Intellectual disability and craniofacial anomalies explained: one more gene associated with Potocki-Shaffer syndrome

References


Translocations disrupting PHF21A in the Potocki-Shaffer-syndrome region are associated with intellectual disability and craniofacial anomalies


Potocki-Shaffer syndrome (PSS) is characterized by developmental defects including multiple exostoses and parietal foramina, as well as intellectual disability (ID) and craniofacial anomalies (CFAs). This rare syndrome is caused by microdeletions of contiguous genes in the short arm of chromosome 11, and more precisely band p11.2. Genes responsible for multiple exostoses and parietal foramina in this chromosomal region have been identified: deletion of EXT2 has been found to cause multiple exostoses (1) and deletion of ALX4 to cause parietal foramina (2). The causative gene for ID and CFAs remained unknown until now.

To identify a novel causative gene, multiple unrelated subjects with similar phenotypes are required to gather strong enough evidence. In this case, the aim was to identify a single gene disrupted in three patients that presented with distinct de novo translocations. When translocations occur, parts of chromosomes are rearranged with or without exchange of material, and
such exchanges can disrupt the genes at the breakpoint and affect their normal function.

Chromosomal analysis of three subjects revealed apparently balanced translocations. Subject 1 (DGAP012) is a white male with significant global developmental delay and multiple CFAs such as microcephaly and midfacial hypoplasia at 15 months of age. Subject 2 (MCN1762), a white female with speech development delay at 3.5 years and dysmorphic features such as microcephaly, mild midfacial hypoplasia and downturned mouth. Subject 3 (GILLE) was examined as a young girl (3) and displayed ID and CFAs, as well as characteristics suggestive of Gillespie syndrome (3). All three subjects display ID and CFAs, typical PSS features, but do not display multiple exostoses nor parietal foramina, suggesting that a gene in or near the PSS region disrupted in all three translocations is responsible for the ID and CFA phenotypes. Translocations occur between chromosomes 11 and 19 for Subject 1, 1 and 11 for Subject 2, and X and 11 for Subject 3. Deletion mapping (Fig. 2a) confirmed that all translocation breakpoints on chromosome 11 disrupted the PHF21A region, which strongly suggests that PHF21A (also known as BHC80) is responsible for the ID and CFA phenotypes in PSS. The non-translocated allele of PHF21A was sequenced and showed no alterations for all three subjects.

To investigate the function of PHF21A, in situ hybridization experiments were performed for the orthologous mouse gene (Fig. 2b). Phf21a expression was detected in the developing central nervous system (CNS) at early stages as well as in cranial bones, suggesting a particular function for Phf21a in craniofacial development. In the adult mouse brain, abundant expression was observed in the hippocampus and cerebellum. These findings suggest that expression of Phf21a is important in CNS and craniofacial development, which is consistent with the proposed role of this protein.

These findings were further supported by experiments performed on zebrafish (Fig. 2c). An antisense morpholino used to inhibit processing of the PHF21A transcript, was injected into zebrafish. This procedure caused a small-head phenotype and facial dysmorphism, which are features reminiscent of microcephaly and dysmorphism seen in the translocation subjects. Importantly, this phenotype can be rescued by introduction of the wild-type human PHF21A mRNA, which is well conserved, supporting the critical role of PHF21A in normal neuronal function and development.

The murine and zebrafish models enhance the likelihood that PHF21A is required for normal neurofacial and craniofacial development. In this case, only one functional copy of the gene is available as the other copy is disrupted by the translocation, but the phenotype is visible. This means that the protein produced by the functional copy is not enough to ensure normal function. The ID and CFA phenotypes in PSS are therefore caused by haploinsufficiency of PHF21A.

Furthermore, immunofluorescence in cultured cells with antibody for PHF21A revealed that the protein is mainly present in the nuclei, consistent with a role in nuclear processes such as chromatin remodeling. Thus, PHF21A adds to a growing list of genes associated with ID that support a critical role for epigenetics in normal neuronal and intellectual development.

**Fig. 2.** Summary of strategies used for identifying PHF21A as the gene responsible for intellectual disability and craniofacial anomalies phenotypes in Potocki-Shaffer syndrome. (a) Human subjects presented with variable intellectual disability and craniofacial anomalies such as microcephaly and midfacial hypoplasia. Deletion mapping strategy employed FISH (fluorescence in situ hybridization), BACs (bacterial artificial chromosomes) and PCR amplification to narrow down the breakpoint region down to one gene. (b) Probes of murine phf21a transcript labeled with uridine 5′-triphosphate for hybridization were used to visualize the sites where the protein is expressed. Expression was detected in the central nervous system and major cranial bones. (c) Antisense morpholinos of phf21a were injected into zebrafish. Repression of phf21a caused a small-head phenotype and facial dysmorphism. This phenotype can be rescued by wild-type human PHF21A.

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