studies on AIS and to conduct multiple pathway analyses on statistically significant SNPs in pursuit of identifying a potential biomolecular pathway that is involved in the development of AIS. If a biomolecular pathway is identified, then it will be analyzed in the context of AIS development and will be flagged as a potential subject of future AIS development research. These genetic pathways have the potential to be involved in the fundamental cause of AIS, and their identification could drastically influence the etiology, future research questions, and medical intervention of AIS.

METHODS

WEB-based GEne SeT AnaLysis Toolkit (WebGestalt) - a Pathway Analysis Program

In order to identify enriched pathways represented by a particular gene list, the program WEB-based GEne SeT AnaLysis Toolkit (WebGestalt) was utilized. WebGestalt identifies the pathways that each gene in the gene list is a part of and then determines if any of those pathways are represented within the gene list more than one would expect by chance. If so, then that pathway is identified as enriched, and further analysis could be conducted to investigate if that genetic pathway affects the development of AIS (Kirov, Snoddy, and Zhang, 2005).

WebGestalt utilizes the Hypergeometric Test and the Fisher's Exact Test to identify enriched pathways. The Kyoto Encyclopedia of Genes and Genomes (KEGG) Table displays the number of genes in each pathway and the false discovery rate adjusted *p* value for the enrichment of each pathway (Kirov, Snoddy, and Zhang, 2005).

First presented in 2005, WebGestalt was one of the original applications that integrated many different forms of analysis and steps to organize data pertaining to large groups of genes. From 2005 to 2013, WebGestalt has acquired more than 400 citations, as reported by Google Scholar (Duncan et al., 2013). There are four main modules that WebGestalt encompasses: gene set management, information retrieval, organization and visualization, and statistics (Kirov, Snoddy, and Zhang, 2005).

Methods for Using WebGestalt

Each gene list was analyzed with WebGestalt using the following parameters and statistical tests:

Parameter	**Selection**
Organism of Interest	hsapiens
Method of Interest	Overrepresentation Enrichment Analysis
Functional Database	pathway
Functional Database Name	KEGG, Panther, Reactome, or Wikipathway
Gene ID Type	genesymbol
Reference Set for Enrichment Analysis	genome_protein_coding
Minimum Number of Genes for a Category	2
Maximum Number of Genes for a Category	2,000
Multiple Test Adjustment	Bonferroni
Significance Level	Top 10
Number of categories visualized in the report	40

Paper Candidates for Intra-GWAS Pathway Analysis

Since conducting a GWAS is outside of the resources and time capability of this project, it was important to find previous GWAS that publish long lists of SNPs that are significant relative to a lower threshold. Cheng et al. (2015), Ward et al. (2010), and Ogura et al. (2015) meet this requirement, so they were used as data sources to conduct intra-GWAS pathway analyses.

Cheng et al. (2015) describes a GWAS of AIS in a female Chinese Han population. In the first stage, or the discovery stage, 43 SNPs were found to surpass $p <$

1.0×10^{-5}, which they identified as the threshold of suggestive whole genome significance. The researchers chose a representative SNP from each haplotype block, totaling 16 SNPs, based on linkage disequilibrium between SNPs in the haplotype blocks. In the replication stages, the researchers further genotyped patients and controls for only those 16 SNPs while utilizing $p < 5.0 \times 10^{-8}$ as a higher threshold of whole-genome significance. The four SNPs whose $p < 5.0 \times 10^{-8}$ were then concluded to be the significant SNPs found in the study. They were further analyzed for their roles in musculoskeletal development and their potential contribution to AIS (Cheng et al., 2015).

Ogura et al. (2015) is an extension of a previously conducted GWAS that identified susceptibility loci in genes LBX1 and GPR126 (Takahashi et al., 2011; Kou et al., 2013). Ogura et al. (2015) increased the number of cohorts in the study, expanded the GWAS to a whole-genome imputation, and conducted replication studies of Japanese and Chinese populations. Ogura et al. (2015) identified significant SNPs in intron 3 of BNC2 (Ogura et al., 2015).

In a similar fashion as Cheng et al. (2015), Ogura et al. (2015) utilized its Supplementary Table 2 (Table S2) to list SNPs from the GWAS with $p < 1.0 \times 10^{-5}$. Both Table S2 of Ogura et al. (2015) and Supplementary Tables 2 and 3 of Cheng et al. (2015) identified SNPs whose $p < 1.0 \times 10^{-5}$ (Ogura et al., 2015). This format of publication matches the requirements of intra-GWAS pathway analysis, assuming that the researcher also does not have the means to conduct their own GWAS.

Ward et al. (2010) created a prognostic test that claims to predict spinal curve progression in Caucasian AIS patients. This test uses 53 SNPs that they claim to be significant from their GWAS. However, the GWAS cited in Ward et al. (2010) has not

yet been published. Considering that it has been seven years since the Ward et al. (2010) publication, it is very unlikely that this GWAS will be published in the future.

Since the Ward et al. (2010) publication, there have been three replication studies that explore the significance of the 53 SNPs (Ward et al., 2010). Of the 53 SNPs identified by Ward et al. (2010), the first two replication studies found none of them to be significantly associated with AIS or spinal curve progression of AIS patients (Ogura et al., 2013; Tang et al., 2015). The most recent replication study found only four of the 53 SNPs to be significant (Xu et al., 2015). These replication studies are far from close to finding 53 significant SNPs, so it is likely that Ward et al. (2010) used a lower threshold for statistical significance. However, the intra-GWAS pathway analysis allows for a lower statistical threshold, so it is likely that this list of SNPs can be adequately utilized in an intra-GWAS pathway analysis. Therefore, regardless of the potential credibility of the list of 53 SNPs, or lack thereof, it will be used to explore potentially significant pathways.

Gene Lists from Cheng et al. (2015), Ogura et al. (2015), and Ward et al.(2010)

In order to limit variability of control factors and genetics of the study populations, statistically significant SNPs were retrieved from within each publication. These SNPs were analyzed in the National Center for Biotechnology Information (NCBI) in order to identify which gene they are most associated with, whether they are within a gene's coding region of if they are in close proximity to a particular gene (Database resources of the National Center for Biotechnology Information, 2016). The gene lists were then analyzed using the methods described in *Methods for using WebGestalt*.

