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Genetics

Genomic Analyses of Patients With Unexplained Early-Onset Scoliosis

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Abstract

Study Design: To test for rare genetic mutations, a cohort of patients with unexplained early-onset scoliosis (EOS) was screened using high-density microarray genotyping. A cohort of patients with adolescent idiopathic scoliosis (AIS) was similarly screened and the results were compared.

Summary of Background Data: Patients with scoliosis in infancy or early childhood (EOS) are at high risk for progressive deformity and associated problems including respiratory compromise. Early-onset scoliosis is frequently associated with genetic disorders but many patients present with nonspecific clinical features and without an associated diagnosis. The authors hypothesized that EOS in these patients may be caused by rare genetic mutations detectable by next-generation genomic methods.

Methods: The researchers identified 24 patients with unexplained EOS from pediatric orthopedic clinics. They genotyped them, along with 39 connecting family members, using the Illumina OmniExpress-12, version 1.0 beadchip. Resulting genotypes were analyzed for chromosomal changes, specifically copy number variation and absence of heterozygosity. They screened 482 adolescent idiopathic scoliosis (AIS) patients and 744 healthy controls, who were similarly genotyped with the same beadchip, for chromosomal changes identified in the EOS cohort.

Results: Copy number variation and absence of heterozygosity analyses revealed a genetic diagnosis of chromosome 15q24 microdeletion syndrome in 1 patient and maternal uniparental disomy of chromosome 14 in a second one. Prior genetic testing and clinical evaluations had been negative in both cases. A large novel chromosome 10 deletion was likely causal in a third EOS patient. These mutations identified in the EOS patients were absent in AIS patients and controls, and thus were not associated with AIS or found in asymptomatic individuals.

Conclusions: These data underscore the usefulness of updated genetic evaluations including high-density microarray-based genotyping and other next-generation methods in patients with unexplained EOS, even when prior genetic studies were negative. These data also suggest the intriguing possibility that other mutations detectable by whole genome sequencing, as well as epigenetic effects, await discovery in the EOS population.

Keywords: Early-onset scoliosis; Microarray; Genotyping; Copy number variation

Introduction

By definition, early-onset scoliosis (EOS) affects children up to 5 years of age. In surgical cohorts, reported mortality rates vary but are as high as 18% compared with 0.08% in the general United States population [1,2]. Children with EOS can pose a significant and challenging clinical problem because they are at risk for pulmonary compromise as well as other growth disturbances [3]. In

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extreme cases, EOS can lead to thoracic insufficiency syndrome, in which the thorax is unable to support normal lung growth and function [4]. Consequently, intense effort has been given to developing surgical methods and devices that preserve lung function and growth while controlling deformity [5-7]. The pathogenesis of EOS is heterogeneous because these patients represent numerous underlying diagnoses that generally divide into 3 classes. One class of EOS is congenital scoliosis, in which deformity is caused by vertebral anomalies or segmentation defects. Although congenital scoliosis can be clearly heritable, it is often sporadic and may result from gene–environment interactions [8]. A second class of EOS is due to known heritable syndromes, many of which are well-recognized and diagnosed by clinical genetic testing, such as Ehlers-Danlos and Larsen syndrome [9]. However a significant fraction, roughly one third of surgical cases, is without an identifiable diagnosis and is therefore described as idiopathic. Historically, idiopathic scoliosis (IS) has been described by the terms ''infantile'' (onset age 0–3 years), ''juvenile'' (onset age 4–9 years), or ''adolescent'' (onset age 10 years or older) [10]. However, EOS nomenclature derives more from the natural history of spinal growth and deformity. Here, the term ''unexplained EOS'' is used to avoid confusion with previous nomenclature and to include all EOS children who may have associated growth issues but have not been ascribed a clear underlying diagnosis.

Unlike later-onset AIS, unexplained EOS rarely presents with a positive family history of scoliosis and may affect boys more than girls [11]. The perception of low heritability in EOS has invoked environmental explanations, including fetal crowding in the womb or positioning of the child in the crib [10,11], but these theories have not been substantiated. For many patients, postnatal disease onset, coupled with particularly malignant deformity progression, argues that EOS is likely genetically driven. Although comprehensive population studies are few, the prevalence of unexplained EOS has been cited as less than 1% of the total IS population [10]. The authors hypothesized that EOS could arise from rare *de novo* mutations: in other words, mutations that are absent in the parents and the general population but are present in the affected offspring. They also hypothesized that such mutations are likely to be heterogeneous: that is, to correspond to many different causal genes, reflecting the clinical heterogeneity observed in this population.

Many mutations that would be missed by traditional techniques are discoverable using methods that search the chromosomes more comprehensively. One method, high-density microarray-based genotyping, enables testing of greater than 1 million single nucleotide polymorphisms (SNPs) spaced across the genome. Measuring SNP content and signal intensity reveals gains and losses of genetic material known as copy number variations (CNVs), typically at higher resolution than a traditional karyotype [12,13]. Microarray-based genotyping also yields information about SNP heterozygosity. A heterozygous SNP harbors different sequences at the same location, indicative of the inheritance of 2 chromosomes. Absence of heterozygosity (AOH) may be an indication of genetic aberration. Large, contiguous regions of AOH genome-wide suggest parental consanguinity. Absence of heterozygosity in specific chromosomal regions may indicate abnormal chromosomal inheritance, as in Prader-Willi and Angelman syndromes [14], or loss of genetic material owing to deletion. These applications of microarray-based genotyping have been shown to increase the likelihood of finding the genetic cause of congenital structural anomalies or neurocognitive disorders, and the method is typically the first-tier genetic testing approach in these populations [15]. Therefore, the authors assessed a cohort of 24 probands with unexplained EOS using microarray-based genotyping to test the hypothesis of rare causal mutations. Subsequently, they screened a large cohort of AIS patients and controls to assess whether variation in EOS genes are more generally associated with IS.

Materials and Methods

Study design

This was a genome-wide microarray genotyping discovery study to assess rare *de novo* CNVs and regions of AOH in a cohort of 24 EOS cases. A follow-up screening study tested EOS-associated CNVs and regions of AOH in 482 AIS patients and 744 controls.

Patients

The Texas Scottish Rite Hospital for Children (TSRHC) Idiopathic Scoliosis DNA registry was established in 1997. From 1997 to 2013 87 EOS and 4,292 AIS patients were treated in TSRHC pediatric orthopedic clinics. A total of 41 EOS and 1,913 AIS probands, plus additional family members, were enrolled into the registry during that time. Informed consent was obtained from all participating research subjects as specified by the University of Texas Southwestern Medical Center Institutional Review Board. Twenty-four unrelated EOS probands and 39 parents were included in the current study. The ethnic composition of the EOS cohort was: Asian/Pacific Islander (1), Hispanic/Latino (4), black, non-Hispanic (2), white, non-Hispanic (16), and other (1). Follow-up studies included 482 AIS cases, all non-Hispanic white ethnicity. All affected subjects in these cohorts met the standard criterion for a positive diagnosis of IS: lateral deviation from the midline greater than 10°, as measured from standing spinal radiographs, axial rotation toward the side of the deviation, and exclusion of all other etiologies. For the purposes of this study, the authors elected to require a minimum Cobb angle of 15°, given the known inter/intra-observer variance.

Controls

Unaffected control individuals (n = 744) were identified from within the local Texas population or nonorthopedic