Chapter 7
Genetics and Functional Pathology of Idiopathic Scoliosis

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Introduction

Idiopathic scoliosis (IS) is a structural lateral curve of the spine that affects approximately 2–3% of pediatric populations, with girls more severely affected than boys. Treatment options are currently limited to bracing, physical therapy, and spinal fusion surgery for severe progressive curves. The variability in clinical presentation, limited treatment options, and inability to detect those at risk for curve progression have confounded physicians as well as IS patients and their families. IS has long been recognized to have a familial or genetic component; however, the mechanisms underlying this heritability are largely unknown. Multiple studies to date have identified genetic variants that are associated with IS in specific cohorts. However, most of these associations, with the exception of variants in or near *LBX1* and *GPR126*, have not been able to be reproduced. The varied results of these studies are indications of the extreme genetic and phenotypic heterogeneity of this disorder. New technologies, including next-generation sequencing and improved animal models, hold promise for the discovery of additional mechanisms that cause IS. Identifying the genetic factors underlying IS may aid in the development of diagnostic screening tools and more effective treatment options for affected children.

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A Genetic Basis for Idiopathic Scoliosis (IS)

A Familial Basis of IS

The hereditary basis of IS was established as early as the 1930s, when scoliosis was identified in five generations in one family [1]. Decades later, clinical observations and population studies documented a higher prevalence of scoliosis among relatives of affected individuals compared to the general population [1–7]. Specifically, Wynne-Davies observed that relatives of individuals with IS were at a higher risk for developing the disease, reporting that IS was present in 11% of first-degree, 2.4% of second-degree, and 1.4% of third-degree relatives [2, 8]. In another study, Bonaiti et al. observed that approximately 40% of IS cases were familial across multiple populations [9]. More recently, in an analysis of a unique database of a Mormon population in Utah (GenDB), 97% of IS patients were determined to be of familial origin [10]. Many researchers within the IS research community have since hypothesized that *IS is likely due to multiple inherited risk alleles in tandem with environmental risk factors.*

Twin Concordance Rates

Studies of monozygotic and dizygotic twins have provided further evidence supporting a genetic basis of IS. Concordance is defined as both twins having the disease, and higher concordance rate in monozygotic twins compared to dizygotic twins is an indication that a disease has a genetic component. The concordance for IS is approximately 73% for monozygotic twins and 36% for dizygotic twins [7, 11–17], indicating there is a strong genetic component to the disease. At first, this may appear confusing and contradictory, as dizygotic twins appear to have a threefold higher concordance rate than that reported by Riseborough and Wynne-Davies for first-degree relatives [8]. However, upon further examination, the higher rate in dizygotic twins may be due to the differences in rates of radiographic confirmation of the scoliotic curve. Radiographic confirmation of scoliosis may be higher among twins, as the diagnosis of one twin may lead to inquiries into the curvature status of the other twin. This radiographic confirmation may be less likely within first-degree relatives, and thus first-degree relatives may have an artificially low concordance rate. There could also be an in utero environmental component to IS that has not yet been identified. Although these factors may confound the interpretation of the twin data, the high concordance rates in both monozygotic and dizygotic twins are an indication of a strong genetic component to IS.

Multiple Modes of Inheritance

To date, multiple modes of genetic inheritance have been reported in investigations of IS families, including X-linked [18, 19], multifactorial [2, 20, 21], and autosomal dominant [22–25]. Rather than presenting conflicting information, these reports serve as examples of the *diverse spectrum of inheritance models for IS* that can exist between families. Figure 7.1 presents two examples of commonly seen IS

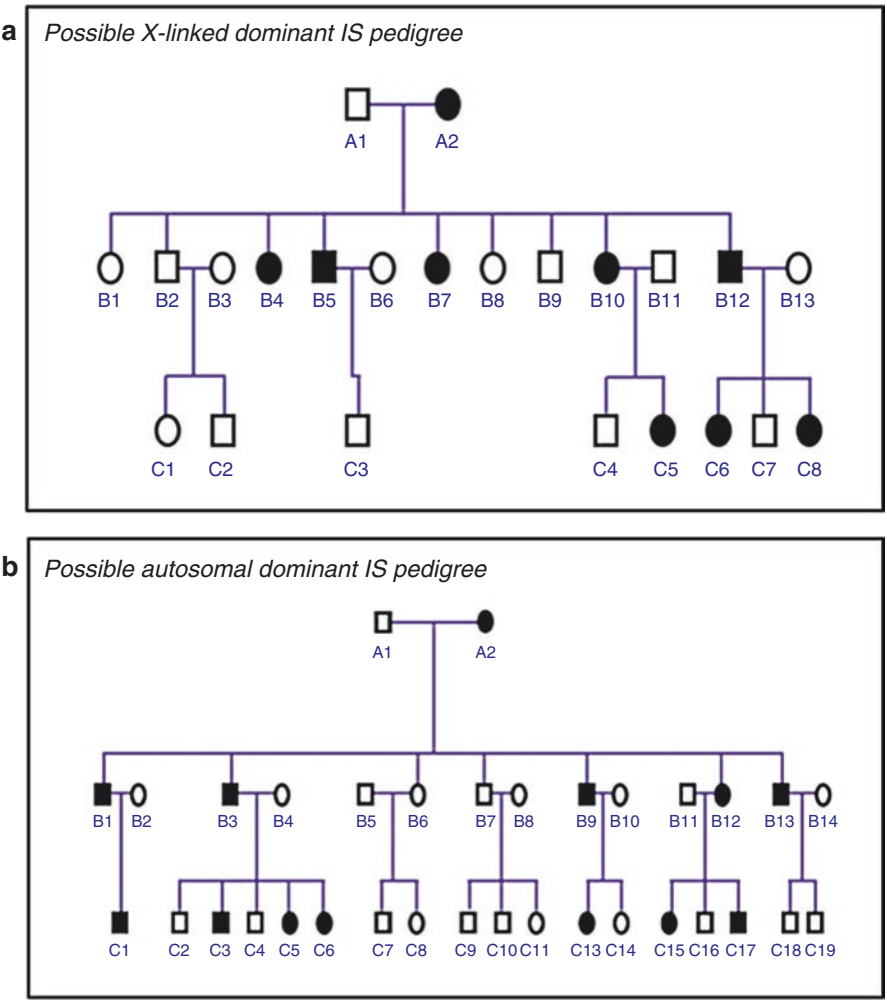

Fig. 7.1 Examples of distinct but typical IS pedigrees. (**a**) A likely X-linked dominant pedigree of a multigenerational IS family. Note the lack of male-to-male transmission. (**b**) IS pedigree displaying male-to-male transmission and a possible autosomal dominant inheritance pattern

pedigrees— one exhibiting an apparent X-linked dominant mode of inheritance and the other showing an apparent autosomal dominant mode of inheritance.

Wynne-Davies suggested that IS was likely inherited in either a dominant or multifactorial mode of inheritance, based on their early studies in the 1960s–1970s with over 2000 individuals [2, 8]. Aksenovich [26] also suggested an autosomal dominant inheritance pattern for IS with sex-dependent penetrance, after conducting an analysis of families with probands with moderate to severe scoliosis (>25 degrees). However, when the authors analyzed probands with mild scoliosis (<25 degrees), multiple damaging alleles (genetic heterogeneity) were attributed as the likely cause for the IS. This same research group later conducted a complex segregation analysis (CSA), a technique used to determine whether a major gene underlies the distribution of a phenotypic trait, of 101 IS families (703 individuals) with spinal curves of at least 5 degrees [27]. This analysis initially did not lead to a clear inheritance pattern, but after excluding individuals with curves under 11 degrees, the results supported an autosomal dominant pattern of inheritance with significant rates of incomplete penetrance. It is important to note that a clinical diagnosis of IS typically requires a spinal curvature of at least 10 degrees.