Traditional Pathway Analysis

A pathway analysis that uses statistically significant SNPs from multiple research projects was conducted in addition to the intra-GWAS pathway analysis. A systematic review of previous publications of GWAS and gene variant association studies regarding AIS in the genetic epidemiological literature was utilized in order to create a gene list from data among previous GWAS publications. Google Scholar searches were performed for keywords "genome-wide association study", "GWAS" or "genetic association" in conjunction with "adolescent idiopathic scoliosis", "AIS", or "scoliosis". There was no time of publication excluded from the search because genetic epidemiology is a relatively new field and the fundamental methods of executing GWAS and gene variant association studies have remained relatively constant throughout their continued utilization. Any study matching these criteria was utilized if it had a sufficiently high study population and was published in a reputable journal.

Creating a Cumulative Gene List from Intra-GWAS Data and Data from Among GWAS and Gene Variant Association Studies

Genes lists from within Cheng et al. (2015), Ogura et al. (2015) and Ward et al. (2010) were combined with the gene list created from among GWAS and gene variant association studies in order to create a final list of genes that have been found to be statistically significant in at least one population. This method ignores variables including

but not limited to restrictions on Cobb angle, ethnicity, age, or gender of study participants.

Combinations and Divisions of Gene Lists for Pathway Analysis

Gene lists from each of the preceding methods sections were combined and divided in the following manner:

- Gene List 1 – Cheng et al. (2015) – all genes associated with significant SNPs
- Gene List 2 – Cheng et al. (2015) – only genes associated with significant SNPs within their coding regions
- Gene List 3 – Cheng et al. (2015) – only genes associated with significant SNPs within their coding regions or within 100,000 base pairs of the SNP
- Gene List 4 – Ogura et al. (2015) – all genes associated with significant SNPs
- Gene List 5 – Ogura et al. (2015) – only genes associated with significant SNPs within their coding regions
- Gene List 6 – Ogura et al. (2015) – only genes associated with significant SNPs within their coding regions or within 100,000 base pairs of the SNP
- Gene List 7 – Ward et al. (2010) – all genes associated with significant SNPs
- Gene List 8 – Cheng et al. (2015), Ogura et al. (2015), Ward et al. (2010) – all genes associated with significant SNPs
- Gene List 9 – Among GWAS and association studies – all genes associated with significant SNPs

- Gene List 10 – Cheng et al. (2015), Ogura et al. (2015), Ward et al. (2010), Among GWAS and association studies – all genes associated with significant SNPs

Pathways were then identified as significant if both their *p* value and false discovery rate corrected *p* value (FDR) were below 0.05. These pathways are displayed in the RESULTS section and are analyzed for potential involvement in the development of AIS in the DISCUSSION section.

RESULTS

Gene Lists 1-8 did not identify any significant pathways. However, Gene Lists 9 and 10 identified multiple significant pathways. These genetic pathways highlight a variety of biochemical mechanisms and diseases that AIS may be related to including cancer, the endocrine system, lung and heart stress response and repair, stem cell differentiation, and skeletal tissue growth, development, and repair. The charts below present statistical information for each identified pathway.

Gene List 9:

Pathways identified using the Kegg database:

ID:hsa01522 Name: Endocrine Resistance		
C=98; O=4; E=0.22; R=17.8; PValue=5.85e-05; FDR=1.81e-02		
Gene Symbol	Gene Name	Entrez Gene
ESR1	estrogen receptor 1	2099
ESR2	estrogen receptor 2	2100
IGF1	insulin like growth factor 1	3479
BCL2	BCL2, apoptosis regulator	596

Pathways identified using the Wikipathways database:

ID:WP2377 Name: Integrated Pancreatic Cancer Pathway		
C=191; O=6; E=0.65; R=9.3; PValue=2.41e-05; FDR=8.38e-03		
Gene Symbol	Gene Name	Entrez Gene
ESR1	estrogen receptor 1	2099
ESR2	estrogen receptor 2	2100
IGF1	insulin like growth factor 1	3479
NTF3	neurotrophin 3	4908
BCL2	BCL2, apoptosis regulator	596
TGFB1	transforming growth factor beta 1	7040

ID:WP474 Name: Endochondral Ossification		
C=64; O=4; E=0.22; R=18.51; PValue=5e-05; FDR=8.69e-03		
Gene Symbol	Gene Name	Entrez Gene
IGF1	insulin like growth factor 1	3479
SOX9	SRY-box 9	6662
TGFB1	transforming growth factor beta 1	7040
CALM1	calmodulin 1	801

ID:WP615 Name: Senescence and Autophagy in Cancer		
C=105; O=4; E=0.35; R=11.28; PValue=3.45e-04; FDR=3.75e-02		
Gene Symbol	Gene Name	Entrez Gene
IGF1	insulin like growth factor 1	3479
IL6	interleukin 6	3569
BCL2	BCL2, apoptosis regulator	596
TGFB1	transforming growth factor beta 1	7040

ID:WP2848 Name: Differentiation Pathway		
C=48; O=3; E=0.16; R=18.51; PValue=5.04e-04; FDR=3.75e-02		
Gene Symbol	Gene Name	Entrez Gene
IGF1	insulin like growth factor 1	3479
IL6	interleukin 6	3569
TGFB1	transforming growth factor beta 1	7040

ID:WP2431 Name: Spinal Cord Injury		
C=118; O=4; E=0.4; R=10.04; PValue=5.39e-04; FDR=3.75e-02		
Gene Symbol	Gene Name	Entrez Gene
EPHA4	EPH receptor A4	2043

IL6	interleukin 6	3569
SOX9	SRY-box 9	6662
TGFB1	transforming growth factor beta 1	7040

