Deleterious mutation in GPSM2 identified as cause for nonsyndromic deafness

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Whole exome sequencing and homozygosity mapping identify mutation in the cell polarity protein GPSM2 as the cause of nonsyndromic hearing loss DFNB82

Walsh et al. (2010)
The American Journal of Human Genetics 87: 90–94

Nonsyndromic deafness is one of the most prevalent sensory disabilities in humans and, in contrast to syndromic deafness, it is not associated with malfunctions in other parts of the body (1). An estimated number of 278 million people worldwide are affected by syndromic or nonsyndromic deafness. Most cases occur in regions of the Middle East and India, where consanguinity leads to larger numbers of inherited hearing loss. Impaired hearing has a profound impact on people’s life; it leads to educational and developmental delays and often to social stigmas and exclusion.

Most cases are caused by genetic factors and are mainly autosomal recessive.

Defects in the inner ear or degeneration of the cochlear sensory neuroepithelial hair cells are known to be the most frequent cause for hearing loss, but identifying the responsible mutations has been a major challenge. To date, a high degree of genetic heterogeneity has been found for deafness and reflects the vast diversity of hearing associated genes.

So far, more than 100 loci have been linked to hereditary nonsyndromic deafness, which largely segregates as a monogenic trait. These chromosomal loci are usually several megabases in size and comprise numerous genes. Identifying the critical mutation from a large chromosomal region has therefore been difficult.

A new cost-effective and promising approach for determining disease-causing alleles is sequencing the exome, the protein-coding portion of the genome. In most cases, it is sufficient to focus on the exome because many of the variants underlying genetic disorders are located in protein-coding sequences or splice donor and acceptor sites. Mutations in these coding regions are predicted to be deleterious, in contrast to variants in noncoding sequences, which generally lead to weaker phenotypes.

In a recent study, Walsh et al. (2) aimed to identify the causative mutation for nonsyndromic hearing loss DFNB82 using exome sequencing in combination with homozygosity mapping in a consanguineous Palestinian family. Among multiple polymorphisms in the 3.1-megabase homozygous region on chromosome 1p13.1, which contains 50 annotated protein-coding genes, the authors determined a single deleterious mutation by exome sequencing (Fig. 1). This nonsense mutation was located in the G protein signaling modulator, GPSM2, also known as LGN (Leu-Gly-Asn repeat-enriched protein) and Pins (human homolog of Drosophila, Partner of Inscuteable). The DFNB82 mutation is predicted to produce a premature truncation and malfunction of the protein (Fig. 1). It is known that GPSM2 is crucial for establishing and maintaining cell polarity and spindle orientation together with the nuclear mitotic apparatus protein NuMA (3). Actins and the planar polarity of hair bundles enable coordinated reaction to mechanical triggers in hair cells. GPSM2 acts by modulating the activity of heterotrimeric G proteins, which transduce extracellular inputs received by cell surface receptors into integrated responses in the cell.

To evaluate the expression patterns of GPSM2 in the inner ear, the authors measured the distribution of GPSM2 in inner ear sections of mice in different developmental stages using immunohistochemistry. In the early stages of development, GPSM2 was located at the apical surfaces of hair and supporting cells of the cochlea, utricle, saccule and cristae. GPSM2 was most frequently transcribed during embryonic development, and its expression decreased in later stages on development. In adult mice, the protein disappeared at the apical surface of the cells, but persisted in pillar cells, in which it was specifically concentrated in the head region cells. The authors conclude that GPSM2...
is of crucial importance for the development of hearing and suggest that its misregulation leads to inadequate cell polarity, impaired hair cell transduction, and ultimately to hearing loss.

The recognition of a deleterious mutation in the signaling modulator GPSM2 as the major cause for hearing loss in patients with DFNB82 will help diagnose nonsyndromic deafness and adds to the understanding of the hearing process.

One of the important challenges in hearing research is to find approaches to recover impaired hair cells. Work so far has focused on gene therapy (4) as well as on cell replacement therapy based on inner ear stem cells that can differentiate to hair cells and neurons (5).

Recently, it has become clear that despite mutations in the coding regions of genes being the determining factors for nonsyndromic hearing loss in many cases, epigenetic mechanisms such as DNA methylation, histone modifications, and miRNA expression are also essential for normal hearing (6). Therefore, it seems crucial to study the exome as well as the noncoding sequence to fully understand mechanisms of the inner ear and pave the road to develop therapies for nonsyndromic deafness.

