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Adolescent Idiopathic Scoliosis – Future Molecular-Based Diagnostic and Prognostic Testing

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ABSTRACT

Adolescent idiopathic scoliosis (AIS) is a three-dimensional deformity of the spine mainly affecting the younger population. Earlier detection of the disorder leads to appropriate treatment and better outcomes, thus avoiding highly invasive surgical treatments. The currently available tests for the disease identification have lost their reliability and validity with time. In the past few decades, efforts have been directed towards developing a highly reliable prognostic test for AIS. Towards this end, several strategies have been employed including biochemical, biomechanical and gene-based tests. Among the three, the gene-based technology has received much attention in recent past. Notably, this is due to the fact that the human genome project, followed by genome-wide association studies (GWAS), facilitated the identification of candidate genes for disorders like AIS. Several promising biomarker genes have been identified. However, their global validations were disappointing as these genes were shown to be limited to a particular group of people/ethnicities. Such observations limit the development of a reliable global molecular/biochemical test for AIS. The currently used AIS ScoliScoreTM also has several limitations. With continued disappointments in the identification of biomarkers for AIS and lack of appropriate tests, researchers have diverted their efforts towards several alternative avenues. A ray of hope is emerging from recent observations on the association of non-coding microRNAs and epigenetic factors that might arise as future reliable markers for AIS, thus paving the way for appropriate clinical management of this disorder.

Key words: idiopathic scoliosis, genetics, adolescent idiopathic scoliosis prognostic test, genome-wide association studies

BACKGROUND

There are three different types of idiopathic scoliosis based on the age dimension, i.e., infantile, juvenile and adolescent. The infantile form is manifested between birth to 3 years of age, juvenile between 4 and 9 years of age and adolescent (AIS) in the age group 10-16 years. The overall pathogenesis and diagnosis of AIS has been challenging due to the fact that the spinal deformity is three dimensional in nature and includes other pathologies, such as changes in the lateral curvature and axial rotation [1,2]. AIS sufferers manifest several symptoms, such as uneven shoulders, a rib hump that becomes prominent during forward bending, and abnormal waist or hip height. Clinically, the Cobb angle, which measures the curvature of the spine in degrees, is a measure of the severity of the disease, although under certain circumstances this particular feature is not sufficient for predicting the disease and treatment outcomes [3,4].

No specific findings are available as far as the etiology of AIS is concerned. The etiology of AIS is still unexplained and, consequently, the bulk of the research is dedicated to understanding the factors involved in causing the disease and overall pathogenesis. The nature of this condition is so complex that several approaches focusing on genetic, biochemical, and biomechanical factors in causing the disease are being evaluated [5-8]. However, none of these can conclusively provide information about the factors causing the disease.

Among the three approaches described above, the genetic approach has become highly attractive in the post-genomic era. The human genome project has led to genetic research and discovery of biomarkers for a number of infectious and non-infectious diseases, including complex disorders such as AIS. Although several studies have tried to identify biomarkers or molecular moieties linked with AIS, no single gene has been conclusively linked to the disease and the mode of inheritance is also inconclusive [5,9,10]. This article discusses existing molecular methodologies related to AIS, ongoing research in this area and future prospects associated with this disorder.

PATHOGENESIS OF ADOLESCENT IDIOPATHIC SCOLIOSIS AND TREATMENT OPTIONS

AIS is difficult to identify early and, accordingly, treatment is late in most patients. If the procedure/management is delayed further subsequent to the identification of the symptoms, AIS has detrimental effects on both the physical and psychological levels [6,7]. Furthermore, the scoliotic curvature is also associated with minor pulmonary effects and back pain although certain studies suggest that back pain is not related to the disease [34,11]. The AIS-associated physical changes, including an asymmetric trunk, uneven shoulders, prominent rib hum and hip height, deteriorate over time (Fig. 1) if proper management strategies are not followed [12]. It has been observed

Fig. 1. A 13-year-old female with scoliosis shows curvature of the spine and rib-hum sign of rotation

that individuals suffering from the disorder also manifest psychological concerns.