ID:WP2795 Name: Cardiac Hypertrophic Response		
C=55; O=3; E=0.19; R=16.15; PValue=7.53e-04; FDR=4.07e-02		
Gene Symbol	Gene Name	Entrez Gene
IGF1	insulin like growth factor 1	3479
TGFB1	transforming growth factor beta 1	7040
CALM1	calmodulin 1	801

ID:WP3624 Name: Lung Fibrosis		
C=63; O=3; E=0.21; R=14.1; PValue=1.12e-03; FDR=4.88e-02		
Gene Symbol	Gene Name	Entrez Gene
IGF1	insulin like growth factor 1	3479
IL6	interleukin 6	3569
TGFB1	transforming growth factor beta 1	7040

Gene List 10:

Pathways identified using the Wikipathways database:

ID:WP474 Name: Endochondral Ossification		
C=64; O=5; E=0.51; R=9.83; PValue=1.32e-04; FDR=4.61e-02		
Gene Symbol	Gene Name	Entrez Gene
IGF1	insulin like growth factor 1	3479
SLC38A2	solute carrier family 38 member 2	54407
SOX9	SRY-box 9	6662
TGFB1	transforming growth factor beta 1	7040
CALM1	calmodulin 1	801

Identification Symbols: ID: identification number for the pathway; Name: name of the pathway; Gene Symbol: Human Genome Organization (HUGO) Gene Nomenclature Committee approved gene symbol; Gene Name: Human Genome Organization (HUGO) Gene Nomenclature Committee approved gene name; Entrez Gene: National Center for Biotechnology Information (NCBI) GeneID number.

Statistical Symbols: C: number of reference genes in the category; O: number of genes in the user gene list and also in the category; E: expected number in the category;

R: ratio of enrichment; PValue: *p* value for hypergeometric test; FDR: false discovery rate adjusted *p* value using the Bonferroni correction.

DISCUSSION

Key Findings

Pathway analysis identified multiple significant genetic pathways to consider for further analysis. These pathways include biochemical interactions involving cancer, the endocrine system, lung and heart stress response and repair, stem cell differentiation, and skeletal tissue growth, development, and repair. While significant SNPs have been identified within these genetic pathways, this study emphasizes the need to further analyze these genetic pathways in search for an underlying cause of AIS. While this study did not identify a single significant pathway, it does identify multiple candidates for further analysis and outlines suggested research topics that future researchers can utilize in order to build upon the findings of this study.

Potential Future Research Questions

There are a variety of potential research questions that may be investigated in regard to the many statistically significant pathways identified in this project. Future researchers may perform genetic variation analysis on the genes involved in any of the pathways in search of potentially expanding the list of genes within a pathway that is associated with AIS. It is also important to note that conducting one's own GWAS would

allow a research team to have more freedom in selecting the threshold of gene significance and the SNPs that undergo sequencing replication analysis.

Future researchers may also consider that many pathways are involved in the development of cancer. This could suggest the need to reevaluate past association studies that have associated AIS patient thoracic diagnostic X-ray procedures with the development of cancer by considering a potential genetic factor instead (Ronckers et al., 2010; Levy et al., 1994). There are also overexpression and knockout methods available using a model organism such as a teleost that could investigate the roles of genes within a pathway or the pathway as a whole (Gorman and Breden, 2007). Researchers who are investigating the following genetic pathways should take these suggestions into consideration and should consider the information provided in the following pathway descriptions.

Endocrine Resistance Pathway

This pathway describes the biological mechanisms behind resistance to the treatment of hormonal-responsive breast cancer. Resistance to hormone treatments such as selective estrogen receptor modulators, estrogen synthesis inhibitors, and selective estrogen down-regulators negatively affects the responsiveness of a patient to hormone therapy when suffering from this disease (Kanesha Laboratories, 2016). Over 75% of breast cancers express Estrogen Receptor α and heightened expression of Estrogen Receptor β is associated with increased resistance to tamoxifen, a hormone-responsive breast cancer treatment (Miller, 2017; Speirs et al., 1999). Problems in regulating and responding to hormones could be the underlying cause of AIS. This pathway and other

pathways involving the endocrine system warrant future investigation. Genes from Gene List 9 included in the Endocrine Resistance pathway include ESR1, ESR2, IGF1, and BCL2.

Females with AIS are more likely than males to have a spinal curve that progresses in severity (Gao et al., 2008). This may be an indication that a manifestation of problems in estrogen regulation is an underlying cause of AIS since females tend to have higher levels of estrogen than males (Ashbee and Goldberg, 2006). Genes involved in estrogen, such as ESR1 and ESR2, should be further investigated.

IGF1 is heavily involved in growth and development. IGF1 is a growth hormone that is secreted mainly by the liver. However, it is also secreted by cartilaginous cells and acts as a paracrine hormone in this manner (Nilsson et al., 1986; Laron, 2001). IGF1 involvement in cartilaginous development may play a role in the development of AIS during times of rapid growth and development of the spinal column. Problems in responses to this protein could also be involved in other endocrine system issues.

BCL2 is a gene that influences apoptosis of cells and is essential for development, homeostasis, and immunity (Czabotar et al., 2014). Problems with BCL2 expression may lead to misregulation of apoptosis during spinal column development and the development of AIS as a result.

In conclusion, genes and hormones involved in the endocrine system, whose inability to function correctly is often related to the resistance to treatments for hormone-responsive breast cancer, may play a role in the development of AIS during life stages of highly regulated growth and development. While many studies have analyzed the relationships between AIS and various hormones that are involved in the endocrine

system (Tang et al., 2006; Karasugi et al., 2011; Wu et al., 2006; Zhang et al., 2009; Janusz et al., 2013; Kotwicki et al., 2014; Qiu et al., 2009), these studies focus specifically on estrogen and largely ignore the relationship between AIS and the endocrine system as a whole. Studying the various gene participants of this pathway and analyzing their potential relationship to the development of AIS would provide insight into AIS etiology.

