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Genes and Cognition in Williams Syndrome

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The study of genes and cognition has become an exciting field. However, genes that significantly affect cognition and behavior have been notoriously hard to locate within the human genome. Williams syndrome (WS) is a chromosome deletion disorder with interesting behavioral and cognitive phenotypic components, and the loss of genes within the WS deletion is responsible for these phenotypic characteristics. Accordingly,

the study of WS gives us the opportunity to identify, first-hand, genes that influence behavior and cognition. The identification of such genes will not only help us understand the molecular basis of WS, but will also expand our knowledge of how genes shape normal cognition and behavior.

WS was first reported in 1961 by Williams, who described children with "unusual" facial features in addition to growth
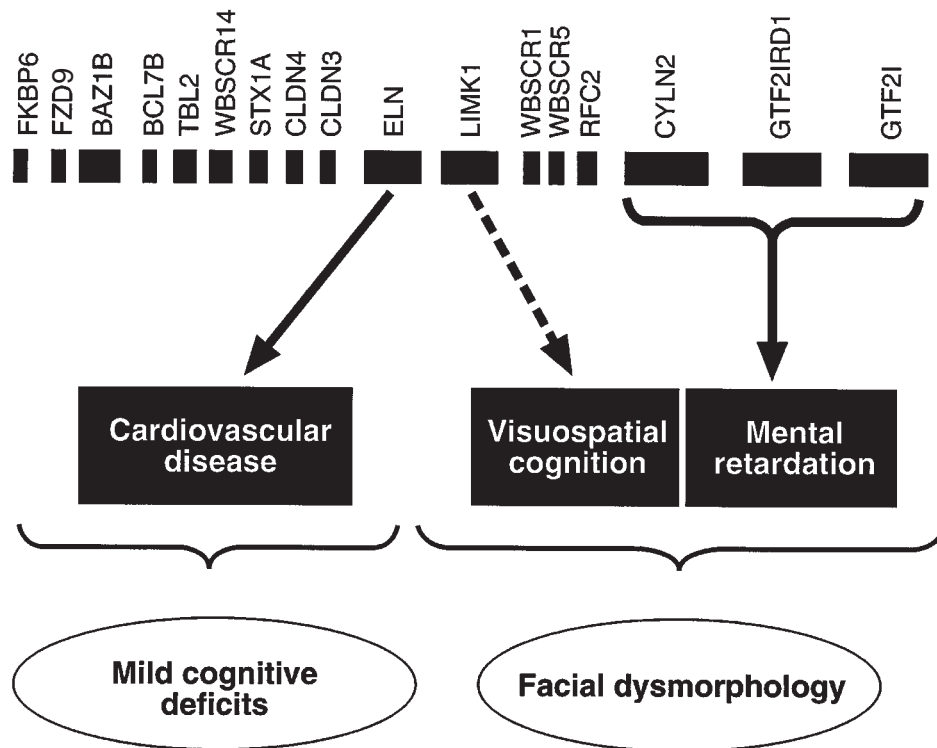
Fig. 1 Genotype–phenotype map for Williams syndrome. The 17 genes within the 1.5 Mb region commonly deleted in Williams syndrome are shown in the order in which they lie on the long arm of chromosome 7, oriented from the centromere (left) to the telomere (right). The approved symbols are shown for each gene and are as follows: *FKBP6* (FK506 binding protein 6); *FZD9* (frizzled 9); *BAZ1B* (bromodomain adjacent to a zinc finger domain 1B); *BCL7B* (B-cell lymphoma 7B); *TBL2* (transducin β-like 2); *WBSCR14* (Williams-Beuren syndrome chromosome region 14); *STX1A* (syntaxin 1A); *CLDN4* (claudin 4); *CLDN3* (claudin 3); *ELN* (elastin); *LIMK1* (LIM kinase 1); *WBSCR1* (Williams-Beuren syndrome chromosome region 1); *WBSCR5* (Williams-Beuren syndrome chromosome region 5); *RFC2* (replication factor 2, subunit C); *CYLN2* (cytoplasmic linker 2); *GTF2IRD1* (general transcription factor 2-I repeat domain containing protein 1); *GTF2I* (general transcription factor 2-I). [For a description of each gene, see the review articles by Francke (1999) and Osborne (1999).] The major phenotypic aspects of WS are shown underneath. The relationship between the genes and the clinical symptoms is shown by arrows indicating a firm relationship and by brackets indicating a less precise link. These relationships are deduced from the study of individuals with smaller deletions of the region (see text).

retardation, supravalvular aortic stenosis (SVAS), and mild mental retardation. Shortly afterward, another report by Beuren described a similar series of children with these features plus dental anomalies and friendly personalities. These characteristic symptoms are present in virtually all individuals with WS, a relatively rare disorder that is now known to be caused by a microdeletion on the long arm of chromosome 7 at 7q11.23.

