Novel pathogenic EIF2S3 missense variants causing clinically variable MEHMO syndrome with impaired eIF2γ translational function, and literature review

Urania Kotzaeridou | Sara K. Young-Baird | Vanessa Suckow
Alexis G. Thornburg | Matias Wagner | Inga Harting
Stine Christ | Tim Strom | Thomas E. Dever | Vera M. Kalscheuer

1Division of Child Neurology and Inherited Metabolic Diseases, Centre for Paediatrics and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany
2Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, Maryland
3National Institute of General Medical Sciences, NIH, Bethesda, Maryland
4Research Group Development and Disease, Max Planck Institute for Molecular Genetics, Berlin, Germany
5Institute of Human Genetics, Technical University München, Munich, Germany
6Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany
7Institute for Neurogenomics, Helmholtz Zentrum München, Neuherberg, Germany
8Department of Neuroradiology, University Hospital Heidelberg, Heidelberg, Germany

Correspondence Vera M. Kalscheuer, Max Planck Institute for Molecular Genetics, Ihnestrasse 63-73, D-14195 Berlin, Germany. Email: kalscheu@molgen.mpg.de

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1 | INTRODUCTION

The eukaryotic initiation factor 2 subunit 3 (EIF2S3) gene encodes the γ subunit of the heterotrimeric translation initiation factor 2 (eIF2) complex, crucial for initiation of protein synthesis and regulation of the integrated stress response (ISR). Pathogenic EIF2S3 variants have been linked with different clinical disorders, ranging from a severe neurological phenotype with severe intellectual disability (ID) and extreme microcephaly, usually as part of MEHMO (mental deficiency, epilepsy, hypogenitalism, microcephaly and obesity) syndrome (OMIM 300148),1-3 to a novel phenotype of hypopituitarism with glucose dysregulation and very mild neurological involvement.4 While severely....

Abstract

Rare pathogenic EIF2S3 missense and terminal deletion variants cause the X-linked intellectual disability (ID) syndrome MEHMO, or a milder phenotype including pancreatic dysfunction and hypopituitarism. We present two unrelated male patients who carry novel EIF2S3 pathogenic missense variants (p.(Thr144Ile) and p.(Ile159Leu)) thereby broadening the limited genetic spectrum and underscoring clinically variable expressivity of MEHMO. While the affected male with p.(Thr144Ile) presented with severe motor delay, severe microcephaly, moderate ID, epileptic seizures responsive to treatments, hypogenitalism, central obesity, facial features, and diabetes, the affected male with p.(Ile159Leu) presented with moderate ID, mild motor delay, microcephaly, epileptic seizures resistant to treatment, central obesity, and mild facial features. Both variants are located in the highly conserved guanine nucleotide binding domain of the EIF2S3 encoded eIF2γ subunit of the heterotrimeric translation initiation factor 2 (eIF2) complex. Further, we investigated both variants in a structural model and in yeast. The reduced growth rates and lowered fidelity of translation with increased initiation at non-AUG codons observed for both mutants in these studies strongly support pathogenicity of the variants.

KEYWORDS
eIF2gamma, EIF2S3, intellectual disability, MEHMO, X-linked
affected patients present with all clinical features, less affected patients exhibit only a subset of these features. It remains largely unknown why clinical severity varies between patients and how the pathogenic variants impact eIF2γ function; and it is becoming clear that the EIF2S3 variants map to distinct regions of eIF2γ, suggesting that they may impact different functions of eIF2γ.1-6

2 | METHODS

2.1 | Subjects

The study was carried out in accordance with the Declaration of Helsinki. Genetic studies were approved by the local ethical committee of the Technical University Munich (#5360/12S). Written informed consent for publication was obtained from the parents.

2.2 | Mutation identification, western blot analysis, and yeast methods

For the index patient from family 1 (Fam1) exome sequencing was performed using a SureSelect Human All Exon Kit (Agilent, 50 Mb V5) for target enrichment and a HiSeq2500 device (Illumina) for sequencing as paired end reads of 100 bp. The average coverage was 130x with more than 97% of the targeted sequence covered >20x. Segregation analysis of the EIF2S3 variant was performed by Sanger sequencing.

For the index patient from family 2 (Fam2) diagnostic genetic testing was performed at the Medical Genetics Center, Munich, Germany. Following NGS only EIF2S3 exons as well as flanking five nucleotides of intronic sequences were analyzed.

Lymphoblastoid cell lines from one affected male (Fam2, II:1) and controls were established by EBV transformation. Details on protein cell lysate preparation and antibodies used for western blot analysis, as well as all yeast methods, are given in the Supporting Information, Appendix S1.