Integrated Pancreatic Cancer Pathway

This pathway describes protein interactions relevant to the development of Pancreatic Cancer (Integrated Pancreatic Cancer Pathway WP2377, 2017). The genes within this pathway that are associated with AIS, including ESR1, ESR2, IGF1, NTF3, BCL2, and TGFB1, all have a role in development. ESR1, ESR2, BCL2, and IGF1 were described in *Endocrine Resistance Pathway*. NTF3 is involved in the differentiation of neurons and is related to a nerve growth factor and a similar brain-derived neurotrophic factor (NTF3 neurotrophin 3 Gene, 2017). TGFB1 is a growth factor that is involved in gene regulation, proliferation, and differentiation of many cell types, especially in bone remodeling and osteoblast stimulation (TGFB1 Gene, 2017).

No study has attempted to identify an association between the development of AIS and Pancreatic Cancer. It would be interesting to test for an association between AIS and Pancreatic Cancer and to test for similarities and differences regarding variations in the genes within the Integrated Pancreatic Cancer pathway between patients with AIS, Pancreatic Cancer, both, and neither.

Senescence and Autophagy in Cancer Pathway

Senescence of cells describes the state of cell cycle arrest where cellular division is stalled. This is often due to stresses such as toxins, DNA damage, or oncogene activation (Pathway Ontology - Cellular Senescence Pathway, 2017). Autophagy of cells describes the degradation process of proteins and organelles within a cell. It describes the process by which these cellular parts are delivered to the lysosome for degradation (Pathway Ontology – Autophagy Pathway, 2017).

Again, IGF1, BCL2, and TGFB1 are identified within this pathway and are all involved in growth and development of the spinal column. IL6 is a protein that is involved in inflammation regulation, immunity, and maintenance of bone homeostasis (IL6 interleukin 6 (*Homo sapiens*), 2017; Dikova et al., 2015). Again, it appears that genes involved in growth and development, which are often associated with problems in growth and development that eventually cause various forms of cancer, may have a role in the development of AIS. This pathway highlights another potential link between the development of AIS and cancer.

Differentiation Pathway

Genes in the Differentiation Pathway are involved in the differentiation of stem cells into specialized cells throughout the body. IGF1 is involved in the development of insulin-secreting beta cells from Pancreatic progenitors, cells involved in the Immune System, and otic hair cells in the inner ear. IL6 is involved in the development of erythropoietic cells, lymphoid progenitors, and osteoclasts. TGFB1 inhibits the

development of multipotent lung progenitors and endothelial cells (Salomonis et al., 2012).

The Differentiation Pathway is a viable topic of interest for future researchers, as the development of osteoclasts, osteoblasts, and the many other cells that influence the development of the spinal column may have a role in the development of AIS.

Spinal Cord Injury Pathway

This pathway outlines the cells, drugs, and genes involved in the response to trauma inflicted on the spinal cord. This involves immune system responses such as inflammation, excitotoxicity, cell death, the formation of glial scarring, and suppression of axonal regeneration (Kutmon and Rieswijk, 2016).

Significant genes involved in this pathway include EPHA4, IL6, SOX9, and TGFB1. Within the Spinal Cord Injury pathway, EPHA4 is involved in the assembly of focal adhesions, which are macromolecular assemblies that function to mechanically link a cell to its extracellular matrix and help to regulate signals with adjacent cells. IL6 an inflammation-promoting factor. SOX9 is involved in glial scar formation, promotion of genes involved in neurofilament formation, promotion of genes that produce Chondroitin Sulfate Proteoglycans, and promotion of genes that inhibit axonal growth. TGFB1 is one of many proteins that activates Macrophages (Kutmon and Rieswijk, 2016).

This is another pathway that includes genes that are involved in regulating biomolecular interactions within the spinal column. While this particular pathway pertains to repair after an injury, the genes involved are also involved in pathways that pertain to spinal column development. Also, there is the possibility that damage to the

spinal column and misregulation of its healing during rapid growth and development may result in the development of AIS. A study conducted on rabbits supports the notion that spinal cord injury and loss of nerve function can induce the development of scoliosis, so early and undetected spinal cord damage may play a role in the later development of AIS (Barrios et al., 1987).

Cardiac Hypertrophic Response Pathway

Cardiac Hypertrophic Response describes the biological response to physiological or pathological stimuli that results in an enlargement of individual myocytes, causing an enlargement of the heart. IGF1 and TGFB1 are growth factors that are involved in numerous biological processes. CALM1 is a gene that encodes a calcium-binding protein that mediates many enzymes, aquaporins, and ion channels (CALM1 – Calmodulin – Homo sapiens, 2017). Genes involved in many biological processes, including Cardiac Hypertrophic Response, may be involved in the development of AIS.

Lung Fibrosis Pathway

Lung Fibrosis, or Pulmonary Fibrosis, is a disease that is caused by the excessive scarring of lung tissue. The scar tissue thickens the lung tissue, making breathing more difficult (MAYO Clinic Staff, 2017). This pathway describes the response to lung damage and includes many immune system and repair processes. IGF1 and TGFB1 are growth factors, while IL6 is an inflammation agent (Fehrhart et al., 2017). These are genes that are involved in the response to trauma and the eventual repair of many tissue

types, so it is possible that their involvement in lung tissue repair may mirror their involvement in the response to and repair of damaged tissue within the spinal column.

Endochondral Ossification Pathway

The Endochondral Ossification Pathway was enriched in both Gene Lists 9 and 10. The pathway describes genes involved in the replacement of cartilaginous tissue by bone tissue. Regulation of this process includes growth hormones, thyroid hormones, and transcription factors, among other molecules (Eijssen et al., 2016). This process is very prominent during rapid growth and development of the skeleton when much of the cartilaginous tissue is replaced by bone (Gilbert, 2000).