Importantly, a meta-analysis taking into consideration the currently used ScoliScore single-nucleotide polymorphism (SNP)-based testing of AIS and other single-nucleotide polymorphism-based approaches showed that AIS has several defining characteristics, including [13]:

- increase in the Cobb angle;
- decrease in the rib-vertebral angle at the apical level of the convex side;
- initial Cobb angle severity (>25^{o});
- osteopaenia;
- patient less than 13 years of age at diagnosis;
- premenarche status;
- skeletal immaturity;
- thoracic deformity;
- brain stem vestibular dysfunction;
- multiple indices combining radiographic, demographic, and psychological characteristics.

Data extracted from this meta-analysis shows that AIS is not a "simple" condition; instead, it involves various body systems and organs. Such an extensive array of features linked with the disease have raised clinical diagnostic issues and the question of staging.

There are several classification systems in place for staging of AIS. However, the Lenke classification system is preferred by clinicians. This classification system is sometimes referred to as the triad classification system as it focuses on the curve type (1-6), a lumbar spine modifier (A, B, C) (Fig. 2a-c), and a sagittal thoracic modifier (-, N, +) (Fig. 3a-c) [14,15].

Treatment options for AIS include both surgical and non-surgical interventions. The decision regarding the treatment or surgical intervention is, however, arbitrary. Still, patients exhibiting mild curves

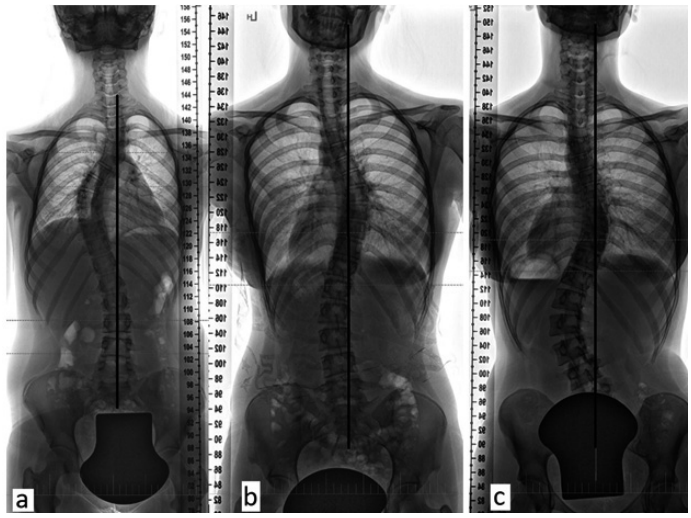

Fig. 2a-c. a – A modifier (Cervicosacral line goes between pedicles of apical lumbar vertebra), b – B modifier (Cervicosacral line touches the pedicle of apical lumbar vertebra), c – C modifier (Cervicosacral line goes medial to apical lumbar vertebra)

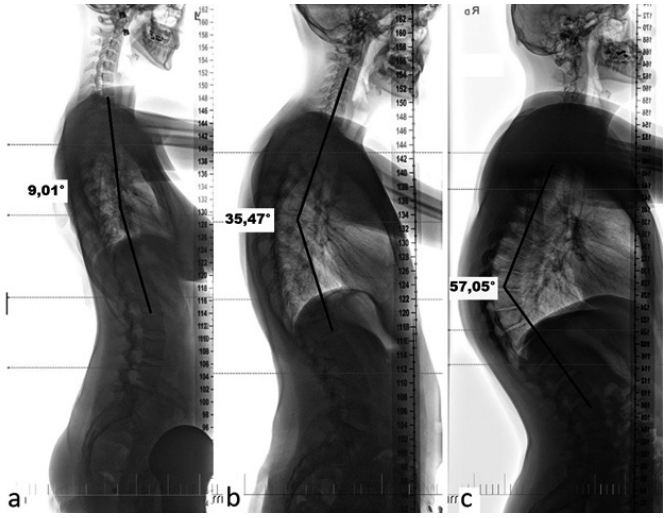

Fig. 3a-c. a – Thoracic hypokyphosis (9°), b – Thoracic normokyphosis (35°), c – Thoracic hyperkyphosis (57°)

