The power of the Mediator complex—Expanding the genetic architecture and phenotypic spectrum of MED12-related disorders


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MED12 is a member of the large Mediator complex that controls cell growth, development, and differentiation. Mutations in MED12 disrupt neuronal gene expression and lead to at least three distinct X-linked intellectual disability syndromes (FG, Lujan-Fryns, and Ohdo). Here, we describe six families with missense variants in MED12 (p.(Arg815Gln), p.(Val954Gly), p.(Glu1091Lys), p.(Arg1295Cys), p.(Pro1371Ser), and p.(Arg1148His), the latter being first reported in affected females) associated with a continuum of symptoms rather than distinct syndromes. The variants expanded the genetic architecture and phenotypic spectrum of MED12-related disorders. New clinical symptoms included brachycephaly, anteverted nares, bulbous nasal tip, prognathism, deep set eyes, and single palmar crease. We showed that molecular modeling (Yasara Structure) performed to model the functional effects of the variants strongly supported the pathogenic character of the variants examined. We showed that molecular modeling is a useful method for in silico testing of the potential functional effects of MED12 variants and thus can be a valuable addition to the interpretation of the clinical and genetic findings.
**1 | INTRODUCTION**

The Mediator is a large multiprotein complex that regulates gene expression in all eukaryotes and interacts with RNA polymerase II. The role of the complex involves transcriptional elongation and termination, mRNA processing, noncoding RNA activation, super enhancer formation, and epigenetic regulation. Thus, the Mediator was called an "integrative hub" for transcriptional processes and emerged as a master coordinator of cell growth and homeostasis, development, and differentiation.1 The Mediator complex (MED) consists of 31 subunits (MED1-MED31) and can be divided into four distinct modules termed as the head, middle, tail, and CDK8 kinase module. The latter module contains CDK8, cyclin C, MED12, and MED13. MED12 is a critical transducer of regulatory information essential for organogenesis. At least, three different intellectual disability (ID) conditions that are associated with MED12 mutations have been described. These conditions include Opitz-Kaveggia syndrome (FG syndrome type 1, FGS1), Lujan-Fryns syndrome, and X-linked Ohdo syndrome (OHDOX).2 These syndromes are allelic disorders that share clinical findings including ID, hypotonia, and some physical features, such as tall prominent forehead, open mouth, or high narrow palate. Probands with nonsyndromic X-linked ID (XLID), including females with variable cognitive impairment, have also been described as harboring MED12 mutations. Here, we present clinical and genetic details of six families with missense variants in the MED12 gene, as well as molecular modeling results for four variants, and expand the phenotypic spectrum of MED12-related disorders.

**2 | MATERIALS AND METHODS**

Six ID families with MED12 variants (NM_005120) were included in the study (Figure 1). Patients' phenotypes are described in File S1, Supporting information and summarized in File S2. Families 1 to 4 were collected as part of the X-exome resequencing project of unresolved families with assumed XLID, while Families 5 and 6 were sequenced in routine diagnostics. For none of the families, a clinical suggestion had been made prior to next-generation sequencing (NGS). The procedures employed were reviewed and approved by the appropriate institutional review committees. DNAs were isolated from peripheral blood according to standard procedures. Written informed consent was obtained from all individuals participating in this study. For the index probands, NGS was performed in three laboratories (Berlin, Tübingen, and Warsaw) as described in File S3. Segregation analysis was performed by Sanger sequencing for the other available family members. The pathogenicity of variants obtained from NGS was assessed by molecular modeling (Yasara Structure) as described in File S3.

**3 | RESULTS**

We report on 13 affected males and two affected females from six families with variants in the MED12 gene (Figure 1). Clinical symptoms of the affected males and females along with a comparison with symptoms described in published MED12-related syndromes are presented in Table 1. All probands had ID and developmental delay, and most of them had macrocephaly, long narrow face, prominent forehead, small ears, dental abnormalities, prognathism, and hypertelorism. The remaining clinical features were variable. The new clinical findings associated with MED12 variants (present in at least two families) were as follows: brachycephaly, anteverted nares, bulbous nasal tip, prognathism, deep set eyes, and single palmar crease. In the families, we identified three novel missense variants in MED12 (p.(Val954Gly), p. (Glu1091Lys), p.(Pro1371Ser)), one known variant but first reported in affected females (p.(Arg1148His)), as well as two variants (p. (Arg815Gln) and p.(Arg1295Cys)) which have already been described as a cohort data by the authors of the current paper, but without clinical details of the presented families.3,4

All MED12 variants identified in the families perfectly cosegregated with the phenotype, except for Family 5 in whom the MED12 variant present in the affected daughters was inherited from the healthy mother. Most of the variants were absent in the gnomAD database (except for c.2444G>A present in one heterozygous female with a minor allele frequency [MAF] of 5.599e-6 and c.4111C>T present in six hemizygous individuals with a MAF of 0.00012). All the variants were predicted as damaging by Polyphen2, MutationTaster, as well as SIFT, except for c.4111C>T identified in patient III:1 from Family 6 which had conflicting interpretations of pathogenicity (Polyphen2—benign, Mutation Taster—disease causing, SIFT—tolerated). Additionally, this variant had been reported to ClinVar as probably benign. WES (whole exome sequencing) performed subsequently in the patient revealed additionally a de novo heterozygous missense variant c.367C>T in the PUF60 gene (NM_078480.2) resulting in the p.(Arg123Trp) substitution. The variant was predicted as damaging (Polyphen2, MutationTaster, and SIFT) and it was absent in the gnomAD database.