The significant genes involved in this pathway include IGF1, SLC38A2, SOX9, TGFB1, and CALM1. IGF1 promotes the proliferation of chondrocytes. Chondrocytes create the bone's extracellular matrix, die, and then are replaced by blood vessels, osteoclasts, osteoblasts, and bone marrow cells that build on the cartilaginous matrix formed by the chondrocytes and eventually convert it into bone (Eijssen et al., 2016). SLC38A2 is a sodium-dependent amino acid transporter that is involved in supplying amino acids to cells during bone formation (Eijssen et al., 2016; SLC36A2 Gene, 2017). SOX9 promotes proliferation of chondrocytes while it inhibits chondrocyte hypertrophy. Thus, it promotes the creation of cartilaginous tissue (Eijssen et al., 2016). TGFB1 is a growth factor that regulates cell proliferation, differentiation, and growth. It also regulates other growth factors (TGFB1 Gene, 2017). TGFB1 also promotes chondrocyte proliferation (Eijssen et al., 2016). CALM1 is a gene that encodes a calcium-binding protein that mediates many enzymes, aquaporins, and ion channels (CALM1 –

Calmodulin – Homo sapiens, 2017). Its upregulation has been shown to be associated with osteoarthritis of the hip in Japanese patients, so it may also be involved in structural issues of the spinal column (Mototani et al., 2005). The pertinence of this pathway to spinal column development makes it a promising subject for future research.

Efficacy of Methods and Project Limitations

While the traditional methods of pathway analysis produced a gene list long enough to have genes within the list that inhabited the same pathways, intra-GWAS pathway analysis, under the limitations of the inability to collect one's own GWAS data, seems to struggle to produce gene lists long enough to adequately conduct a pathway analysis. Gene Lists 1-8 did not produce any statistically significant pathways and the pathways that were presented rarely had more than one or two genes represented from the gene list within them.

The most statistically significant pathways came from Gene List 9, which included all statistically significant genes from the traditional pathway analysis. Thus, it would be optimal for future researchers to conduct an intra-GWAS pathway analysis using their own genomic data in order to have control over the threshold of inclusion in the gene list, best maintain gene list length, and allow for confounding variable control. While this method was not within the capacity of this project, this project was able to identify the limitations of using other publications' data in respect to the traditional pathway analysis and the intra-GWAS pathway analysis. Future researchers should take the methods presented in this project into consideration when conducting their own

GWAS and pathway analyses. While it is still unclear whether intra-GWAS methods are more insightful than traditional GWAS methods, this project was successful in generating a model by which to conduct future intra-GWAS pathway analyses.

In regard to the fundamental limitations of genetic analysis and in the context of the biomolecular community's current understanding of the Central Dogma of molecular biology, there are many limitations in understanding the influence of SNPs and DNA as a whole on phenotype. Many processes involved in gene regulation and expression remain unclear, so it does not suffice to distinguish with certainty that an SNP close to a particular gene actually influences said gene. It also does not suffice to distinguish with certainty that an SNP far from a particular gene does not influence said gene. Making these distinctions would require adequate experimental data. Thus, many inferences are made in the molecular biology literature, including this project, regarding the connection between a particular SNP and the gene that it is a part of or closest to. Regulatory loci for a particular gene can be located throughout the genome, so the link or lack thereof between a particular SNP and a gene, without adequate experimental data, is inferred and based on the increased probability that SNPs closer to a gene are more likely to be in its promoter region or a regulatory region (Maston, Evans, and Green, 2006).

While genetic processes may be the fundamental cause of the development of AIS, the possibility that AIS may also be influenced by environmental factors that affect gene mutation, biomechanical mechanisms, or variations of growth and development remains. The need to investigate environmental influences, especially prior to or during periods of rapid growth and development, is still profound in the genetic epidemiology community's search for the cause of AIS.

Statistically, the Hypergeometric Distribution Test used in a pathway analysis does not take differences between magnitudes of statistical significance of genes into consideration. This sacrifice of information was not especially important in the intra-GWAS pathway analyses because the gene lists were so short to begin with. The sacrifice was necessary for the traditional pathway analysis because the variability among study populations would be too great to assign weighted power of input genes with confidence that the weights accurately and equally portray all of the diverse populations from the different GWAS. However, future researchers conducting an intra-GWAS pathway analysis could apply weight to the statistical significance of the genes included in their gene lists in order to better distinguish between, for example, fewer in number but more influential genes within one pathway and greater in number but less influential genes within another pathway.

Another fundamental limitation to pathway analysis is that it does not consider the interaction between different pathways in its analysis. These interactions can be observed afterward but are not a part of the statistical analysis that identifies significant pathways.

Conclusion

In conclusion, this study has identified multiple genetic pathways that are statistically significant with AIS. These pathways can serve as subjects for further investigation into the etiology of AIS. While there were limitations to the procedure of the intra-GWAS pathway analyses, specifically the inability to perform a GWAS, this study provides suggestions for repetition pathway analysis studies and insight into the procedural benefits and drawbacks of pathway analysis. AIS affects children across the

world, so insight into the etiology of AIS can lead future researchers towards a potential cure. This could profoundly affect the way that physicians treat AIS and help to improve the health and well-being of future generations.

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SUPPLEMENTAL MATERIALS

APPENDIX #1: Gene Lists

List #	Description of Gene List	Genes Included in List
1	Cheng et al. (2015) – all genes	ABCA13 AJAP1 BCL2 CASC20 CBX1P3 CNST CYB5AP5 DCBLD1 EBF1 HNRNPA1P57 HSPA9P1 KREMEN1 LBX1-AS1 RBMXP1 SESN3 SLC38A2
2	Cheng et al. (2015) – only genes with SNP in coding region	BCL2 CASC20 DCBLD1 EBF1 KREMEN1 LBX1-AS1
3	Cheng et al. (2015) – only genes with SNP in coding region and genes within 100,000 bp of SNP	ABCA13 BCL2 CASC20 CBX1P3 CYB5AP5 DCBLD1 EBF1 HNRNPA1P57

		HSPA9P1 KREMEN1 LBX1-AS1 RBMXP1
4	Ogura et al. (2015) – all genes	ADGRG6 ATG14 BNC2 BTRC CALN1 CYP51A1P1 FTO HSP90AB5P KCTD16 LBX1 LOC100131680 LOC100528063 LOC729316 LRRC55 MSC NT5DC1 PAX8 PTPRD RNF186 RPL41P1 TNFSF12-TNFSF13 TTC23 WWOX
5	Ogura et al. (2015) – only genes with SNP in coding region	ATG14 BNC2 FTO KCTD16 NT5DC1 TNFSF12-TNFSF13 TTC23 WWOX
6	Ogura et al. (2015) – only genes with SNP in coding region and genes within 100,000 bp of SNP	ADGRG6 ATG14 BNC2 BTRC CALN1

