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Basic Science

Understanding the Basis of Genetic Studies: Adolescent Idiopathic Scoliosis as an Example

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Abstract

Study Design: A review of the general concepts of genetics studies with specific reference to adolescent idiopathic scoliosis (AIS).
Objectives: To equip the average spine surgeon with the vocabulary and understanding needed to understand the genetics of scoliosis and the approaches used to identify risk genes.
Summary of Background Data: Adolescent idiopathic scoliosis is a multifactorial disease. Increasing evidence from families and monozygotic twins suggests the involvement of genetic factors. An estimation of heritability also indicates a strong influence of genetics on the disease. Increasing focus has been placed on identifying genes and genetic variants associated with AIS.
Review: This is a review of genes and genetic variations, the phenotype definition of AIS in genetics studies, concepts and approaches to identifying associated genes, and the evaluation of results. Different types of genetic variations are present in the genome. These variations may modulate the expression or function of protein products, which in turn alter individuals' susceptibility to disease. Identifying the variants related to AIS requires an objective and clearly defined phenotype, among which the Cobb angle is commonly used. The phenotype helps classify subjects into cases and controls. By selecting candidate genes of growth factors and hormonal receptors, which are speculated to be involved in the mechanism of disease, the variants within these genes were compared between cases and controls to identify any differences. Another approach was to use large families and inspect the co-segregation of variants and phenotypes. Recently, arrays covering the variants of the whole genome were developed and assist in high-throughput screening for associated genes.
Conclusions: Genetic factors have an important role in AIS. Deciphering the genes and genetic variants associated with AIS can improve our understanding of the mechanisms of the disease, as well as assist in designing treatment methods and preventive measures.

Keywords: Adolescent idiopathic scoliosis; Genetics; Association studies; Linkage

Introduction

Adolescent idiopathic scoliosis (AIS) affects about 3% of children from 10 to 16 years of age [1]. Despite many years of research, the etiology of AIS remains unknown. Neurological mechanisms, hormonal influence, growth imbalance, and biomechanical and genetic factors have been proposed to contribute to this abnormality [2]. In particular, genetics are believed to have a major role, owing to the increased incidence of AIS in families [3,4] and the high concordance rate in monozygotic twins [5,6]. The heritability of AIS was estimated to be around .88 in a Chinese sibling cohort [7] and .38 in a Swedish twin cohort [8]. Despite differences in estimated heritability, these 2 studies confirmed the presence of the effect of genetic in AIS. Focus is currently placed on locating the associated genes. This review aims to provide the basic concepts of genetics and current approaches to identifying associated genes and their applications in AIS research.

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Genes, Mutations and Polymorphisms

What are chromosomes, deoxyribonucleic acid, and genes?

Deoxyribonucleic acid (DNA) stores the instructions for development, survival, and reproduction. Deoxyribonucleic acid coils around histones in an organized manner, forming a threadlike structure of chromosomes inside the nucleus. Chromosomes exist in pairs, with 1 set originating from the father and the other originating from the mother. In humans, there are 23 pairs of chromosomes, including 22 pairs of autosomes and 1 pair of sex chromosomes. If one unwinds a chromosome, DNA appears as a long chain of 2 polymers running in opposite directions. The basic building blocks of DNA are nucleotides. Each nucleotide consists of a sugar, a phosphate, and a base. For DNA, the base can be adenine, guanine, cytosine, or thymine. Typically, adenine pairs with thymine and guanine pairs with cytosine to form the 2 complementary strands. The segment of DNA carrying information for making a protein is called a gene. Within a gene, regions of DNA sequence encoding the protein or part of the protein are called exons. Exons are flanked by introns, which may have regulatory roles. The human genome is estimated to contain 3 billion base pairs, encoding for 20,000 to 30,000 genes.