References


TMEM216 joins its ciliary cousins in ciliopathies

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Mutations in TMEM216 perturb ciliogenesis and cause Joubert, Meckel and related syndromes
Valente et al. (2010)
Nature Genetics 42(7):619–625

Joubert syndrome (JBTS) was first described in 1969 by Dr Marie Joubert of Montreal, Canada, in four siblings in a large French-Canadian family. Based on this initial report and many subsequent findings, classic JBTS and Joubert syndrome and related disorders (JSRDs) are described as autosomal recessive disorders that are mainly characterized by absence or underdevelopment of the cerebellar vermis, resembling the cross section of a molar tooth (the molar tooth sign), as well as abnormal eye movements, ataxia, hyperpnea, hypertonia, retinal dystrophy, nephronophthisis (NPH), liver fibrosis, polydactyly, and mental retardation (1, 2). Several of these phenotypes are similar to those of Meckel syndrome (MKS), a lethal, recessive disorder characterized by renal cystic dysplasia, central nervous system malformations, polydactyly, and liver fibrosis. The syndrome has a mortality rate of 100% as infants are either stillborn or die shortly after birth (3). MKS has a prevalence rate of 1 in 13,250 to 1 in 140,000 live births, with a much higher prevalence of 1:6000 in Belgium and 1:9000 in Finland (3). JSRDs have a prevalence of 1:100,000 in the United States (1) but is probably an underestimate due to the wide range of symptoms.

Joubert, Meckel and related syndromes are of an emerging class of genetic disorders called ciliopathies, as the underlying cause lies in the primary cilia/basal bodies of the cell, organelles that are present in almost all cell types in the human body. These organelles are essential to many signal transduction pathways that are involved in numerous developmental processes and may explain the multisymptom nature of JSRDs and MKS. There are eight known causative genes for JSRDs, including INPP5E, AHII, NPHP1, CEP290, TMEM67/MKS3, RPGRIP1L, ARL13B, and CC2D2A, all of which are ciliary/basal body genes (2).

In this study, Valente et al. report that a previously mapped locus for Joubert syndrome, JBTS2/CORS2 on chromosome 11p12-q13.3, is allelic to the MKS2 locus mapped for MKS (Fig. 2). They determined that the two loci are in fact alleles of the gene TMEM216, which encodes for a previously uncharacterized transmembrane protein. Of their overall cohort, 20 individuals with JSRDs and 11 fetuses with MKS carried mutations in TMEM216. All nonsynonomous amino acid substitutions were in evolutionarily conserved residues and resulted in unstable proteins. Missense mutations at Arg73 were the most common mutation identified, with the R73L substitution being a founder mutation that has a carrier frequency of 1:100 in an Ashkenazi Jewish population. The G77A mutation leads to an alternative splice site usage.

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**Fig. 2.** Ideogram of chromosome 11 with location of the MKS2 and JBTS2/CORS2 loci. On the right is the genomic organization of the TMEM216 gene, depicting the start and stop codons, exons/introns, as well as locations of identified base changes that result in the corresponding amino acid substitution in brackets.
that results in premature protein termination. None of these mutations was found in over 500 controls from ethnically matched cohorts, further supporting the causal relationship between the gene TMEM216 and the two syndromes JSRDs and MSK. Intriguingly, Valente et al. found that mutations in TMEM216 also causes orofacio-digital type VI (or Varadi-Papp) syndrome (OMIM 277170), which is characterized by polydactyly and either tongue tumors or multiple oral frenula.

TMEM216 is a tetraspan transmembrane protein, which generally has four hydrophobic putative transmembrane domains that form two extracellular loops and one intracellular loop and usually participates in signaling and trafficking processes. TMEM216 localized to the base of the primary cilia or adjacent basal body in most cells and the knockdown of its expression resulted in reduced ciliogenesis and docking of centrosomes at the apical cell surface. The ciliogenesis defects of the TMEM216 knockdown in human fibroblasts were similar to the knockdown of TMEM67 (encoding the protein Meckelin), another JSRD and MKS causative gene, leading to the determination that the two proteins indeed formed a complex in vivo. The authors also discovered that losing either component of this complex resulted in actin cytoskeleton remodeling and hypeactivation of RhoA and Dishevelled, two critical components of the planar cell polarity (PCP) pathway that is essential for cell polarity, cellular morphogenesis and ciliogenesis.

In conclusion, Valente et al. provide evidence that TMEM216, as a previously uncharacterized transmembrane protein, forms a complex with Meckelin to mediate PCP signaling and is required for proper ciliogenesis, centrosome docking and actin cytoskeleton remodeling. Therefore, identification of TMEM216 mutations as a cause of JSRDs and MKS further supports the importance of signal transduction pathways that affect cell polarity and cellular morphogenesis in the pathogenesis of ciliopathies.