Other groups observed pedigrees that appear to display an X-linked inheritance pattern. This pattern is defined by a lack of male-to-male transmission, as males are unable to pass their X chromosome onto their male offspring. In 1972, Cowell et al. reported on 17 families with this mode of inheritance [18]. Later, Miller [19] and Justice [28] analyzed a subset of families that displayed characteristics of X-linked inheritance and found that the Xq23–26 region may be linked to IS in this subset.

Section Summary

A genetic basis for IS has been well-established for decades, beginning with observational familial studies in the 1930s by Garland. Studies of inheritance patterns of IS have proven to vary both between and within families, with pedigrees supporting autosomal dominant, X-linked, and multifactorial inheritance patterns. Taken together, data acquired over the last century suggest that IS is a complex genetic disorder with variations in inheritance patterns in affected families.

Heterogeneity and Confounding Factors

Phenotypic Heterogeneity and Overlapping Conditions

Significant phenotypic and genetic heterogeneity across patient cohorts have caused significant challenges for determining the genetic factors underlying IS. Non-idiopathic scoliosis is part of the disease phenotype for several musculoskeletal

conditions, including Marfan syndrome and osteogenesis imperfecta, and early studies of IS may not have fully excluded these individuals from their cohorts [1, 3, 29, 30]. Other studies did not detail specific criteria for affected versus unaffected scoliosis status or were unable to consistently obtain spinal radiographs from study participants [2, 7, 8, 20, 22, 31]. Well-documented diagnostic criteria and radiographic confirmation of curve magnitude can aid in the distinction between true affected and unaffected individuals, and radiographic confirmation can potentially reduce the rate of false-negative diagnoses made from clinical observations alone. In addition, thorough patient records are necessary for phenotypic subtype analyses within large study cohorts, and desired clinical information may include age of onset, curve severity, curve progression, family history of IS, and lifestyle or environmental factors. Detailed phenotypic characterization, including radiographs, is important for assigning both affected and unaffected status and will allow for a better understanding of IS.

Genetic Heterogeneity

Likewise, genetic heterogeneity of patient populations is a significant challenge for IS researchers to overcome. Genetic heterogeneity is defined as a phenotype that arises by different genetic mechanisms. In the context of IS, this means that mutations in multiple genes could each give rise to a similar phenotype— a lateral spinal curvature that is currently labeled as IS. However, once the underlying genetic causes of IS are determined, it is possible that "idiopathic scoliosis" will be broken down into different subtypes based on phenotypic and genetic differences. In practice, it can be difficult to identify a clear inheritance pattern in families with high degrees of genetic heterogeneity, and these families may also appear to have high rates of incomplete penetrance. A major concern with genetic heterogeneity in IS is that individuals are treated as having the exact same condition for experimental and analysis purposes. This could confound both linkage and association studies. A full understanding of the genetic spectrum of IS may also be important for treatment options for the different disease subsets.

Section Summary

IS is marked by a high degree of genetic and phenotypic heterogeneity, creating a significant challenge for researchers trying to unravel the genetic regions and mechanisms underlying this condition. Strict diagnostic criteria, particularly radiographic confirmation of IS status, are required to distinguish true IS from other conditions, as well as to identify true negative controls.

Overview of IS Genetic Findings

Candidate Gene Studies

Prior to the availability of next-generation sequencing technologies, candidate gene approaches were used in order to analyze protein-coding genes thought to be important to the physiological basis of IS. As scoliosis is a phenotypic component of many connective tissue disorders, several extracellular matrix (ECM) genes including collagens, elastin, fibrillin, and aggrecan were studied in IS individuals and families [25, 32]. However, these studies largely resulted in negative findings, as identified variants in these genes often did not segregate with the disease phenotype. Later studies using more modern sequencing technologies, however, did reveal certain significant associations between variants in ECM genes and the IS phenotype (see *Next-Generation Sequencing*).

Linkage Analysis

After advances in genetic technologies in the early 1990s, researchers were able to screen the entire genome for known genetic markers or polymorphisms evenly spaced throughout the chromosome. One such analysis resulting from this advancement was *linkage analysis,* which relies on the concept of genetic *linkage*, or the tendency of certain genes or genetic regions to be inherited together due to physical proximity on a chromosome. Linkage studies typically analyze certain genetic markers in large families with a disease and seek to identify marker alleles that are only present in affected individuals. From there, the candidate region can be narrowed down further with additional fine mapping within the family members, including within candidate genes that may be present in the region, often using single nucleotide polymorphisms (SNPs). In parametric linkage analysis, LOD (*l*ogarithm of the *od*ds) scores are reported to assess whether the allele segregating with the disease phenotype is due to linkage or due to chance. Parametric linkage requires specification of allele frequencies, penetrance, and an inheritance pattern. Nonparametric linkage analysis does not make any assumptions about the disease model and reports the probability of family members sharing alleles identical by descent. It should be noted that the presence of linkage or association of a locus with a disease does not prove causality. Results need to be validated within independent cohorts and then studied for functional importance.

In 2000, Wise et al. conducted nonparametric linkage analysis on a multiplex IS family, which suggested four regions, on chromosomes 3, 6, 12, and 18, as potentially important for IS. After further study of regions 6, 10, and 18 in a second family, region 18q was determined to be the most important region for linkage, with a secondary area on chromosome 6p. Additionally, both families supported a common candidate on distal chromosome 10q [33]. In 2002, Chan et al. analyzed the

three regions identified by Wise et al. in one multiplex family but were not able to replicate the linkage. The Chan group conducted a second genome-wide scan of seven families, which identified two regions of interest, a primary area on chromosome 19p13.3 and a secondary area on chromosome 2q [34]. Salehi et al. also conducted linkage analysis on one large multiplex family, which yielded a candidate region on chromosome 17p11 [35]. This region was of particular interest, as it contained several ECM genes.

In 2005, Miller et al. reported a large genetic linkage screen of 202 families (1198 individuals), and stratified families based on phenotypic subtypes and the apparent mode of inheritance, to decrease the heterogeneity within the population. Linkage analysis of families with an apparent autosomal dominant inheritance pattern yielded primary regions on chromosomes 6p, 6q, 9, 16, and 17, as well as secondary regions on chromosomes 1, 3, 5, 7, 8, 11, and 12. Similarly, in families with an apparent X-linked inheritance pattern, the Xq23–26 candidate region was identified. Stratification of the samples into families with an individual with *kyphoscoliosis* yielded significant regions on chromosomes 5 and 13, and analysis of families with an individual with a severe curve (>40 degrees) yielded a region on chromosome 19 [36].

Gao et al. produced evidence of linkage and association of the 8q12 region. Fine-mapping association studies of this region revealed evidence of IS-associated haplotypes centered over exons 2–4 of the *CHD7* gene [37]. Interestingly, mutations in *CHD7* are associated with CHARGE syndrome, for which scoliosis is frequently part of the disease phenotype. However, an independent association study of 22 single nucleotide polymorphism (SNPs) in the *CHD7* gene in 244 IS families failed to replicate the *CHD7* finding [38].