		FTO HSP90AB5P KCTD16 LBX1 LOC100528063 LOC729316 LRRC55 MSC NT5DC1 PAX8 RNF186 TNFSF12-TNFSF13 TTC23 WWOX
7	Ward et al. (2010) – all genes	AIM1L AMY1C APOB APOL1 ARHGAP24 ARID1B ARL4C ARPP21 BMP3 BRIP1 C4orf22 CALCR CBLN4 CDH11 CDH13 CDH2 CLSTN2 CMPK2 CUX2 EED EEPD1 EFNA3 EXOC5 FAM83B FOXB1 GPBP1

		GPM6B GYPC HMCN1 ID2 INTS2 ITGA1 KCNC4 KIAA1671 KLHL29 LAPTM4B MICALL2 MMP3 MUC2 MYH9 NEDD1 NLRP1 NRG1 RIMS2 RRAGC SCAF8 SERPINB8 SETBP1 SH3BP4 SLC16A2 SORCS1 SORCS1 SPATA21 TMEM132D TMEM161B TNIP1 TUSC1 ZBTB20
8	Cheng et al. (2015), Ogura et al. (2015), and Ward et al. (2010) – all genes	ABCA13 ADGRG6 AIM1L AJAP1 AMY1C APOB APOL1 ARHGAP24 ARID1B

		ARL4C ARPP21 ATG14 BCL2 BMP3 BNC2 BRIP1 BTRC C4orf22 CALCR CALN1 CASC20 CBLN4 CBX1P3 CDH11 CDH13 CDH2 CLSTN2 CMPK2 CNST CUX2 CYB5AP5 CYP51A1P1 DCBLD1 EBF1 EED EEPD1 EFNA3 EXOC5 FAM83B FOXB1 FTO GPBP1 GPM6B GYPC HMCN1 HNRNPA1P57 HSP90AB5P HSPA9P1 ID2

		INTS2 ITGA1 KCNC4 KCTD16 KIAA1671 KLHL29 KREMEN1 LAPTM4B LBX1 LBX1-AS1 LOC100131680 LOC100528063 LOC729316 LRRC55 MICALL2 MMP3 MSC MUC2 MYH9 NEDD1 NLRP1 NRG1 NT5DC1 PAX8 PTPRD RBMXP1 RIMS2 RNF186 RPL41P1 RRAGC SCAF8 SERPINB8 SESN3 SETBP1 SH3BP4 SLC16A2 SLC38A2 SORCS1 SPATA21 TMEM132D TMEM161B

		TNFSF12-TNFSF13 TNIP1 TTC23 TUSC1 WWOX ZBTB20
9	Among GWAS publications – all genes	ADGRG6 AJAP1 BCL2 BIN1 BNC2 CALM1 CDH13 CHD7 CHL1 CNTNAP2 DSCAM EPHA4 ESR1 ESR2 FBN1 FBN2 IGF1 IL17RC IL6 KCNJ2 LAPTM4B LBX1 LBX1AS1 MATN1 NTF3 PAX1 PAX3 SETBP1 SOX9 SPATA21 TGFB1 TPH1
10	Among GWAS publications, Cheng et al. (2015), Ogura et al. (2015), and Ward et al. (2010) – all genes	ABCA13 ADGRG6 AIM1L

		AJAP1 AMY1C APOB APOL1 ARHGAP24 ARID1B ARL4C ARPP21 ATG14 BCL2 BIN1 BMP3 BNC2 BRIP1 BTRC C4orf22 CALCR CALM1 CALN1 CASC20 CBLN4 CBX1P3 CDH11 CDH13 CDH2 CHD7 CHL1 CLSTN2 CMPK2 CNST CNTNAP2 CUX2 CYB5AP5 CYP51A1P1 DCBLD1 DSCAM EBF1 EED EEPD1 EFNA3

		EPHA4 ESR1 ESR2 EXOC5 FAM83B FBN1 FBN2 FOXB1 FTO GPBP1 GPM6B GYPC HMCN1 HNRNPA1P57 HSP90AB5P HSPA9P1 ID2 IGF1 IL17RC IL6 INTS2 ITGA1 KCNC4 KCNJ2 KCTD16 KIAA1671 KLHL29 KREMEN1 LAPTM4B LBX1 LBX1-AS1 LOC100131680 LOC100528063 LOC729316 LRRC55 MATN1 MICALL2 MMP3 MSC MUC2 MYH9

		NEDD1 NLRP1 NRG1 NT5DC1 NTF3 PAX1 PAX3 PAX8 PTPRD RBMXP1 RIMS2 RNF186 RPL41P1 RRAGC SCAF8 SERPINB8 SESN3 SETBP1 SH3BP4 SLC16A2 SLC38A2 SORCS1 SOX9 SPATA21 TGFB1 TMEM132D TMEM161B TNFSF12-TNFSF13 TNIP1 TPH1 TTC23 TUSC1 WWOX ZBTB20

***Note: Tang et al. (2015) identified genes associated with six of the SNPs that Ward et al. (2010) did not identify: rs10168146 - ARL4C; rs10004901 - C4orf22; rs10787096 - SORCS1; rs16945692 - INTS2; rs8093693 - SERPINB8; rs136187 - MYH9. These genes were included in the gene list with the genes that Ward et. al. (2010) associated with the same SNPs.