References

Cognitive deficits in Down syndrome: narrowing ‘Down’ to Olig1 and Olig2

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Olig1 and Olig2 triplication causes developmental brain defects in Down syndrome

Chakrabarti et al. (2010)
Nature Neuroscience 13(8):927–934

Down syndrome (DS) is a chromosomal disorder caused by trisomy 21 with prevalence as high as one in 700–800 live births (1). Patients with DS show complex phenotypes of variable severity, including reduced birth weight, craniofacial abnormalities, perinatal lethality, cardiovascular malformations, immune deficiencies, neurological structural deficiencies, increased risk of leukemia and early development of Alzheimer’s disease (2, 3). Although many phenotypes observed in patients with DS are variable, cognitive deficit is common among all patients and makes trisomy 21 the most common genetic cause of intellectual disability. The average IQ of patients is significantly lower than in the control population and cognitive impairments include deficits in spatial and long-term memory as well as difficulties acquiring new skills (4). Currently, little is known about the molecular mechanism underlying these cognitive deficits. However, individuals with DS have cellular and anatomical abnormalities in the prenatal and perinatal forebrain and cerebellum, suggesting an early neural developmental problem (5).

Given that the long arm of human chromosome (Chr) 21 contains over 300 genes, it has been challenging to identify critical genes responsible for specific disease phenotypes, including cognitive impairment. Several mouse models have aided in uncovering the genetic and molecular mechanisms underlying cognitive impairments in DS, of these, the Ts65Dn transgenic mice is the most commonly used. The Ts65Dn model was generated by a reciprocal translocation between murine Chr 16 and Chr 17 resulting in segmental trisomy for distal Chr 16 that is orthologous to the long arm of the human chromosome 21 (6). Previous expression studies have shown that trisomic genes in Ts65Dn mice have increased expression compared to control mice, similarly seen in patients with trisomy 21 (7). Furthermore, Ts65Dn mice exhibit relevant DS-like phenotypic
features, including learning and behavioral deficits, alterations in the morphology, density and distribution of synapses and a reduction in the number of excitatory synapses per neuron in the hippocampus and temporal cortex (2, 8), suggesting that dosage imbalance of genes in this trisomic region contributes to the underlying neuropathology of cognitive deficits in patients with DS.

Recent functional and pharmacological studies on Ts65Dn mice identified the imbalance between excitatory and inhibitory synapses as the functional deficit contributing to learning deficits in these mice and perhaps also in patients with DS (9, 10). In this study, Chakrabarti et al. showed that prenatal defects in neurogenesis result in a long-lasting increase in the number of specific forebrain inhibitory neurons in the dorsal neocortex, including the hippocampus, a region important in learning and memory. In addition to having more inhibitory interneurons in Ts65Dn mice compared with control mice, Ts65Dn interneurons also have the propensity to be more active under basal conditions, leading to alterations in hippocampal circuitry. The expression of Lhx6, a transcription factor preferentially expressed in progenitor cells in the medial ganglionic eminence (MGE), a region where a majority of mouse forebrain interneurons originate, was expanded in the prenatal Ts65Dn brain and results in increased progenitor pool compared with controls.

To examine the effects of genes triplicated in DS and Ts65Dn mice on neurogenesis to identify molecular targets giving rise to the imbalance of excitatory/inhibitory neurons in Ts65Dn mice, Chakrabarti et al. focused on genes that are triplicated and involved in neurogenesis. They identified that transcription of Olig1 and Olig2, basic-helix-loop-helix transcription factors implicated in generation of oligodendrocytes and neurons, was increased in the Ts65Dn MGE. Interestingly, Lhx6 transcription was regulated by Olig2 expression, thereby making overexpression of Olig1 and Olig2 strong genetic factors for increased interneuron production in Ts65Dn mice. To test the hypothesis that overexpression of Olig1/Olig2 results in overproduction of inhibitory interneurons in Ts65Dn mice, Chakrabarti et al. crossed Ts65Dn mice with Olig1+/−;Olig2+/− double heterozygous mice to generate Ts65Dn Olig1+/−;Olig2+/− rescue mutants. The number of Olig2− and Lhx6-positive cells in the MGE and the level of MGE neurogenesis in prenatal rescue mutants returned to control levels. Furthermore, the numbers of interneurons and the electrophysiological profile of these neurons were comparable with those in control mice.

These experiments provide evidence for increased dosage of just two genes, Olig1 and Olig2, as the major underlying genetic cause of altered neurogenesis in the MGE and the inhibitory neuron phenotype in Ts65Dn mice. This misbalance of excitatory/inhibitory neurons observed in Ts65Dn mice may represent the cellular dysfunction involved in cognitive impairment in individuals with DS. Interestingly, imbalance of excitation and inhibition is also implicated in other neurodevelopmental disorders, including epilepsy, autism and Fragile X syndrome. Although future studies on Ts65Dn Olig1+/−;Olig2+/− rescue mice are needed to show an actual improvement in cognitive function as well as to confirm this dosage imbalance in patients; results from this study sets the stage for potential use of Olig1 and Olig2 as biomarkers to develop appropriate early intervention for cognitive impairments and to establish molecular tools that can modulate Olig1 and Olig2 levels to ameliorate cognitive deficits in patients with DS.

References