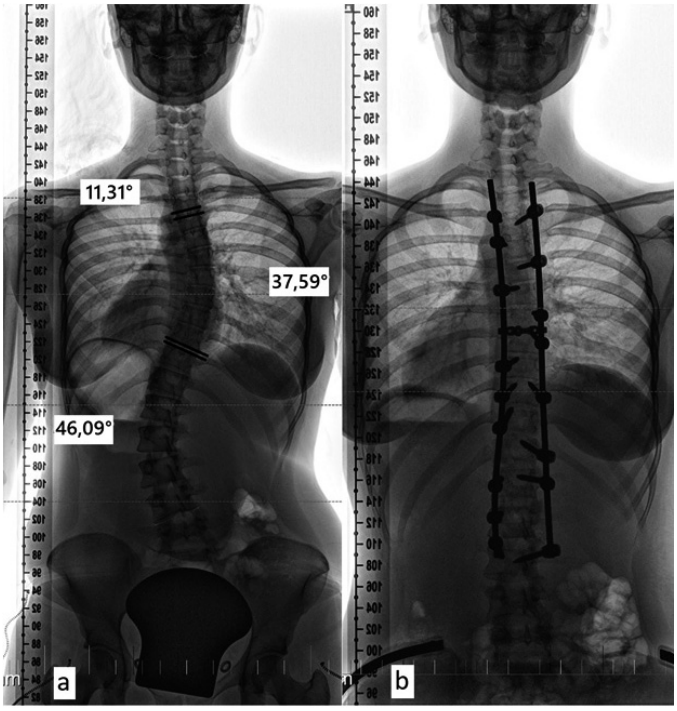

Fig. 4a-b. a – Preoperative PA X-ray: AIS, Lenke type 6C-, b – Postoperative PA X-ray: AIS, Lenke type 6C-

(<25º) are monitored, those displaying moderate curves (25º-45º) are treated conservatively through bracing and physical therapy, and severe curves (>45º) are treated either through spinal fusion or surgical correction (Fig. 4a-b) [16-18]. Earlier identification of AIS leads to appropriate management, avoiding further complications.

PROGNOSTIC GENETIC TESTING OF IDIOPATHIC SCOLIOSIS

Current advances in human genome research and ensuing genomic technologies have led to prognostic detection for several genetic and non-genetic disorders [19,20]. Particularly for a disease like AIS, the etiological factors are unknown; the only defining feature is the age factor linked with this disorder and 80% of the patients fall in this category [21]. Early detection of AIS can allow clinicians to develop management plans earlier. ScoliScore™, a currently available test for AIS, is considered one of the most authentic, first and only genetic test proven to give physicians and parents insight [22-24]. This DNA-based test takes into consideration 53 single nucleotide variants and the spinal curve (Cobb angle) ultimately generating a score in the range of 1-200. Based on the ScoliScore™, patients undergo a treatment programme. However, the validity of this scoring matrix has always been questioned. Furthermore, it only applies to Caucasian patients between 9 and 13 years of age and relatively milder values [25].

As far as prognosis in AIS is concerned, the ScoliScore™ coupled with the SNPs is the only option in hand. However, its global implications are limited. There are several studies suggesting that this test is insufficient in predicting or defining the disease. Towards, this end, an evaluation of the ScoliScore™ algorithm among 126 AIS sufferers revealed that the test scores did not correlate with curve progression, a major indication of AIS [26,27].