From genes to proteins

The functional unit of most genes is protein. Turning genes into proteins involves a number of well-coordinated steps (Fig. 1). A gene is transcribed to single-stranded pre−messenger ribonucleic acid (mRNA). Introns within the pre-mRNA are spliced out, and exons are joined to form mature mRNA. Various mature mRNAs can be formed from the same pre-mRNA by using different combinations of exons, a process called alternative splicing. This process gives rise to different protein products. The mature mRNA is transported out of the nucleus to ribosomes, where translation occurs. Every 3 nucleotides (codon) code for a specific amino acid. After locating the start codon, the next 3 nucleotides are matched with the anti-codon of transfer RNA, which carries along the corresponding amino acid. The same process occurs in the subsequent 3 nucleotides, and the 2 amino acids are joined together. This continues until the stop codon is reached. The amino acid chain (polypeptide) is posttranslational-modified and folded to form protein. Correct folding is essential for a protein to function properly. Any failure would result in inactive products or even lead to disease. Alzheimer disease and Parkinson disease are examples of conditions caused by the accumulation of misfolded proteins. Although each of these steps is critical for producing the desired protein products, they depend on the nucleotide sequences. Variations in nucleotide sequences may therefore affect the resultant proteins.

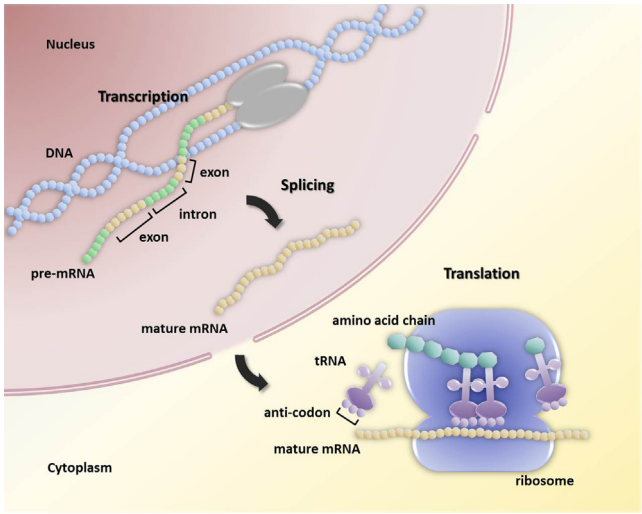

Fig. 1. Converting genes to proteins. During transcription, the genetic sequence is copied to form single-stranded pre−messenger ribonucleic acid (mRNA). The introns inside the pre-mRNA are removed and the remaining exons are joined together to form mature mRNA. This process is called splicing. The mature mRNA is transported out of the nucleus to ribosomes, where translation to protein occurs. A codon consists of 3 nucleotides in the mRNA sequence. It matches with the anti-codon of tRNA, which carries the corresponding amino acid. These amino acids are linked when reading along the mRNA. Protein is formed after posttranslational modification and folding of the amino acid chain.

Types of genetic variation

Among the genetic variations (Fig. 2), the most common type is the single nucleotide polymorphism (SNP), which involves a single nucleotide change in a defined genetic location. On average, SNP occurs every 300 nucleotides throughout the genome. It can be found in any position, and when it is present within the coding region of a gene and causes amino acid substitution, it is classified as nonsynonymous. An SNP generally has 2 alleles, meaning that 2 different nucleotides would be detected in a population. Single nucleotide polymorphisms serve as important markers for locating genetic regions associated with disease. They are also used in most case-control association studies in AIS (see below).

Another type of genetic variation is a variable number of tandem repeats (VNTR) or microsatellites. This refers to a short nucleotide sequence that repeats a variable number of times. Thus, in contrast to SNP, a microsatellite can have tens of alleles in a population. This highly polymorphic nature of microsatellites makes them informative genetic markers for linkage analysis in families, as well as for DNA fingerprinting. Whole-genome scanning of microsatellites has been applied to locate genetic regions associated with AIS.

Less common variations include deletion and insertion. They generally involve 1 or a few nucleotides, although large chromosomal segments can also be affected. Other genetic variations include duplication and inversion of DNA segments, as well as translocation of genetic materials between 2 chromosomes.