Edery et al. performed a genome-wide scan of three large multigenerational IS families compatible with an autosomal dominant inheritance pattern [39]. The group was not able to replicate the previous findings for 19p13.3, 17p11.2, 9q34, and 18q in any of the three families. However, they observed disease co-segregation in the 3q12.1 and 5q13.3 loci in one family. Subsequent exome sequencing in this family narrowed the disease gene to *POC5*, and injection of patient-specific *POC5* mRNA into zebrafish embryos led to the development of an IS-like phenotype [40] (see *Functional Studies and Animal Models*).

Genome-Wide Association Studies

Genome-wide association studies (GWAS) use genetic data from large cohorts to test the association of a phenotype with a genotype, typically a SNP. The first GWAS for IS was reported in 2011 by Sharma et al. [41], which assayed 419 adolescent IS families with 327,000 SNPs. The authors found the strongest evidence for association with chromosome 3p26.3 SNPs in the proximity of the *CHL1* gene, which encodes an axon guidance protein related to ROBO3. Later that year, a GWAS of 1376 Japanese females with adolescent IS and 11,297 controls revealed a significant

association with variants near the gene *LBX1* [42]. *LBX1* encodes the transcription factor ladybird homeobox 1, which is an important determinant of spinal cord neuron migration and cell fate choice [42]. Significant associations of SNPs near *LBX1* have since been reported in several additional studies, including those from Chinese, European, and French-Canadian cohorts [43–52]. The same research group who performed the Takahashi et al. GWAS expanded their original study cohort to 1819 cases and 25,939 controls of Japanese ancestry, which revealed a susceptibility locus within the G-protein-coupled receptor gene *GPR126* [53]. This association was replicated in two independent studies within IS cohorts of Chinese ancestry [54, 55]. Other IS GWAS have reported associations within *BCN2* [56], between *SOX9* and *KCNJ2* [57], near *PAX1* [58], and with several loci in or near genes involved in Wnt signaling [59]. Table 7.1 provides a summary of IS GWAS, linkage, and other association studies to date.

High-Throughput Sequencing

The advent and adoption of high-throughput ("next-generation") sequencing technologies in the 2000s spurred a genetic revolution by allowing researchers to sequence whole genomes or exomes in a fraction of the time and cost of traditional sequencing methods. Exome sequencing captures the 1–2% of the human genome that is predicted to be protein-coding and allows for the identification of both rare and common variants in these protein-coding genes. Exome sequencing is based on the hypothesis that variants in protein-coding regions are more likely to have functional effects that could cause the disease and has been used successfully to identify causative variants for many diseases, particularly those that are monogenic.

Exome sequencing has been used in multiple IS studies to identify candidate variants and genes. In 2014, Buchan et al. reported an exome sequencing study of 91 unrelated individuals of European ancestry with severe scoliosis (>40 degrees), which revealed the variant burden in *FBN1* as most associated with adolescent IS. Subsequent sequencing of both *FBN1* and *FBN2* in a larger cohort showed a significant enrichment of rare variants in both genes within Caucasian individuals with severe scoliosis (7.6%) compared with in-house controls (2.4%) ($p = 5.46 \times 10^{-4}$) and exome sequencing project controls (2.3%) ($p = 1.48 \times 10^{-6}$) [60]. These findings were also replicated in an independent Han Chinese cohort ($p = 0.0376$), suggesting that these rare variants might be useful markers of curve progression. In 2015, Baschal et al. reported that rare variants in *HSPG2*, which encodes the ECM protein perlecan, were associated with the IS phenotype in a multigenerational AIS family. One particular rare variant, p.Asn786Ser, was also overrepresented in an additional cohort of 100 unrelated IS cases as compared to controls ($p = 0.024$) [61]. ECM variants were further implicated by Haller et al. in an exome sequencing study of 391 severe AIS cases and 843 controls of European ancestry [62]. Novel non-synonymous/splice-site variants in ECM genes were significantly enriched in cases versus controls ($p = 6 \times 10^{-9}$); furthermore, novel variants in

Table 7.1 Genetic studies of idiopathic scoliosis (IS) cohorts using linkage or association analyses

Study	Approach	Number and type of sample	Region	Candidate gene(s)/ marker(s)	Significance (*p* value)
Carr et al. 1992 [23]	Candidate gene, linkage	4 families	17q21	*COL1A1*	NS
			7q22	*COL1A2, COL2A1*	NS
Miller et al. 1996 [25]	Candidate gene, linkage	11 families	15q21.1	*FBN1*	NS
			7q11	*ELN*	NS
			7q22	*COL1A2*	NS
Wise et al. 2000 [33]	Linkage	2 families	6q	NA	0.023, NS
			Distal 10q	NA	0.0193, 0.033
			18q	NA	0.0023, NS
Morcuende et al. 2003 [75]	Candidate gene, linkage	47 families	4q	*MTNR1A*	NS
Inoue et al. 2002 [88]	Candidate gene	304 cases	6q25	*ESR1*	0.002
Chan et al. 2002 [34]	Linkage	7 families	19p13.3	D19S894–D19S1034	0.00001 (4.48)[4]
			2q13–2q22.3	D2S160–D2S151	0.0049 (1.72)[4]
Salehi et al. 2002 [35]	Linkage	1 family	17p11–17q11.2	D17S799–D17S925	0.0001 (3.2)[4]
Justice et al. 2003 [28]	Linkage	51 families	Xq23–Xq26.1	DXS6804–DXS1047	0.0014 (2.23)[4]
Miller et al. 2005 [36]	Linkage	202 families	6p	F13A1–D6S2439	0.01215
			6q16	D6S1031–D6S1021	0.00215
			9q32–9q34	D9S938–D9S1838	0.00055
			16q11–16q12	D16S764–D16S3253	0.00025
			17p11–17q11	D17S1303–D17S1293	0.0025
Alden et al. 2006 [89]	Linkage	72 families	19p13	D19S591–D19S1034	0.013565
Miller et al. 2006 [89]	Linkage, association	7 families	5q13	D5S417–D5S807	0.00173
			13q13.3	D13S305–D13S788	0.00013
			13q32	D13S800–D13S779	0.00013
Yeung et al. 2006 [90]	Candidate gene	506 cases	12q22	*IGF1*	NS

(continued)

Table 7.1 (continued)