Molecular modeling was applied to assess the functional effects of the MED12 (four of the six identified MED12 substitutions are located within the protein regions, the structure of which may be reasonably modeled by homology) and PUF60 variants identified by NGS.

**KEYWORDS**

FG syndrome, Lujan-Fryns syndrome, MED12, molecular modeling, Ohdo syndrome, X-linked intellectual disability

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1. The Mediator is a large multiprotein complex that regulates gene expression in all eukaryotes and interacts with RNA polymerase II.
2. MED12 is a critical transducer of regulatory information essential for organogenesis.
3. All MED12 variants identified in the families perfectly cosegregated with the phenotype.
4. WES performed subsequently in the patient revealed an additional de novo heterozygous missense variant in the PUF60 gene.
All the variants are expected to have substantial structural effects on proteins (Figure 2).

X chromosome inactivation (XCI) analysis performed in Family 4 revealed that the mother (II:1) of the index patient (III:2) who also carried the mutation had mildly skewed XCI (83:17) in her blood cells. In Family 5, XCI analysis revealed a mildly skewed pattern in the mother (82:18) and a skewed pattern in her two affected daughters (100:0 and 85:15). In the three females, the same allele of the androgen receptor locus was inactive.
TABLE 1  Phenotypic comparison between known MED12 related syndromes and the families presented in the study. Clinical symptoms specific for a given syndrome are coloured accordingly (FG, red; L-F, green; Ohdo, blue). Symptoms not described previously that expand the phenotypic spectrum of the MED12 variants are marked in bold. “+” in family columns denotes that at least one patient per family was diagnosed with the given feature, and “blank” denotes that none of the family members presented the given feature.

<table>
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<th>Feature category</th>
<th>Feature</th>
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<th>LFS</th>
<th>Ohdo syndrome</th>
<th>Family 1</th>
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DISCUSSION

The phenotypic spectrum of MED12-related disorders is still being expanded as new families with MED12 variants are being identified due to the improvement of new sequencing technologies. Moreover, it was pos-
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toms. Thus, it is more appropriate to indicate only a ‘MED12-related disor-
der’ than to attribute a definite syndrome for a majority of the subjects.

To date, over 20 MED12 genetic variants have been reported, which are evenly distributed with no apparent hot spots in specific exons (Figure 1). All but one were missense variants causing FGS1, Lujan-Fryns, or Ohdo, but they rather present, similar to recently published data, a continuum of symp-
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<td>Sparse eyebrows</td>
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<td>Hyperextensible joints</td>
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<td>Urinary incontinence</td>
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<td>Behaviour</td>
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<td>Hyperactive, aggressive, shy</td>
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Abbreviations: ID, intellectual disability; EEG, electroencephalogram; LFS, Lujan-Fryns syndrome; MED12, Mediator complex subunit 12.
molecular modeling and combined they better explain the phenotype than each individually. Thus, it is possible that the proband from Family 6 presents a complex phenotype caused by these two variants. Such digenic inheritance has recently been postulated as an important cause of ID.

Currently, the reports on MED12-related clinical phenotypes in females are rather scarce. With the current knowledge, pathogenicity of the first reported MED12 p.(Arg1148His) substitution in affected females (Family 5) and assumed as causing Ohdo syndrome in males only, by clinical, genetic, and functional findings remains to be established.\(^7\) Segregation analysis revealed that the variant was inherited from their healthy mother. Although MED12 is subject to X-inactivation, there was no clear correlation between the clinical phenotypes of the MED12 carrier females from Family 5 and their XCI pattern in blood cells, similar to the results published previously.\(^8\) It seems that blood DNA-based X-inactivation does not predict clinical outcome in MED12-related carrier females and different affection statuses in females might be related to varying X-inactivation in the brain. Thus, it is possible that similar to other genes initially implicated in X-linked recessive disorders in males, missense variants in MED12 may predict a potential risk for phenotypic expression in carrier females. This assumption needs to be more firmly established in the future, and if confirmed would be important for genetic counseling and prognostic evaluation.

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**Conflict of interest**

The authors declare no potential conflict of interests.

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**REFERENCES**


SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section at the end of the article.