The incidence of WS is estimated at approximately 1 per 20,000. It usually occurs in a sporadic manner, although rare cases of autosomal dominant transmission have been described. WS is associated with a recognizable facies, including stellate irides, flat nasal bridge, short up-turned nose with anteverted nostrils, long philtrum, full lips and lower cheeks, and a small chin. Cardiovascular lesions are very characteristic and are found in 80% of individuals. These present as a generalized arteriopathy leading to vascular stenoses, most frequently of the ascending aorta (SVAS) or peripheral pulmonary arteries, and also to hypertension. SVAS is the major life-threatening component of the WS phenotype but can be corrected with surgery. Other symptoms include dental problems such as malocclusion, small and missing teeth; growth deficiency; hypersensitivity to sounds such as sirens, vacuum cleaners, and thunder; hypercalcemia, vomiting, constipation, and colic in infancy; musculoskeletal abnormalities; impaired visual acuity; and a hoarse, low voice.

Individuals with WS also tend to have mild mental retardation (average IQ is between 55 and 60). In addition to impaired cognition, patients show hyperreactivity, sensory integration dysfunction, delayed expressive and receptive language skills, and multiple developmental motor disabilities affecting balance, strength, coordination, and motor planning. Approximately 70% of individuals with WS suffer from attention-deficit/hyperactivity disorder, and there is a high incidence of anxiety and simple phobias. A striking aspect of the WS phenotype is the coexistence of an anxiety disorder with a friendly, socially engaging personality (Pober and Dykens, 1996).

The most common cardiovascular lesion found in WS (SVAS) also exists as a distinct autosomal dominant disease. In 1993, the elastin gene was implicated in the pathogenesis of SVAS by the identification of a family with SVAS and a disruption of the elastin (*ELN*) gene at 7q11. The deletion of the same gene in two unrelated SVAS families and the subsequent identification of point mutations in sporadic cases supports the hypothesis that mutations in *ELN* are the cause of SVAS. Analysis of the region surrounding *ELN* in patients with WS demonstrates that the majority of individuals harbor a large deletion spanning approximately 1.5 Mb of DNA. This deletion spans many genes that contribute to the additional clinical symptoms seen in WS, compared with individuals with isolated SVAS.

Since the WS deletion was first identified, various groups have built a framework of genomic clones across the region and used these as the basis for gene discovery. A total of 17 genes have now been shown to lie within the common deletion, but none except *ELN* has been definitively shown to contribute to any of the symptoms. The genes code for proteins that span a large range of cellular functions, including some whose function remains unclear. Efforts to link individual genes with specific parts of the WS clinical picture have followed two paths: the study of individuals with atypical deletions of the region and the study of the protein products themselves.

In the vast majority of individuals with WS, the deletion breakpoints cluster within a small stretch of DNA, resulting in the same-sized deletion. This homogenous-sized deletion is thought to occur because of unequal crossover during meiosis, a mechanism that has been shown to be responsible for other deletion disorders, including DiGeorge syndrome, Smith-Magenis syndrome, and some forms of neurofibromatosis type I. As a result of the presence of a "common" deletion, genotype–phenotype correlation in WS has been problematic. Groups throughout the world have been searching for the rare individuals who harbor smaller deletions of the region, and they have succeeded in identifying only a few. These individuals can be separated into two distinct subgroups: individuals who have classic WS but have shorter deletions and individuals who do not have classic WS but have deletions involving the WS region.

Two individuals with WS have been reported whose deletions are smaller than the common one (Botta et al., 1999). Both children were from Italy and, although young, they appeared to exhibit most of the main features of WS (typical facies, cardiovascular abnormalities, cognitive impairment, hyperactivity). The elder of the two, who was 6 years old upon examination, showed the characteristic cognitive and behavioral profile, with more pronounced deficits in visual-spatial skills and a friendly but anxious personality. These children had a similar-sized deletion of the WS region that shared a telomeric breakpoint with the common deletion but had a unique centromeric breakpoint. At least six genes that were usually deleted in WS were not deleted in these children, suggesting that these genes do not contribute to the major features of WS.

There have been nine identified in the other group of atypical individuals, with aspects of the WS phenotype and deletions involving the WS region. The deletions remove from 2 to 15 of the commonly deleted genes, but none of the individuals has a classic WS phenotype. In an effort to try to dissect the parts of the WS behavioral or cognitive profile, psychologists have developed tests designed to pick up cognitive deficits that are characteristic of WS, such as poor visuospatial skills (Mervis et al., 1999). It is postulated that by reducing the complexity of the cognitive profile, a genotype–phenotype relationship may be established for some aspects of WS.

