Short Report

A novel mutation in MED12 causes FG syndrome (Opitz–Kaveggia syndrome)


Opitz–Kaveggia syndrome is a rare X-linked multiple congenital anomalies and intellectual disability disorder caused by the recurrent p.R961W mutation in the MED12 gene. Twenty-three affected males from 10 families with this mutation in the MED12 gene have been described so far. Here we report on a new family with three affected cousins, in which we identified a novel MED12 mutation (p.G958E). This is the first demonstration that other mutations in this gene can also lead to Opitz–Kaveggia syndrome. The clinical phenotype of these three new cases is reviewed in detail and compared with the previous reported cases.

Opitz–Kaveggia syndrome (also known as FG syndrome; MIM #305450) is characterized by intellectual disability, a distinctive facial appearance and behavioral phenotype, hypotonia, anal anomalies, macrocephaly, and an X-linked mode of inheritance (1). A single mutation in the MED12 gene (p.R961W) has been shown to cause Opitz–Kaveggia syndrome in six families, including the original family described by Opitz and Kaveggia (2). More families with exactly the same mutation have been described (3, 4). So far, only one other MED12 mutation (p.N1007S) has been reported; this is in Lujan–Fryns syndrome (MIM #309520), a clinically distinctive disorder that has some overlapping features (5). Here we present a Dutch family with three cousins affected by Opitz–Kaveggia syndrome, in which we have identified a novel MED12 mutation leading to p.G958E. This is the first report of another MED12 mutation that also causes Opitz–Kaveggia syndrome.

Clinical reports

The family’s pedigree and photographs of the affected cousins are shown in Fig. 1. The proband (case 1) was referred to our clinic at the age of 13 years by a child psychiatrist for further diagnostic evaluation of his developmental delay. He had been delivered by Caesarean section after a 44-week pregnancy complicated by maternal hypertension. He had no feeding problems or hypotonia during infancy. His developmental milestones were mildly delayed. During childhood he had frequent ear infections, once complicated by a febrile seizure. His full-scale IQ score was 52, with a verbal IQ of 48 and a performance IQ of 61. He had an affable personality but with...
anxiety and behavioral problems; for example, he had temper tantrums and screaming attacks, sometimes associated with aggressive and destructive behavior. Obsessive and compulsive components are also present, such as repetitive hand washing. His family history revealed that both his mother and younger sister had mild learning problems. His mother also had down-slanting palpebral fissures. His height was 164 cm (0 SD) and his head circumference was 56 cm (+0.7 SD). He had short blond hair with a frontal upsweep and a high forehead. He also had a bilateral ptosis of the eyelids, mildly down-slanting palpebral fissures, epicanthal folds, a high nasal bridge, small ears with an over-folded superior helix, full lips, a short philtrum, narrow palate, mild pectus excavatum, and one café-au-lait spot on his back. He had a normally placed anus and normal male genitalia. An ophthalmological and neurological evaluation, echocardiogram, and metabolic screen in blood and urine were all normal. He had a normal male karyotype (46,XY). A deletion 22q11 was excluded using fluorescent in situ hybridization (FISH). Analysis of the \textit{FMR1} and \textit{PTPN11} genes involved in Fragile-X (MIM #309550) and Noonan syndrome (MIM #163950), respectively, revealed no mutations.

Case 2 was 17 years old when he was first referred to us by his psychiatrist for further examination. He was delivered at term by a primary Caesarean section after an uncomplicated pregnancy. He had been born with an anal stenosis, cryptorchid testes, and a patent ductus arteriosus. He had feeding problems for the first 4 years. At the age of 3 years he was diagnosed with acute
lymphatic leukemia (T-cell ALL, FAB class L1), for which he was treated with chemotherapy. He had frequent ear infections during childhood. His motor and speech developments were delayed, and at the age of 4 years his full-scale IQ score was 67. He had a friendly personality, was cooperative, and reacted strongly to compliments. From the age of 10 years, he had recurrent episodes with a lowering of consciousness, which were diagnosed as epileptic and for which he was treated with valproic acid. An attention deficit hyperactivity disorder was diagnosed at 12 years of age and treated with methylphenidate. He also had temper tantrums and could be aggressive during such outbursts. Sometimes his behavior also had compulsive components, such as repetitive hand washing. His family history revealed that his mother had two miscarriages. At the age of 17 years his height was 179 cm (−0.2 SD) and his head circumference 58.5 cm (+1 SD). He had a strabismus, hypertelorism, down-slanting palpebral fissures, small ears, a broad nasal bridge, and a high narrow palate. There were two café-au-lait spots on his right leg. There was no hypospadias and his anus was normally placed. An ophthalmological and neurological evaluation, echocardiogram, brain computed tomography scan, metabolic screen in blood and urine, and an oral glucose tolerance test were all normal. He had a normal male karyotype (46,XY). A deletion 22q11 was excluded by FISH, and analysis of the \textit{FMRI} gene and the \textit{MID1} gene, involved in Opitz–Frias syndrome (G/BBB syndrome; MIM #300000), revealed no mutations. A whole-genome comparative genomic hybridization using a customized BAC array with an average resolution of 1 Mb also showed no abnormalities.