APPENDIX #2: Publications for traditional pathway analysis

#	Paper Name	Year	Journal	SNP	Gene	*p* value	OR (95%CI)
1	Genome-wide association study identifies new susceptibility loci for adolescent idiopathic scoliosis in Chinese girls	2015	Nature Communications	rs241215	AJAP1	2.95E-09	0.83 (0.78 - 0.89)
1				rs4940576	BCL2	2.22E-12	1.23 (1.03 - 1.46)
1				rs13398147	EPHA4	7.59E-13	1.28 (1.20 - 1.37)
1				rs678741	LBX1AS1	9.68E-37	1.44 (1.37 - 1.52)
1				rs13398147	PAX3	7.59E-13	1.28 (1.20 - 1.37)
2	Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes	2011	Human Molecular Genetics	rs1400180	CHL1	1.33E-06	1.30 (1.14 - 1.48)
2				rs10510181	CHL1	8.22E-07	1.37 (1.20 - 1.58)
2				rs11770843	CNTNAP2	6.20E-05	1.75 (1.32 - 2.30)
2				rs2222973	DSCAM	1.46E-06	0.59 (0.48 - 0.74)
3	Lack of association between the CHL1 gene and adolescent idiopathic scoliosis susceptibility in Han Chinese: a case-control study	2014	BMC Musculoskeletal Disorders	none	N/A	N/A	N/A
4	Investigation of the 53 Markers in a DNA-Based Prognostic Test Revealing New Predisposition Genes for Adolescent Idiopathic Scoliosis	2015	Spine	rs12618119	BIN1	<0.001	1.29 (1.15 - 1.46)
4				rs4782809	CDH13	<0.001	0.82 (0.72 - 0.92)
4				rs9945359	SETBP1	<0.001	1.29 (1.12 - 1.50)
4				rs4661748	SPATA21	<0.001	0.77 (0.66 - 0.90)
5	A genome-wide association study identifies common variants near *LBX1* associated with adolescent idiopathic scoliosis	2011	Nature Genetics	rs11190870	LBX1	1.24E-19	1.56 (1.41 - 1.71
5				rs625039	LBX1	8.13E-15	1.49 (1.34 - 1.64)
5				rs11598564	LBX1	5.98E-14	1.42 (1.30 - 1.56)
6				Rs12946942	KCNJ2	4.00E-08	2.05 (1.58 - 2.66)
6	Identification of a Susceptibility Locus for Severe Adolescent Idiopathic Scoliosis on Chromosome 17q24.3	2013	PLOS ONE	Rs12946942	SOX9	4.00E-08	2.05 (1.58 - 2.66)
7	Genetic Variants in Melatonin Synthesis and Signaling Pathway are not Associated with Adolescent Idiopathic Scoliosis	2011	Spine	none	N/A	N/A	N/A
8	Study Testing Association of Genetic Markers Of Melatonin Signaling and Biosynthesis to Predict Adolescent Idiopathic Scoliosis (AIS) or Curve Severity	2009	Spine	none	N/A	N/A	N/A

9	A Replication Study for Association of 53 Single Nucleotide Polymorphisms in a Scoliosis Prognostic Test With Progression of Adolescent Idiopathic Scoliosis in Japanese	2013	Spine	none	N/A	N/A	N/A
10	Lack of association between suppressor of cytokine signaling-3 gene polymorphism and susceptibility and curve severity of adolescent idiopathic scoliosis	2014	European Spine Journal	none	N/A	N/A	N/A
11	Genome-wide Association Study of Adolescent Idiopathic Scoliosis in Southern China	2012	Spine	none	N/A	N/A	N/A
13	SNP rs11190870 near LBX1 is associated with adolescent idiopathic scoliosis in southern Chinese	2012	Journal of Human Genetics	rs11190870	LBX1	9.10E-10	1.85 (1.52 - 2.25)
14	Melatonin Receptor 1B (MTNR1B) Gene Polymorphism Is Associated With the Occurrence of Adolescent Idiopathic Scoliosis	2007	Spine	none	N/A	N/A	N/A
15	Association of rs11190870 near LBX1 with adolescent idiopathic scoliosis susceptibility in a Han Chinese population	2013	Spine	rs11190870	LBX1	1.80E-09	1.51 (1.33 - 1.71)
17	A PAX1 enhancer locus is associated with susceptibility to idiopathic scoliosis in females	2015	Nature Communications	rs6137473	PAX1	5.58E-07	1.53 (1.29 - 1.80)
17				rs169311	PAX1	1.25E-06	1.51 (1.28 - 1.78)
18	A Functional SNP in BNC2 is Associated with Adolescent Idiopathic Scoliosis	2015	The American Journal of Human Genetics	rs10738445	BNC2	2.46E-13	1.21 (1.14 - 1.28)
19	Association between Common Variants near LBX1 and Adolescent Idiopathic Scoliosis Replicated in the Chinese Han Population	2013	PLOS ONE	rs625039	LBX1	5.09E-05	1.49 (1.23 - 1.80)
19				rs11190870	LBX1	1.17E-08	1.70 (1.42 - 2.04)
19				rs11598564	LBX1	5.54E-06	1.52 (1.27 - 1.83)
20	Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis	2013	Nature Genetics	rs6570507	ADGRG6	1.27E-14	1.27 (1.20 - 1.35)
21	CHD7 Gene Polymorphisms Are Associated with Susceptibility to Idiopathic Scoliosis	2007	The American Journal of Human Genetics	rs1038351	CHD7	0.00018	N/A
24	A Replication Study for Association of 53 Single Nucleotide Polymorphisms in ScolioScore Test With Adolescent Idiopathic Scoliosis in French-Canadian Population	2015	Spine	none	N/A	N/A	N/A
25	Promoter polymorphism of matrilin-1 gene predisposes to adolescent idiopathic scoliosis in a Chinese population	2009	European Journal of Human Genetics	rs1149048	MATN1	0.0007	1.35 (1.14 - 1.61)
26	Transforming Growth Factor Beta 1 Is a Novel Susceptibility Gene for Adolescent Idiopathic Scoliosis	2013	Spine	rs1800469	TGFB1	0.003	N/A
26				rs1800471	TGFB1	0.0008	N/A
27	Associations between matrilin-1 gene polymorphisms and adolescent idiopathic scoliosis curve patterns in a Korean population	2012	Molecular Biology Reports	rs1065755	MATN1	0.021	2.56 (1.12 - 5.83)
28	Lack of Association between Adolescent Idiopathic Scoliosis and Previously Reported Single Nucleotide Polymorphisms in MATN1, MTNR1B, TPH1, and IGF1 in a Japanese Population	2012	Journal of Orthopaedic Research	none	N/A	N/A	N/A