Study	Approach	Number and type of sample	Region	Candidate gene(s)/ marker(s)	Significance (*p* value)
Wu et al. 2006 [91]	Candidate gene	202 cases	6q25	*ESR1*	0.001
Tang et al. 2006 [92]	Candidate gene	540 cases, 260 controls	6q25	*ESR1*	NS
Qiu et al. 2006 [93]	Candidate gene	473 AIS, 311 controls	11q21	*MTNR1B*	NS
Montanaro et al. 2006 [94]	Candidate gene, linkage	81 trios	1p35	*MATN1*	0.024
Marosy et al. 2006 [32]	Candidate gene, linkage	58 families	15q25–26	*AGC1*	NS
Gao et al. 2007 [37]	Linkage, association	52 families	8q12	*CHD7*	0.005
Ocaka et al. 2008 [95]	Linkage	25 families	9q31.2–q34.2	D9S930–D9S1818	0.00004
			17q25.3-qtel	D17S1806	0.00001
Raggio et al. 2009 [96]	Linkage, association	7 families	12p	D12S1608–D12S1674	Unknown
Marosy et al. 2010 [97]	Linkage, association	3 families (triple curves)	6p	D6S1043-D6S474	<0.001
			10q	D10S2325–D10S1423 and D10S1765–D10S1239	<0.001
Clough et al. 2010 [98]	Linkage, association	17 families (males)	17p	D17S975, D17S2196	<0.05
Edery et al. 2011 [39]	Linkage, association	3 families (1 family with disease co-segregation)	3q12.1	D3S3690–D3S3045,	<0.001
			5q13.3	D5S2851–D5S1397	<0.001
Sharma et al. 2011 [41]	GWAS	419 families	3p26.3	*CHL1*	2.58×10^{-8}
Takahashi et al. 2011 [42]	GWAS	1376 AIS and 11,297 controls	10q24.31	*LBX1*	1.24×10^{-19}
Gao et al. 2013 [44]	Association, replication	513 AIS and 440 controls	10q24.31	*LBX1*	5.09×10^{-5}–1.17×10^{-8}
Kou et al. 2013 [53]	GWAS	1819 AIS and 25,939 controls	6q24.1	*GPR126*	2.25×10^{-10}
Miyake et al. 2013 [57]	GWAS	554 AIS (severe) and 1474 controls	17q24.3	*SOX9, KCNJ2*	4.00×10^{-18}

(continued)

Table 7.1 (continued)

Study	Approach	Number and type of sample	Region	Candidate gene(s)/ marker(s)	Significance (*p* value)
Londono et al. 2014 [46]	Meta-analysis, replication	9 cohorts	10q24.31	*LBX1*	1.22×10^{-43} (*for rs11190870)*
Zhu et al. 2015 [48]	GWAS	4317 AIS and 6016 controls	1p36.32	*AJAP1*	2.95×10^{-9}
			2q36.1	*PAX3, EPHA4*	7.59×10^{-13}
			18q21.33	*BCL2*	2.22×10^{-12}
Ogura et al. 2015 [56]	GWAS	2109 AIS and 11,140 controls	9p22.2	*BNC2*	2.46×10^{-13}
Sharma et al. 2015 [58]	GWAS	3102 individuals	20p11.22	*PAX1*	6.89×10^{-9}
Zhu et al. 2017 [59]	GWAS	5953 AIS and 8137 controls	2p14	*MEIS1*	1.19×10^{-13}

P-value denotes most significant published value.
NS not significant.

musculoskeletal collagen genes were present in 32% of AIS cases versus 17% of controls. Patten et al. combined genetic linkage data with exome sequencing of one large IS family to identify a rare variant in *POC5*, a centrosomal protein, as associated with the phenotype [40]. In 2016, Li et al. performed exome sequencing on a large family with IS to identify *AKAP2*, a gene encoding a cAMP regulatory protein that associates with the actin cytoskeleton [63]. In a recent study, Gao et al. combined linkage data from a three-generation IS family of Chinese decent with exome sequence data in a discovery cohort of 20 AIS individuals and 86 controls, which showed a significant association of the IS phenotype with three missense variants in the *MAPK7* gene [64]. *MAPK7* encodes a nuclear transport protein, and in vitro experiments demonstrated that the three missense variants each disrupted nuclear translocation in cellular models. Table 7.2 provides a non-exhaustive summary of IS studies using next-generation sequencing technologies.

Transcriptomics and Other Approaches

Although an individual's genomic DNA is generally identical across tissues, their messenger RNA (mRNA) will vary from tissue to tissue to create unique expression signatures, collectively defined as the *transcriptome*. Several groups have compared the gene expression in relevant cell types between IS and control individuals, with the aim of identifying biological differences that may more accurately reflect what is occurring at the protein level of the cell. Osteoblasts, bone cells that form mineralized matrix, have been analyzed by several IS research groups due to their importance to skeletal growth and maintenance, as well as their ability to be collected

Table 7.2 Genetic studies of idiopathic scoliosis (IS) cohorts using next-generation sequencing (NGS)

Study	Approach	Number and type of samples	Candidate gene(s)	Significance (*p* value)
Buchan et al. 2014b [60]	Exome	852 AIS and 669 controls	*FBN1, FBN2*	5.46×10^{-4}
Baschal et al. 2015 [61]	Exome	1 family with validation in 240 AIS/4679 controls	*HSPG2*	0.024
Patten et al. 2015 [40]	Exome	1 family with validation in 40 families	*POC5*	0.045, 0.0273
Haller et al. 2016 [62]	Exome	391 AIS and 843 controls, targeted seq of 919 AIS	ECM (multiple), musculoskeletal collagens (multiple), *COL11A2*	6×10^{-9} (*ECM enrichment*), 1×10^{-9} (*musculoskeletal collagen enrichment*), 6×10^{-9} (*COL11A2*)
Li et al. 2016 [63]	Exome	1 family with validation in 503 controls	*AKAP2*	*Unknown*
Gao et al. 2017 [64]	Exome, linkage	1 family with targeted seq in 20 AIS families and 86 simplex patients, validation in 1038 AIS simplex and 1841 controls	*MAPK7*	2.8×10^{-5}

P-value denotes most significant published value.

during spinal surgeries. Fendri et al. conducted a microarray of IS and control osteoblasts and observed differential expression of multiple homeobox genes in IS cells [65]. Additionally, clustering analysis of differentially expressed genes showed that these genes functioned within biological pathways important in bone development. The Moreau group has also observed differences in IS osteoblasts compared to controls, including altered melatonin signaling [66, 67] and longer cilia, which they believe may affect the cell's mechanotransduction capabilities [68].

Other groups have analyzed gene expression within the paraspinal or paravertebral muscles, which extend and bend the spine and are able to be collected during spinal fusion surgery. Microarray and RT-qPCR analysis of the paraspinal muscles of IS versus control individuals revealed increased activity in TGF-β signaling, which localized to the muscle's extracellular region [69]. Paravertebral muscles were also shown to have asymmetric expression of the MT2 melatonin receptor on the convex versus concave sides of the scoliotic curve [70]. However, this finding was later disputed by Zamecnik et al., who found no difference in the expression of melatonin receptors between either the convex and concave sides of the scoliotic curve, and similarly did not find any differences between IS cases and controls [71].

Lastly, Buchan et al. analyzed genomic copy number variations (CNVs) in 148 IS patients and 1079 controls [72]. The group identified a duplication of chromosome 1q21.1 in 2.1% of IS patients, but only 0.09% of controls, as well as the presence of two chromosomal rearrangements that were previously associated with spinal phenotypes. The group concluded that over 6% of adolescent IS patients in their cohort had a clinically important copy number abnormality and suggested that copy number analysis could be clinically useful to IS patients.

Section Summary

Early genetic studies of IS were marked by specific analyses of genes or regions hypothesized to be biologically important to IS, including within the ECM, although these largely produced negative findings. Linkage analysis studies have revealed several loci associating with the disease phenotype, including SNPs in *CHD7*, although the relevance of these loci is unclear. GWAS have revealed several promising findings, with the *LBX1* and *GPR126* genes being replicated in cohorts of varying ethnicities. Other GWAS loci have not yet been replicated in other studies. The adoption of next-generation sequencing in the late 2000s also spurred several discoveries, including the identification of ECM genes as important for IS, particularly the musculoskeletal collagen genes and *HSPG2*.