The picture derived from these smaller deletions is far from clear, however. In 1996 two large families were identified with deletions involving only *ELN* and a neighboring gene, LIM kinase 1 (*LIMK1*) (Frangiskakis et al., 1996). The majority of individuals from these kindreds had SVAS, as would be pre-

dicted because *ELN* was disrupted, but also showed an impairment in visuospatial skills that was comparable with that seen in WS. This finding led the authors to conclude that the LIMK1 protein was intimately involved in proper visuospatial cognition. The role of *LIMK1* in neurons, as a key molecule in the cycle of building and dismantling the actin cytoskeleton, supported this conclusion. However, three additional individuals with deletions encompassing *LIMK1* were identified in 1999, but none of them showed any visuospatial impairment (by the same testing methods), refuting the *LIMK1* hypothesis (Tassabehji et al., 1999). The contribution of *LIMK1* to WS is, therefore, still under debate. It is clearly an excellent functional and positional candidate for playing an important role in neuronal development and function, but we await the results of further experiments, including an animal model, to define its role in WS.

Taking the deletion data as a whole, a preliminary map of the genotype–phenotype relationships seems to be emerging (Fig. 1). This map suggests that the genes toward the telomeric end of the deletion may play a larger role in the development of many of the classic WS features, and particularly the cognitive profile. Firm conclusions, however, cannot be based on the small number of reported individuals with atypical deletions, particularly when there is the possibility of somatic mosaicism, an occurrence that has recently been reported in DiGeorge syndrome.

Further insight into the contribution of particular genes to the cognitive profile might be gained by studying the deleted genes themselves, in an attempt to identify functional candidates rather than positional candidates. Of the 17 commonly deleted genes, the majority are expressed at some level in the central nervous system. A few are expressed at high levels, or exclusively in the brain, which makes them more attractive candidates, but this does not exclude the remaining genes. Cytoplasmic linker 2 (CYLN2) is found only in the brain, where it is thought to link specific organelles within neurons to the cytoskeleton (De Zeeuw et al., 1997). How a reduction in this protein could affect brain function is still unclear. Syntaxin 1A (STX1A) is another protein that is found almost exclusively in neurons, and its function is better understood (Osborne et al., 1997). STX1A is a key component of a protein complex that mediates the release of neurotransmitters across the synapse, thus conveying chemical signals from one neuron to another. Studies in model organisms such as flies and worms have shown that the amount of this protein is critical to its proper function, suggesting that a 50% reduction, as seen in WS, could cause a clinically relevant phenotype.

Predictions cannot be made about the function of many of the remaining genes, but several are thought to be transcriptional regulators. Most are these are widely expressed, but it is quite possible that reducing the level of a particular transcription factor could have different effects in different tissues, ranging from inconsequential to severe. We must also consider the potentially additive effects of deleting many genes at once. This may be particularly pertinent to a pair of genes adjacent to the telomeric boundary of the deletion, whose protein products are similar in both structure and function, which means that they may be at least partially functionally redundant. Reducing the amount of either gene individually may not produce a noticeable phenotype, but in combination the effect is considerable. These genes, general transcription factor 2I (*GTF2I*) (Cheriyath and Roy, 2000) and *GTF2I* repeat domain containing protein 1 (*GTF2IRD1*) (Bayarsaihan and Ruddle, 2000), code for general transcription factors that mediate the activation of transcription of a wide variety of genes with the help of other spatially or temporally regulated transcription factors.

Research into the basic molecular function of each of the candidate genes will provide valuable information, but perhaps the greatest insight may come from the generation and study of animal models. Mouse models have become powerful tools for investigating both the molecular and physiological basis of human genetic disease. The mouse genome can be easily manipulated to produce either single-gene knockouts or larger alterations that remove several genes at once. This technology gives us the opportunity to engineer WS deletions of choice, instead of relying on the rare atypical deletions that we can identify in humans. In addition, inbred laboratory strains of mice have homogenous genetic backgrounds, eliminating the influence of genes outside the WS deletion region on development of the phenotype. Many sophisticated behavioral analyses can be performed on mice in order to assess their cognitive abilities, so we should be able to model at least some of the WS cognitive and behavioral profile.

In summary, there is still not a clear picture of the genetic basis for the WS cognitive phenotype. No single gene can yet be excluded from a role, however minor, in WS. It is likely that the cognitive impairment is the result of the deletion of several genes, although specific components of the impairment, such as visuospatial deficits, may be attributable to a single gene. The correlation of specific genes from within the WS deletion with cognitive impairment may bring to light some interesting and unsuspected culprits that will give us entry points into novel biological pathways.

WEB SITES OF INTEREST

http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?194050
http://www.williams-syndrome.org/
http://www.neosoft.com/~rlpierce/wgene.htm
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