There are other limitations also related to ScoliScore™ testing besides its utilization being limited to a particular group of patients. Consequently, this test, involving a molecular DNA signature, is not accepted globally and it has not been recognized by regulatory bodies like the US Food and Drug Administration (FDA). The current offering of this test is based on its approval from the Center for Medicare and Medicaid (CMS) [28]. This scenario has left the clinicians in a tedious situation for clinical diagnosis and determination of treatment outcomes.

NEWLY EMERGING PROGNOSTIC GENETIC TESTING FOR ADOLESCENT IDIOPATHIC SCOLIOSIS

AIS is regarded as a complex trait and polygenic disorder. The identification of genes putatively linked with the disease has been an active area of inves-

tigation. There is an unmet need for finding candidate genes linked with AIS and establishing their role in the etiology and pathogenesis of this disorder [29]. Several genetic molecular signatures like oestrogen receptor gene polymorphism linkage with curve severity [30], promoter polymorphism of the matrilin-1 gene in the Chinese population [31], melatonin receptor 1B gene (MTNR1B) [32] and transforming growth factor beta 1 (TGFB1) association with susceptibility to this disorder [33] have been described. However, it was later observed that all these genes could only play a role as prognostic markers for specified ethnicities, thus limiting their development as reliable biomarkers. Worth mentioning is the first genome-wide association study (GWAS) related to AIS. It revealed rs1400180 of CHL1 manifesting a strong association with AIS among Caucasians [34], but failed to show any such association in the Chinese population [35]. Similar findings have been reported for the MTNR1A [35] and DSCAM [36] genes. Such results have posed a dilemma for existing and ongoing studies related to AIS.

FUTURE GENETIC TEST FOR ADOLESCENT IDIOPATHIC SCOLIOSIS

The SoliScore™ is the only prognostic or diagnostic testing available for AIS. However, there are several unanswered questions regarding the ScoliScore™ algorithms, and researchers are striving to identify a unique test that can provide a reliable linkage with the onset of AIS. In this regard, comparative genome-wide association studies (GWAS) of healthy and diseased individuals were expected to generate some conclusive information for the AIS.

With the passage of time, it is becoming clear that it would not be possible to devise a global single prognostic molecular test based on the existing scientific information across the globe. More likely, the prognostic identification of the AIS will be ethnicity-based. This will make clinical management very much challenging for clinicians. This has led to several completed and ongoing molecular diagnostic studies of AIS taking into consideration ethnicity. Researchers are even now sensitive to the sex issue also, with some studies focusing on either adolescent boys or girls. One such GWAS study evaluated AIS in a cohort of 4,317 girls manifesting the disease and 6,016 controls. All of them represented the Chinese Han population. A thorough evaluation of SNPs lead to the identification of three SNP loci at 1p36.32 near *AJAP1*, 2q36.1 between *PAX3* and *EPHA4* and 18q21.33 near *BCL-2* [37]. This study is considered the first one in this area. The findings of this study further add to the complexity of molecular prognosis in AIS.

The continued disappointments in biomarker studies for AIS have compelled the researcher community to look into alternative molecular signatures instead of just focusing on genomic information. Among these changes in the pattern of up- and down-regulation of various molecular moieties among individuals suffering from this disease may provide some useful information. A comparative study evaluated the cartilage oligomeric matrix protein (COMP) expressing gene promoter methylation pattern among 50 AIS patients and 50 healthy controls. Data revealed COMP gene promoter methylation values for the AIS and control individuals values to be 12.26 ± 2.36 and 8.76 ± 1.94, respectively ($p < 0.0001$). Further statistical procedures also confirmed that promoter methylation has important implications and a high correlation with the Cobb angle of the main curve. A higher methylation of the COMP promoter is reflected in the form of reduced gene expression [38, 39]. The authors conclude that such observations show important future molecular prognostic features intended for predicting AIS [40]. This study also alludes that COMP methylation can not only serve as a susceptibility marker. It can also help in the evaluation of curve progression among patients. However, the findings of this study have certain limitations. One is the overall sample size, and the other issue is whether the results of this study can be extrapolated. Although, these findings are quite intriguing, they need further validation.