Functional Studies and Animal Models

Animal modeling is an important step in research that is often required to prove that candidate genetic variations are able to cause disease. As few mammals other than humans are bipedal or develop scoliosis naturally, appropriately modeling IS has represented a significant hurdle for genetic discovery. The chicken, naturally bipedal, was used to create the first animal model of IS upon removal of the pineal gland. This phenotype was rescued upon administration of melatonin, which is secreted by the pineal gland, leading researchers to hypothesize that melatonin deficiencies may underlie IS development [73, 74]. However, subsequent linkage analysis of a region on chromosome 4q, which contained the human melatonin receptor, showed no evidence of association with IS [75]. Rats that have been made bipedal by amputation of the tail and forelimbs, coupled with gradual raising of the food and water sources, have also been used to model IS. Like the chicken, bipedalized rats likewise developed an IS-like phenotype upon pinealectomy [76, 77]. These rats were also observed to have abnormal levels of serum leptin, osteopontin, and calmodulin antagonists that were associated with spinal curve development and severity [78–81]. There is debate in the field, however, over whether these bipedal rodents accurately represent human disease or if the scoliosis is simply the result of degeneration due to an unnatural physiology.

Recently, several bony fish (teleosts) including the guppy (*Poecilia reticulata*) and zebrafish (*Danio rerio*) have emerged as leading models of IS. Scoliosis occurs naturally among several types of fish and is the most common morphological deformity [81]. These fish experience a cranial-to-caudal load, generated by swimming through water, which is hypothesized to mimic the force load experienced by humans during locomotion [81–83]. Additionally, zebrafish possess many advantages as an animal model, including rapid reproduction times, inexpensive care, ease of genetic manipulation, and abundant experimental resources including a well-annotated genome. The first fish model of IS was developed in a guppy lineage termed *curveback* [82]. The majority of the IS susceptibility of this lineage was later mapped to a 5 cM region which contained over 100 genes, including the melatonin receptor *MTNR1B* [84].

More recent studies have modeled IS in *Danio rerio* (zebrafish), a well-studied laboratory animal with a higher abundance of genetic and experimental resources as compared to the guppy. In 2014, Buchan et al. performed a forward mutagenesis screen for IS in zebrafish and isolated a recessive mutant called *skolios,* which developed an isolated spinal curvature without vertebral malformations. The phenotype was caused by a recessive mutation in *kif6*, a kinesin gene [85]. The Ciruna research group discovered that zebrafish with mutations in *ptk7* developed a late-onset spinal curvature reminiscent of adolescent IS (Fig. 7.2) [86]. The group later recreated this phenotype using temperature-sensitive mutations in multiple cilia genes with a motile cilia-specific promotor. Mutant zebrafish exhibited irregularities in cerebrospinal fluid (CSF) flow, leading the group to hypothesize that altered CSF flow may underlie the development of IS [87]. Patten et al. injected zebrafish embryos with three human *POC5* mRNA sequences identified in IS patients [40]. Injection of any of these sequences resulted in a spine deformity, without affecting other skeletal structures. The group concluded that mutations in *POC5*, which encodes a centriolar protein, may contribute to the development of IS.

Section Summary

A significant challenge in IS genetics research has been the identification of an appropriate animal model, which is often required to demonstrate disease causality of candidate genes. Pinealectomized chickens and bipedal rodents were both shown to develop scoliosis by multiple groups, although there is debate on the utility of these models. Recently, bony fishes (teleosts), particularly the zebrafish, have emerged as promising animal models. Zebrafish naturally develop spinal curvatures and may more accurately mimic scoliosis in humans due to the analogous cranial-to-caudal load experienced from swimming. Additionally, zebrafish have a well-annotated genome and fast reproduction times and are easy and inexpensive to care for. Mutations in several genes have been shown to cause an IS-like phenotype in fish models, including human *POC5* and zebrafish *ptk7*, as well as other cilia genes that lead to abnormal CSF flow.

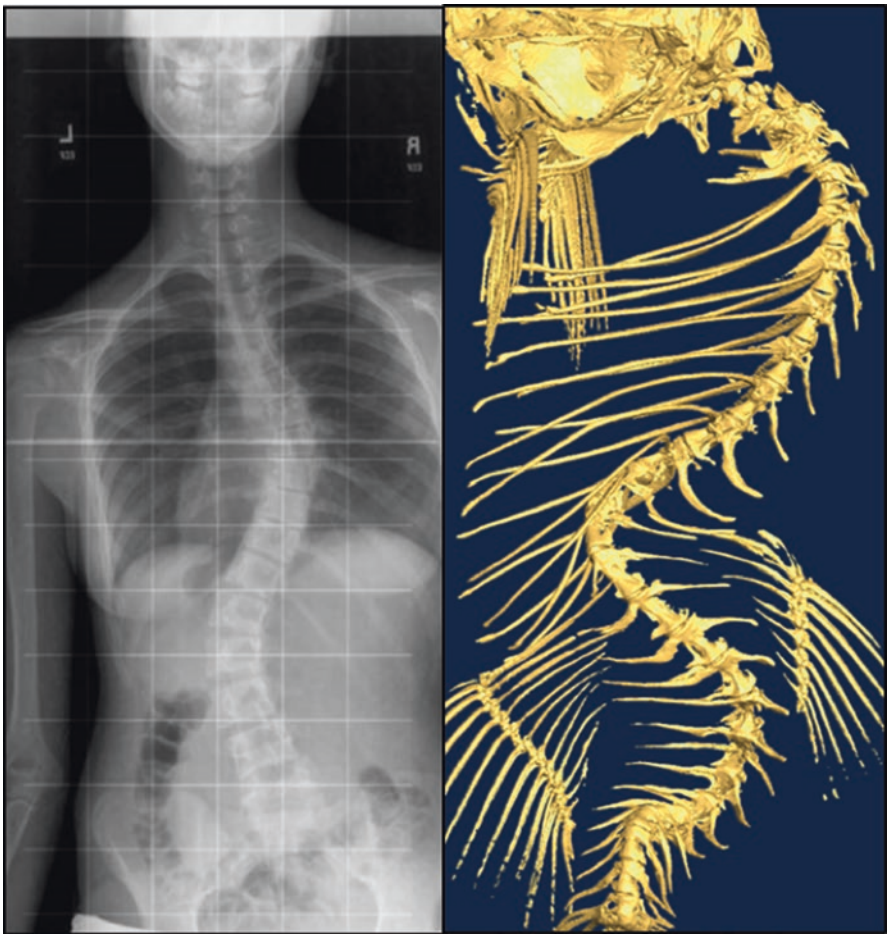

Fig. 7.2 Left, a spinal x-ray of an IS patient. Right, microcomputed tomography (micro-CT) of a *ptk7* mutant zebrafish, presenting late-onset, rotational spinal curvature mirroring defining attributes of human IS

Conclusion

Unraveling the genetic basis of IS has proven to be difficult due to extreme genetic and phenotypic heterogeneity within patient cohorts. Despite this difficulty, several candidate loci have been collectively identified by GWAS, linkage analysis, exome sequencing, and other experimental methods over the past several decades. New and emerging technologies including next-generation sequencing and CRISPR-Cas9 genetic editing present unique opportunities to discover new loci underlying IS etiology. Additionally, bony fish, particularly the zebrafish, have emerged as leading animal models to assist in demonstrating causality of candidate genomic regions in

the etiology of IS. Understanding the genetic causes of IS is an important piece of the puzzle in understanding disease pathogenesis, which will help pave the way for future diagnostics, therapeutics, and perhaps cures for those affected with the disease.