The youngest cousin (case 3) had been referred to our outpatient child neurology clinic at the age of 7.5 months for evaluation of hypotonia and delayed gross motor development. He had been delivered at term by a primary Caesarean section combined with a forceps extraction. During pregnancy his mother was treated for epilepsy with carbamazepine. He was born with a hypospadias, hypoplastic scrotum, cryptorchid testes, and a membranous anal atresia. His appearance at birth was characterized by a large head (head circumference at +2 SD), broad nasal bridge, telecanthus, and small ears with a dysplastic left ear. During infancy he had feeding problems for which he received tube feeding. He also had a mild respiratory insufficiency that was treated with nasal continuous positive airway pressure. An ophthalmological evaluation, echocardiogram, and renal and cerebral ultrasounds showed no abnormalities. Chromosome analysis was normal (46,XY).

At the age of 7 months he had an open mouth with down-turned corners, axial hypotonia with complete head-lag, and a positive slipping-through sign, but normal tendon reflexes. A brain magnetic resonance imaging (MRI) scan showed widened peripheral cerebrospinal fluid spaces and some minor anomalies of the corpus callosum (Fig. 2). Because of constipation and coprostasis, he was treated with clysmas, lactulose, and subsequently with macrogol. At the age of 1 year the hypotonia had improved considerably, his height was 85 cm (0 SD) and his head circumference was 51 cm (+2 SD). In addition to the earlier features, we noted a high forehead with a frontal upsweep, a short upturned nose and two café-au-lait spots on his abdomen. There was a global developmental delay with signs of attention deficit. He had an affable personality and was sensitive to compliments.

Based on these findings, the diagnosis of Opitz–Kaveggia syndrome was considered and we performed sequencing of exon 21 of the \textit{MED12} gene. This analysis revealed a novel c.2873G>A mutation, leading to a replacement of the small, hydrophobic and non-polar amino acid glycine at position 958 by the larger, hydrophilic and charged glutamic acid (Fig. 3). An alignment of the amino acid sequence of this part of the protein indicates that the glycine residue at position 958 is highly conserved in many species (Fig. 3). The
p.G958E mutation was present in all three affected boys. In contrast, this mutation was absent in 140 X-chromosomes of healthy controls. No samples of unaffected male family members were available for molecular analysis.

Discussion

The clinical diagnosis of FG syndrome has previously been difficult because the previous literature inappropriately broadened the characteristic features and failed to emphasize the key features in the absence of a confirmatory molecular marker for FG syndrome. Many subjects with intellectual disability and features such as macrocephaly, constipation, and hypotonia were given a clinical diagnosis of FG syndrome, and the phenotypic spectrum of the syndrome has been broadened over time. After their discovery of the recurrent p.R961W mutation, Risheg et al. (2) proposed that the designation of Opitz–Kaveggia syndrome should be reserved for those with both a clinical phenotype of FG syndrome and a proven MED12 mutation. By re-evaluating 30 patients with a clinical diagnosis of FG syndrome, Lyons et al. (4) showed that only a minority of them...
in fact have a MED12 mutation and that other diagnoses could often be made in those without such a mutation. The recent identification of mutations in other genes further illustrates the heterogeneity among patients previously considered to have FG syndrome (6, 7). By identifying a mutation in the MED12 gene, we were able to diagnose Opitz–Kaveggia syndrome and separate this family from the heterogeneous group of similar phenotypes.