A recent interesting study reports on the association of microRNA (miRNA) with AIS. miRNAs are small non-coding RNA molecules regulating gene expression. A GWAS led to the identification of a genomic locus MIR4300HG, the host gene of micro RNA MIR4300. A decrease in the genomic region of MIR 4300HG, i.e. rs35333564, manifested a strong linkage with AIS progression [41]. These findings are quite intriguing against the background of failure to identify a candidate gene linked with AIS.

As AIS is a developmental abnormality, studies aimed at evaluating molecular moieties relevant to the development pathways will be quite of interest. Towards this end, a GWAS revealed linkage between the Wnt/beta-catenin pathway and the development of AIS. It is essential to take into consideration here that the Wnt family of signaling proteins are involved in cell proliferation, polarity and ultimate commitments towards differentiation besides playing an essential role in cellular homoeostasis [42]. Of importance, the role of Wnt pathway in bone development

further supports its role in AIS, as the disorder mainly involves hard tissue deformities [43].

Genomic data has led to several new options for biomarker discovery. Notably, the issues relevant to AIS genomics and biomarker discovery are entering into new discovery phases and alternative methodologies to predict specific unique biomarkers that can be strongly linked to the disease. Metabolomics involves the study of metabolites within cells, biofluids and body tissues produced as a result of biochemical processes. Importantly, a disease condition is always linked with differential metabolic pathways when compared with healthy controls. A study examining the serum metabolic profile of 30 AIS patients with 31 healthy controls through state of the art methodologies involving high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry identified seven metabolites with differential profiles among AIS patients. These potential diagnostic biomarkers (PC(20:4), 2-hexenoylcarnitine, beta-D-glucopyranuronic acid, DG(38:9), MG(20:3), LysoPC(18:2) and LysoPC(16:0)) suggest that AIS sufferers are subject to disrupted lipid metabolism [44]. Differential metabolite expression information generated from this study corroborates with the elevated expression of triglyceride and hormone-sensitive lipase, further strengthening the validity of these findings. The findings of this study are also interesting and bring the researcher to the point of considering whether biomarkers for AIS should be identified through direct or reverse genomics. The global validity of these observations is yet to be confirmed. Whatsoever will be future outcome of this investigation, it may be concluded that molecular diagnosis for AIS is a quite a possibility; however, the options might differ.

CONCLUSION

In concluding this article, it is essential to keep in mind the severity of the issue. The disease mainly impacts the life of 1-3% children aged between 10 and 16 years [45]. The outcome of AIS is not only a physical deformity and the associated symptoms; it is also related to emotional distress for the adolescent individuals. Furthermore, the controversial nature of the Scolimeter and Adams Forward Bend Test, routinely used for determining the severity of disease, is demanding new methodologies for disease characterization. The level of distrust in these currently used diagnostic methods has been manifested by the United States Preventive Services Task Force and American Academy of Family Physicians [46].

Existing data suggest that it will not be possible to develop a DNA-based unique test for the diagnosis or management of AIS. Recent interest in allied genomic methodologies like the involvement of microRNAs, changes in promoter profile and metabolic changes offers quite interesting avenues. However, the issue still lingers due to the validation of findings globally. It is important to keep in mind that our analyses should not be considered an authoritative statement that DNA/gene-based detection of AIS is impossible. Our conclusion is based on existing data. It is entirely possible that researchers have been missing specific genes or that the available technologies are not able to identify the candidate biomarker gene for AIS. Maybe with future advances, it might be possible to link AIS to a particular gene/s or specific unique DNA sequences. On the forefront of disease genetics, the issue still remains elusive and needs further investigation.

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