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References

1. Garland HG. Hereditary scoliosis. Br Med J. 1934;1:328.
2. Wynne-Davies R. Familial (idiopathic) scoliosis. A family survey. J Bone Joint Surg Br. 1968;50(1):24–30.
3. Faber A. Untersuchungen uber die Erblichkeit der Skoliose. Arch Orthop Unfallchir. 1936;36:247–9.
4. Ma XJ, Hu P. The etiological study of idiopathic scoliosis. Zhonghua Wai Ke Za Zhi. 1994;32(8):504–6.
5. MacEwen GD, Shands AR Jr. Scoliosis--a deforming childhood problem. Clin Pediatr (Phila). 1967;6(4):210–6.
6. Perricone G, Paradiso T. Familial factors in so-called idiopathic scoliosis. Chir Organi Mov. 1987;72(4):355–8.
7. De George FV, Fisher RL. Idiopathic scoliosis: genetic and environmental aspects. J Med Genet. 1967;4(4):251–7.
8. Riseborough EJ, Wynne-Davies R. A genetic survey of idiopathic scoliosis in Boston, Massachusetts. J Bone Joint Surg Am. 1973;55(5):974–82.
9. Bonaiti C, Feingold J, Briard ML, Lapeyre F, Rigault P, Guivarch J. Genetics of idiopathic scoliosis. Helv Paediatr Acta. 1976;31(3):229–40.
10. Ogilvie JW, Braun J, Argyle V, Nelson L, Meade M, Ward K. The search for idiopathic scoliosis genes. Spine. 2006;31(6):679–81.
11. Carr AJ. Adolescent idiopathic scoliosis in identical twins. J Bone Joint Surg Br. 1990;72(6:1077.
12. Gaertner RL. Idiopathic scoliosis in identical (monozygotic) twins. South Med J. 1979;72(2):231–4.
13. Senda M, Harada Y, Nakahara S, Inoue H. Lumbar spinal changes over 20 years after posterior fusion for idiopathic scoliosis. Acta Med Okayama. 1997;51(6):327–31.
14. Kesling KL, Reinker KA. Scoliosis in twins. A meta-analysis of the literature and report of six cases. Spine. 1997;22(17):2009–14. discussion 15
15. McKinley LM, Leatherman KD. Idiopathic and congenital scoliosis in twins. Spine. 1978;3(3):227–9.
16. Murdoch G. Scoliosis in twins. J Bone Joint Surg Br. 1959;41-B:736–7.
17. Scott TF, Bailey RW. Idiopathic scoliosis in fraternal twins. J Mich State Med Soc. 1963;62:283–4.
18. Cowell HR, Hall JN, MacEwen GD. Genetic aspects of idiopathic scoliosis. A Nicholas Andry award essay, 1970. Clin Orthop Relat Res. 1972;86:121–31.
19. Miller NH, Schwab DL, Sponseller P, Shugert E, Bell J, Maestri N. Genomic search for X-linkage in familial adolescent idiopathic scoliosis. In: IRSoSDMn, editor. Research into spinal deformities 2. Amsterdam: IOS Press; 1998. p. 209–13.
20. Czeizel A, Bellyei A, Barta O, Magda T, Molnar L. Genetics of adolescent idiopathic scoliosis. J Med Genet. 1978;15(6):424–7.

21. Kruse LM, Buchan JG, Gurnett CA, Dobbs MB. Polygenic threshold model with sex dimorphism in adolescent idiopathic scoliosis: the Carter effect. J Bone Joint Surg Am. 2012;94(16):1485–91.
22. Robin GC, Cohen T. Familial scoliosis. A clinical report. J Bone Joint Surg Br. 1975;57(2):146–8.
23. Carr AJ, Ogilvie DJ, Wordsworth BP, Priestly LM, Smith R, Sykes B. Segregation of structural collagen genes in adolescent idiopathic scoliosis. Clin Orthop Relat Res. 1992;(274):305–10.
24. Bell M, Teebi AS. Autosomal dominant idiopathic scoliosis? Am J Med Genet. 1995;55(1):112.
25. Miller NH, Mims B, Child A, Milewicz DM, Sponseller P, Blanton SH. Genetic analysis of structural elastic fiber and collagen genes in familial adolescent idiopathic scoliosis. J Orthop Res. 1996;14(6):994–9.
26. Aksenovich TI, Semenov IR, Ginzburg E, Zaidman AM. Preliminary analysis of inheritance of scoliosis. Genetika. 1988;24(11):2056–63.
27. Axenovich TI, Zaidman AM, Zorkoltseva IV, Tregubova IL, Borodin PM. Segregation analysis of idiopathic scoliosis: demonstration of a major gene effect. Am J Med Genet. 1999;86(4):389–94.
28. Justice CM, Miller NH, Marosy B, Zhang J, Wilson AF. Familial idiopathic scoliosis: evidence of an X-linked susceptibility locus. Spine. 2003;28(6):589–94.
29. Berquet KH. Considerations on heredity in idiopathic scoliosis. Z Orthop Ihre Grenzgeb. 1966;101(2):197–209.
30. Shapiro JR, Burn VE, Chipman SD, Velis KP, Bansal M. Osteoporosis and familial idiopathic scoliosis: association with an abnormal alpha 2(I) collagen. Connect Tissue Res. 1989;21(1–4):117–23. discussion 24
31. Levaia NV. Genetic aspect of dysplastic (idiopathic) scoliosis. Ortop Travmatol Protez. 1981;(2):23–9.
32. Marosy B, Justice CM, Nzegwu N, Kumar G, Wilson AF, Miller NH. Lack of association between the aggrecan gene and familial idiopathic scoliosis. Spine. 2006;31(13):1420–5.
33. Wise CA, Barnes R, Gillum J, Herring JA, Bowcock AM, Lovett M. Localization of susceptibility to familial idiopathic scoliosis. Spine. 2000;25(18):2372–80.
34. Chan V, Fong GC, Luk KD, Yip B, Lee MK, Wong MS, et al. A genetic locus for adolescent idiopathic scoliosis linked to chromosome 19p13.3. Am J Hum Genet. 2002;71(2):401–6.
35. Salehi LB, Mangino M, De Serio S, De Cicco D, Capon F, Semprini S, et al. Assignment of a locus for autosomal dominant idiopathic scoliosis (IS) to human chromosome 17p11. Hum Genet. 2002;111(4–5):401–4.
36. Miller NH, Justice CM, Marosy B, Doheny KF, Pugh E, Zhang J, et al. Identification of candidate regions for familial idiopathic scoliosis. Spine. 2005;30(10):1181–7.
37. Gao X, Gordon D, Zhang D, Browne R, Helms C, Gillum J, et al. CHD7 gene polymorphisms are associated with susceptibility to idiopathic scoliosis. Am J Hum Genet. 2007;80(5):957–65.
38. Tilley MK, Justice CM, Swindle K, Marosy B, Wilson AF, Miller NH. CHD7 gene polymorphisms and familial idiopathic scoliosis. Spine (Phila Pa 1976). 2013;38(22):E1432–6.
39. Edery P, Margaritte-Jeannin P, Biot B, Labalme A, Bernard JC, Chastang J, et al. New disease gene location and high genetic heterogeneity in idiopathic scoliosis. Eur J Hum Genet. 2011;19(8):865–9.
40. Patten SA, Margaritte-Jeannin P, Bernard JC, Alix E, Labalme A, Besson A, et al. Functional variants of POC5 identified in patients with idiopathic scoliosis. J Clin Invest. 2015;125(3):1124–8.
41. Sharma S, Gao X, Londono D, Devroy SE, Mauldin KN, Frankel JT, et al. Genome-wide association studies of adolescent idiopathic scoliosis suggest candidate susceptibility genes. Hum Mol Genet. 2011;20(7):1456–66.
42. Takahashi Y, Kou I, Takahashi A, Johnson TA, Kono K, Kawakami N, et al. A genome-wide association study identifies common variants near LBX1 associated with adolescent idiopathic scoliosis. Nat Genet. 2011;43(12):1237–40.
43. Fan YH, Song YQ, Chan D, Takahashi Y, Ikegawa S, Matsumoto M, et al. SNP rs11190870 near LBX1 is associated with adolescent idiopathic scoliosis in southern Chinese. J Hum Genet. 2012;57:244–6.