29	A Relook Into the Association of the Estrogen Receptor [alpha] Gene (PvuII, XbaI) and Adolescent Idiopathic Scoliosis: A Study of 540 Chinese Cases	2006	Spine	none	N/A	N/A	N/A
30	Replication Study of the Association between Adolescent Idiopathic Scoliosis and Two Estrogen Receptor Genes	2010	Journal of Orthopaedic Research	none	N/A	N/A	N/A
31	Assignment of two loci for autosomal dominant Adolescent Idiopathic Scoliosis (AIS) to chromosomes 9q31.2-q34.2 and 17q25.3-qtel	2008	Journal of Medical Genetics	none	N/A	N/A	N/A
32	Identification of Candidate Regions for Familial Idiopathic Scoliosis	2005	Spine	none	N/A	N/A	N/A
33	Association Study of Tryptophan Hydroxylase 1 and Arylalkylamine N-Acetyltransferase Polymorphisms With Adolescent Idiopathic Scoliosis in Han Chinese	2008	Spine	rs10488682	TPH1	0.0003	2.909
34	Association of Estrogen Receptor Gene Polymorphisms With Susceptibility to Adolescent Idiopathic Scoliosis	2006	Spine	none	N/A	N/A	N/A
35	Association of Estrogen Receptor β Gene Polymorphisms With Susceptibility to Adolescent Idiopathic Scoliosis	2009	Spine	N/A	ESR2	0.004	2.09 (1.14 - 3.81)
36	CHD7 Gene Polymorphisms and Familial Idiopathic Scoliosis	2013	Spine	none	N/A	N/A	N/A
37	Haplotypes at LBX1 Have Distinct Inheritance Patterns with Opposite Effects in Adolescent Idiopathic Scoliosis	2015	PLOS ONE	rs11190870	LBX1	5.43E-09	0.66 (0.57 - 0.76)
38	Association of GPR126 gene polymorphism with adolescent idiopathic scoliosis in Chinese populations	2015	Genomics	rs6570507	ADGRG6	0.0035	1.729 (1.021 - 2.543)
38				rs7774095	ADGRG6	0.0078	1.687 (1.112 - 2.562)
38				rs7755109	ADGRG6	0.0078	1.687 (1.112 - 2.562)
39	Rare variants in FBN1 and FBN2 are associated with severe adolescent idiopathic scoliosis	2014	Human Molecular Genetics	N/A	FBN1	0.0041	4.2 (1.5 - 11.7)
39				N/A	FBN2	0.0307	3.0 (1.6 - 7.3)
41	Candidate gene analysis and exome sequencing confirm LBX1 as a susceptibility gene for idiopathic scoliosis	2015	The Spine Journal	rs11190870	LBX1	7.00E-18	1.53 (1.39 - 1.69)
42	Analysis of Single Nucleotide Polymorphism in Adolescent Idiopathic Scoliosis in Korea: For Personalized Treatment	2013	Yonsei Medical Journal	rs5742612	IGF1	0.006	N/A
42				rs2449539	LAPTM4B	0.002	0.35
43	XbaI and PvuII Polymorphisms of Estrogen Receptor 1 Gene in Females with Idiopathic Scoliosis: No Association with Occurrence or Clinical Form	2013	PLOS ONE	none	N/A	N/A	N/A
44	Lack of association between DSCAM gene polymorphisms and adolescent idiopathic scoliosis susceptibility in a Chinese Han population	2015	Journal of Back and Musculoskeletal Rehabilitation	none	N/A	N/A	N/A
45	Estrogen Receptor 2 Gene Polymorphism in Idiopathic Scoliosis	2014	Spine	none	N/A	N/A	N/A
46	Single-Nucleotide Polymorphism in Turkish Patients with Adolescent Idiopathic Scoliosis: Curve Progression Is not Related with MATN-1, LCT C/T – 13910, and VDR Bsml	2012	Journal of Orthopaedic Research	none	N/A	N/A	N/A
47	A single-nucleotide polymorphism rs708567 in the IL-17RC gene is associated with a susceptibility to and the curve severity of adolescent idiopathic scoliosis in a	2012	MBC Musculoskeletal	rs708567	IL17RC	0.023	1.550 (1.062 - 2.261)

	Chinese Han population: a case-control study		Disorders				
48	A Genome Wide Association Study Identifies IL17RC as an Adolescent Idiopathic Scoliosis Locus	2011	Spine	rs708567	IL17RC	1.18E-09	2.28
62	A Promoter Polymorphism of Neurotrophin 3 Gene Is Associated With Curve Severity and Bracing Effectiveness in Adolescent Idiopathic Scoliosis	2012	Spine	rs11063714	NTF3	0.008	N/A
64	Association Study between Idiopathic Scoliosis and Polymorphic Variants of VDR, IGF-1, and AMPD1 Genes	2015	Genetics Research International	none	N/A	N/A	N/A
65	Adolescent Idiopathic Scoliosis and the Single-nucleotide Polymorphism of the Growth Hormone Receptor and IGF-1 Genes	2009	Orthopedics	none	N/A	N/A	N/A
66	Association between adolescent idiopathic scoliosis with double curve and polymorphisms of calmodulin1 gene/estrogen receptor-α gene	2009	Orthopaedic Surgery	rs12885713	CALM1	0.034	N/A
66				rs2234693	ESR1	0.0185	N/A
67	Role of the IL-6 Gene in the Etiopathogenesis of Idiopathic Scoliosis	2015	Analytical Cellular Pathology	rs1800795	IL6	0.0006	2.02 (1.35 - 3.03)
68	Association Between IL-6 and MMP-3 Gene Polymorphisms and Adolescent Idiopathic Scoliosis	2007	Spine	N/A	IL6	0.001	10.54 (2.9 - 37.1)
68				N/A	MMP3	0.001	3.34 (1.3 - 8.3)