Graham et al. recently described the behavioral phenotype, and Clark et al. delineated the clinical features and natural history of Opitz–Kaveggia syndrome in those with the p.R961W mutation (3, 8). The latter study reviewed 10 families (with a total of 23 affected males) and developed an algorithm with criteria to help identify cases with a likely diagnosis of Opitz–Kaveggia syndrome and to select patients for MED12 analysis. Our family also met these criteria (Table 1), and the three affected cousins share many features with the cases reported with a p.R961W mutation. However, we also observed some features that separate our family from the families with the recurrent p.R961W mutation. Hypoplasia or complete absence of the corpus callosum was noted in all the 13 patients with the recurrent p.R961W mutation for whom brain imaging was available. The brain MRI of case 3 showed an overall normal genesis of the corpus callosum except for some subtle abnormalities. Although genital anomalies such as cryptorchid testes and inguinal hernia are common among patients with the p.R961W mutation, hypospadias (as in our case 3) has not been reported before. Although subjective, the thumbs of the affected males did not seem remarkably broad or flat. Broad and flat thumbs were noted in 14 of the previously reported cases. The facial appearance of cases 1 and 3 is quite characteristic of Opitz–Kaveggia syndrome, but case 2 seems to be different. Moreover, an acute lymphatic leukemia (as seen in case 2) has not been reported in any other cases with Opitz–Kaveggia syndrome. This disorder could well be coincidental. Finally, case 1’s mother, an obligate carrier of the MED12 mutation, also had mild learning problems and down-slanting palpebral fissures. His younger sister had mild learning problems too, but the parents decided against genetic testing so her carrier status is unknown. Whether these features are attributable to the new MED12 mutation is uncertain. So far, no clinical signs of Opitz–Kaveggia syndrome have been reported in females with the recurrent p.R961W MED12 mutation. Previous X-inactivation studies in nine female carriers of the p.R961W mutation have shown marked skewing, moderate skewing, as well as absence of skewing (2).

The recurrent p.R961W mutation and the p.G958E mutation reported here are located close to each other in exon 21 of the MED12 gene. The p.N1007S mutation reported in Lujan–Fryns syndrome is located in the adjacent exon 22. These exons encode part of the large Leu-Ser-rich domain of the MED12 protein. MED12 is a component of the mediator complex which has a regulatory role in RNA polymerase II activity (9). Mediator acts as a facilitator or repressor of gene transcription and is involved in many developmental processes (2, 10). MED12 is a subunit of the so-called cyclin-dependent kinase (CdK) 8 module.

Table 1. Algorithm with clinical criteria for MED12 molecular testing applied to our family

<table>
<thead>
<tr>
<th>Criteriaa</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history</td>
<td>X-linked pattern of inheritance</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Required</td>
<td>1. Male sex</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. Developmental delay or cognitive impairment</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Clinical criteria</td>
<td>1. Small ears</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. Characteristic faceb</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>3. Congenital anomaliesc</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>4. Affable, eager-to-please personality</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5. Macrocephald</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6. Early hypotonia, constipation or feeding problems</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Exclusion criteriae

aWith a positive family history, four clinical criteria are required in children under age 5 years, and five clinical criteria (including criterion 4) are required in older subjects. For more details on this algorithm, see Clark et al. (8).
bDefined as narrow tall head, dolichocephaly, tall forehead, frontal upsweep, long narrow face, puffy eyelids and open mouth.
cDefined as anomalies of corpus callosum, anus, heart, or skeleton in the proband or an affected male relative through the maternal lineage. (The mild pectus excavatum in case 1 was considered a skeletal anomaly.)
dDefined as head circumference percentile greater than height percentile or head circumference above the 98th percentile.
eFemale sex, normal intelligence, affected females in the pedigree, or male-to-male transmission in the pedigree. (The mother and sister of case 1 were not considered affected because they did not have the required clinical criteria.)
of mediator. This module may act as a switch regulating transcription initiation and re-initiation events (11). It has been shown that mediator modulates Gli3-dependent Sonic hedgehog signaling and that it plays a role in the regulation of Nanog and its target genes (12, 13). The MED12/Cdk8 module also seems to be involved in the epigenetic restriction of neuronal gene expression to the nervous system (14). MED12 has been shown to be part of a protein network that links G9a histone methyltransferase activity and the RE1 silencing transcription factor (REST) with neuronal gene expression. MED12 is required for the G9a-dependent chromatin modification and gene silencing that is imposed by REST. The p.R961W and p.N1007S MED12 mutations seem to alter protein conformation (2, 5), and both mutations were shown to impair the recruitment of mediator to RE1 elements and disrupt the REST-specific epigenetic restriction of neuronal gene expression (14). It is conceivable that the new p.G958E mutation has similar effects. A misregulation of REST target gene expression might affect neuronal differentiation and could be the pathological basis for the intellectual disability and behavioral phenotypes in the Opitz–Kaveggia and Lujan–Fryns syndromes (14).

At the moment, targeted analysis for the recurrent p.R961W mutation in MED12 still seems to be a reasonable first step toward confirming the diagnosis of Opitz–Kaveggia syndrome in suspected cases. However, we have shown here that rare mutations other than the recurrent p.R961W may also cause Opitz–Kaveggia syndrome. We therefore recommend performing a broader MED12 analysis in males with a clinical phenotype consistent with Opitz–Kaveggia syndrome but lacking the recurrent p.R961W mutation.

Acknowledgements

We thank the family for their cooperation, Linda Meiners for reviewing the brain MRI of case 3, and Jackie Senior for critically reading this article.

Conflict of interest

Nothing to declare.

References