44. Gao W, Peng Y, Liang G, Liang A, Ye W, Zhang L, et al. Association between common variants near LBX1 and adolescent idiopathic scoliosis replicated in the Chinese Han population. PLoS One. 2013;8(1):e53234.
45. Jiang H, Qiu X, Dai J, Yan H, Zhu Z, Qian B, et al. Association of rs11190870 near LBX1 with adolescent idiopathic scoliosis susceptibility in a Han Chinese population. Eur Spine J. 2013;22(2):282–6.
46. Londono D, Kou I, Johnson TA, Sharma S, Ogura Y, Tsunoda T, et al. A meta-analysis identifies adolescent idiopathic scoliosis association with LBX1 locus in multiple ethnic groups. J Med Genet. 2014;51(6):401–6.
47. Grauers A, Wang J, Einarsdottir E, Simony A, Danielsson A, Akesson K, et al. Candidate gene analysis and exome sequencing confirm LBX1 as a susceptibility gene for idiopathic scoliosis. Spine J. 2015;15(10):2239–46.
48. Zhu Z, Tang NL, Xu L, Qin X, Mao S, Song Y, et al. Genome-wide association study identifies new susceptibility loci for adolescent idiopathic scoliosis in Chinese girls. Nat Commun. 2015;6:8355.
49. Chettier R, Nelson L, Ogilvie JW, Albertsen HM, Ward K. Haplotypes at LBX1 have distinct inheritance patterns with opposite effects in adolescent idiopathic scoliosis. PLoS One. 2015;10(2):e0117708.
50. Cao Y, Min J, Zhang Q, Li H, Li H. Associations of LBX1 gene and adolescent idiopathic scoliosis susceptibility: a meta-analysis based on 34,626 subjects. BMC Musculoskelet Disord. 2016;17:309.
51. Liu S, Wu N, Zuo Y, Zhou Y, Liu J, Liu Z, et al. Genetic polymorphism of LBX1 is associated with adolescent idiopathic scoliosis in northern Chinese Han population. Spine (Phila Pa 1976). 2017;42:1125–9.
52. Nada D, Julien C, Samuels ME, Moreau A. A replication study for association of LBX1 locus with adolescent idiopathic scoliosis in French-Canadian population. Spine (Phila Pa 1976). 2017;43:172–178.
53. Kou I, Takahashi Y, Johnson TA, Takahashi A, Guo L, Dai J, et al. Genetic variants in GPR126 are associated with adolescent idiopathic scoliosis. Nat Genet. 2013;45(6):676–9.
54. Xu JF, Yang GH, Pan XH, Zhang SJ, Zhao C, Qiu BS, et al. Association of GPR126 gene polymorphism with adolescent idiopathic scoliosis in Chinese populations. Genomics. 2015;105(2):101–7.
55. Qin X, Xu L, Xia C, Zhu W, Sun W, Liu Z, et al. Genetic variant of GPR126 gene is functionally associated with adolescent idiopathic scoliosis in Chinese population. Spine (Phila Pa 1976). 2017;42:E1098–103.
56. Ogura Y, Kou I, Miura S, Takahashi A, Xu L, Takeda K, et al. A functional SNP in BNC2 is associated with adolescent idiopathic scoliosis. Am J Hum Genet. 2015;97(2):337–42.
57. Miyake A, Kou I, Takahashi Y, Johnson TA, Ogura Y, Dai J, et al. Identification of a susceptibility locus for severe adolescent idiopathic scoliosis on chromosome 17q24.3. PLoS One. 2013;8(9):e72802.
58. Sharma S, Londono D, Eckalbar WL, Gao X, Zhang D, Mauldin K, et al. A PAX1 enhancer locus is associated with susceptibility to idiopathic scoliosis in females. Nat Commun. 2015;6:6452.
59. Zhu Z, Xu L, Leung-Sang Tang N, Qin X, Feng Z, Sun W, et al. Genome-wide association study identifies novel susceptible loci and highlights Wnt/beta-catenin pathway in the development of adolescent idiopathic scoliosis. Hum Mol Genet. 2017;26(8):1577–83.
60. Buchan JG, Alvarado DM, Haller GE, Cruchaga C, Harms MB, Zhang T, et al. Rare variants in FBN1 and FBN2 are associated with severe adolescent idiopathic scoliosis. Hum Mol Genet. 2014;23:5271–82.
61. Baschal EE, Wethey CI, Swindle K, Baschal RM, Gowan K, Tang NL, et al. Exome sequencing identifies a rare HSPG2 variant associated with familial idiopathic scoliosis. G3 (Bethesda). 2014;5(2):167–74.

62. Haller G, Alvarado D, McCall K, Yang P, Cruchaga C, Harms M, et al. A polygenic burden of rare variants across extracellular matrix genes among individuals with adolescent idiopathic scoliosis. Hum Mol Genet. 2016;25(1):202–9.
63. Li W, Li Y, Zhang L, Guo H, Tian D, Li Y, et al. AKAP2 identified as a novel gene mutated in a Chinese family with adolescent idiopathic scoliosis. J Med Genet. 2016;53(7):488–93.
64. Gao W, Chen C, Zhou T, Yang S, Gao B, Zhou H, et al. Rare coding variants in MAPK7 predispose to adolescent idiopathic scoliosis. Hum Mutat. 2017;38(11):1500–10.
65. Fendri K, Patten SA, Kaufman GN, Zaouter C, Parent S, Grimard G, et al. Microarray expression profiling identifies genes with altered expression in adolescent idiopathic scoliosis. Eur Spine J. 2013;22(6):1300–11.
66. Moreau A, Wang DS, Forget S, Azeddine B, Angeloni D, Fraschini F, et al. Melatonin signaling dysfunction in adolescent idiopathic scoliosis. Spine (Phila Pa 1976). 2004;29(16):1772–81.
67. Azeddine B, Letellier K, Wang da S, Moldovan F, Moreau A. Molecular determinants of melatonin signaling dysfunction in adolescent idiopathic scoliosis. Clin Orthop Relat Res. 2007;462:45–52.
68. Oliazadeh N, Gorman KF, Eveleigh R, Bourque G, Moreau A. Identification of elongated primary cilia with impaired Mechanotransduction in idiopathic scoliosis patients. Sci Rep. 2017;7:44260.
69. Nowak R, Kwiecien M, Tkacz M, Mazurek U. Transforming growth factor-beta (TGF- beta) signaling in paravertebral muscles in juvenile and adolescent idiopathic scoliosis. Biomed Res Int. 2014;2014:594287.
70. Qiu Y, Wu L, Wang B, Yu Y, Zhu Z. Asymmetric expression of melatonin receptor mRNA in bilateral paravertebral muscles in adolescent idiopathic scoliosis. Spine (Phila Pa 1976). 2007;32(6):667–72.
71. Zamecnik J, Krskova L, Hacek J, Stetkarova I, Krbec M. Etiopathogenesis of adolescent idiopathic scoliosis: expression of melatonin receptors 1A/1B, calmodulin and estrogen receptor 2 in deep paravertebral muscles revisited. Mol Med Rep. 2016;14(6):5719–24.
72. Buchan JG, Alvarado DM, Haller G, Aferol H, Miller NH, Dobbs MB, et al. Are copy number variants associated with adolescent idiopathic scoliosis? Clin Orthop Relat Res. 2014;472(10):3216–25.
73. Machida M, Dubousset J, Imamura Y, Iwaya T, Yamada T, Kimura J. Role of melatonin deficiency in the development of scoliosis in pinealectomised chickens. J Bone Joint Surg Br. 1995;77(1):134–8.
74. Bagnall K, Raso VJ, Moreau M, Mahood J, Wang X, Zhao J. The effects of melatonin therapy on the development of scoliosis after pinealectomy in the chicken. J Bone Joint Surg Am. 1999;81(2):191–9.
75. Morcuende JA, Minhas R, Dolan L, Stevens J, Beck J, Wang K, et al. Allelic variants of human melatonin 1A receptor in patients with familial adolescent idiopathic scoliosis. Spine. 2003;28(17):2025–8. discussion 9
76. Machida M, Murai I, Miyashita Y, Dubousset J, Yamada T, Kimura J. Pathogenesis of idiopathic scoliosis. Experimental study in rats. Spine (Phila Pa 1976). 1999;24(19):1985–9.
77. Machida M, Saito M, Dubousset J, Yamada T, Kimura J, Shibasaki K. Pathological mechanism of idiopathic scoliosis: experimental scoliosis in pinealectomized rats. Eur Spine J. 2005;14(9):843–8.
78. Akel I, Kocak O, Bozkurt G, Alanay A, Marcucio R, Acaroglu E. The effect of calmodulin antagonists on experimental scoliosis: a pinealectomized chicken model. Spine (Phila Pa 1976). 2009;34(6):533–8.
79. Wu T, Sun X, Zhu Z, Zheng X, Qian B, Zhu F, et al. Role of high central leptin activity in a scoliosis model created in bipedal amputated mice. Stud Health Technol Inform. 2012;176:31–5.
80. Yadav MC, Huesa C, Narisawa S, Hoylaerts MF, Moreau A, Farquharson C, et al. Ablation of Osteopontin improves the skeletal phenotype of Phospho1 mice. J Bone Miner Res. 2014;29:2369–81.

81. Boswell CW, Ciruna B. Understanding idiopathic scoliosis: a new zebrafish School of Thought. Trends Genet. 2017;33(3):183–96.
82. Gorman KF, Breden F. Teleosts as models for human vertebral stability and deformity. Comp Biochem Physiol C Toxicol Pharmacol. 2007;145(1):28–38.
83. Gorman KF, Breden F. Idiopathic-type scoliosis is not exclusive to bipedalism. Med Hypotheses. 2009;72(3):348–52.
84. Gorman KF, Christians JK, Parent J, Ahmadi R, Weigel D, Dreyer C, et al. A major QTL controls susceptibility to spinal curvature in the curveback guppy. BMC Genet. 2011;12:16.
85. Buchan JG, Gray RS, Gansner JM, Alvarado DM, Burgert L, Gitlin JD, et al. Kinesin family member 6 (kif6) is necessary for spine development in zebrafish. Dev Dyn. 2014;243(12):1646–57.
86. Hayes M, Gao X, Yu LX, Paria N, Henkelman RM, Wise CA, et al. ptk7 mutant zebrafish models of congenital and idiopathic scoliosis implicate dysregulated Wnt signalling in disease. Nat Commun. 2014;5:4777.
87. Grimes DT, Boswell CW, Morante NF, Henkelman RM, Burdine RD, Ciruna B. Zebrafish models of idiopathic scoliosis link cerebrospinal fluid flow defects to spine curvature. Science. 2016;352(6291):1341–4.
88. Inoue M, Minami S, Nakata Y, Kitahara H, Otsuka Y, Isobe K, et al. Association between estrogen receptor gene polymorphisms and curve severity of idiopathic scoliosis. Spine. 2002;27(21):2357–62.
89. Alden KJ, Marosy B, Nzegwu N, Justice CM, Wilson AF, Miller NH. Idiopathic scoliosis: identification of candidate regions on chromosome 19p13. Spine. 2006;31(16):1815–9.
90. Yeung HY, Tang NL, Lee KM, Ng BK, Hung VW, Kwok R, et al. Genetic association study of insulin-like growth factor-I (IGF-I) gene with curve severity and osteopenia in adolescent idiopathic scoliosis. Stud Health Technol Inform. 2006;123:18–24.
91. Wu J, Qiu Y, Zhang L, Sun Q, Qiu X, He Y. Association of estrogen receptor gene polymorphisms with susceptibility to adolescent idiopathic scoliosis. Spine. 2006;31(10):1131–6.
92. Tang NL, Yeung HY, Lee KM, Hung VW, Cheung CS, Ng BK, et al. A relook into the association of the estrogen receptor [alpha] gene (PvuII, XbaI) and adolescent idiopathic scoliosis: a study of 540 Chinese cases. Spine. 2006;31(21):2463–8.
93. Qiu XS, Tang NL, Yeung HY, Qiu Y, Qin L, Lee KM, et al. The role of melatonin receptor 1B gene (MTNR1B) in adolescent idiopathic scoliosis--a genetic association study. Stud Health Technol Inform. 2006;123:3–8.
94. Montanaro L, Parisini P, Greggi T, Di Silvestre M, Campoccia D, Rizzi S, et al. Evidence of a linkage between matrilin-1 gene (MATN1) and idiopathic scoliosis. Scoliosis. 2006;1:21.
95. Ocaka L, Zhao C, Reed JA, Ebenezer ND, Brice G, Morley T, et al. Assignment of two loci for autosomal dominant adolescent idiopathic scoliosis to chromosomes 9q31.2-q34.2 and 17q25.3-qtel. J Med Genet. 2008;45(2):87–92.
96. Raggio CL, Giampietro PF, Dobrin S, Zhao C, Dorshorst D, Ghebranious N, et al. A novel locus for adolescent idiopathic scoliosis on chromosome 12p. J Orthop Res. 2009;27(10):1366–72.
97. Marosy B, Justice C, Vu C, Zorn A, Nzegwu N, Wilson A, et al. Identification of susceptibility loci for scoliosis in FIS families with triple curves. AJMG. 2010;152A:846–55.
98. Clough M, Justice CM, Marosy B, Miller NH. Males with familial idiopathic scoliosis: a distinct phenotypic subgroup. Spine (Phila Pa 1976). 2010;35(2):162–8.