3 | RESULTS

The boy from Fam1 (Figure 1A, III:1) is the first child born to non-consanguineous parents. Pregnancy was uneventful with delivery at 35 + 1 gestational weeks due to pathologic antepartal cardiotocograms. His birth length was 41.5 cm (−1.85 SD), weight 1840 g (−1.85 SD), Apgar score 8/8 at 5 and 10 minutes and occipitofrontal circumference (OFC) 27.5 cm (−3.56 SD). Postnatally he presented with respiratory distress grade 2, poor feeding, coronary hypoplasia, microcephaly and muscular hypotonia. At 7 months he was admitted for generalized tonic-clonic seizures. His development was significantly delayed. At last follow-up, epileptic seizures were well controlled under topiramate monotherapy. Non-autoimmune diabetes mellitus was diagnosed, and insulin treatment was started. He has moderate ID (FSIQ 40, WISC-IV test) with autistic features. Language skills are limited to less than five words. He does not walk independently and cannot perform any daily tasks. Brain magnetic resonance imaging (MRI) at 9 months showed delayed myelination corresponding to that normally seen at 4 to 5 months (Figure 1D, e-h). By 4.5 years myelination had progressed to what is normally seen at 9 to 10 months, but was still incomplete (Figure 1D, i-l). In addition, there was marked atrophy of supratentorial white matter. Details on white matter quantification are given in Appendix S1.

Facial and dysmorphic features include narrow forehead, full cheeks, increase in supraorbital soft tissue, relatively large ears with prominent earlobes, short philtrum, long eyelashes and thick eyebrows, micrognathia (Figure 1B, Fam1 III:1, Table 1), mild edematosus hands and feet, and tapered fingers (not shown).

Genetic testing revealed a novel maternally inherited hemizygous EIF2S3 variant interpreted as likely pathogenic (chrX:g.24078252C>T (hg19), NM_001415.4:c.431C>T; p.(Thr144Ile)) (ClinVar database accession number VCV000488501.1). There were no other potential pathogenic variants identified.

The patient from Fam2 (Figure 1A, II:1) was born at term to non-consanguineous parents after an uneventful pregnancy and normal postnatal adoption at the 39th gestational week. His birth length was 49 cm (−1.26 SD), weight 3210 g (−0.64 SD), Apgar score 10/10 at 5 and 10 minutes and OFC of 33 cm (−1.77 SD). At 3 months mild developmental delay and microcephaly were noticed and at 6 months he developed therapy-resistant epileptic seizures with generalized tonic-clonic, but also myoclonic and absence seizures. EEG showed a severe deterioration in the following year with the complete loss of the physiologic background activity and pathologic sleeping pattern. Seizures were refractory to antiepileptic drugs. He can walk some steps by himself showing ataxic components, has mild motor delay with muscular hypotonia, can sit free and grab for things. He does not speak, is adipsic and suffers from snoring and sleep apnea. MRI at 22 months (Figure 1D, m-p) revealedatrophy of supratentorial white matter with thin corpus callosum, widened ventricles, and increased bicaudate ratio. Details on white matter quantification are given in Appendix S1. The anterior pituitary appeared relatively small. Facial features include relatively large ears, epicantthus, full cheeks, increase in supraorbital soft tissue, thin upper lip and short philtrum (Figure 1B, Fam2 II:1, Table 1). His ID is moderate and he has behavioral problems.

EIF2S3 sequence analysis revealed a novel variant interpreted as variant of uncertain significance (ACMG class 3) (chrX:g.24078296A>T (hg19), NM_001415.4:c.475A>T; p.(Ile159Leu)). There were no other potential pathogenic variants identified.

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Both variants affect highly conserved amino acids (Figure S1A) and are not present in control databases including 1000 Genomes and gnomAD.

In addition, by western blot analysis of protein cell lysate from lymphoblastoid cells of the affected male from Fam2 we could show that mutant eIF2γ protein is present (Figure 1C).

Consisting of distinct α, β, and γ subunits, the stable eIF2 heterotrimer binds GTP and the initiator Met-tRNAiMet to form a ternary complex, which then binds to the small ribosomal subunit.7 The eIF2γ subunit consists of an N-terminal G domain followed by two β-barrel domains (Figure 2A,B). The residue I159 (yeast I218) lies at...
the end of strand β6 (Figure 2B), which helps buttress the position of the NKxD motif that contributes to guanine specificity and nucleotide binding affinity. Mutation of this residue could alter the position of the NKxD motif, and thereby affect GTP binding. The T144 residue (yeast T203) is located at the C-terminus of the Switch 2 (Sw2) element (Figure 2B) that responds to GTP vs GDP binding. Mutation of T203 might impair eIF2 function by weakening GTP binding or by disrupting structural transitions necessary for binding Met-tRNA\textsuperscript{Met}.

To test if the I159L and T144I mutations impair eIF2 function, analogous mutations were introduced into yeast eIF2γ. Like the eIF2γ-I318M and eIF2γ-V281K mutations, corresponding to the MEHMO mutations I259M and I222T,\textsuperscript{1,5} the yeast I218L (human I159L) mutation conferred a significant slow-growth phenotype in yeast (Figure 2C, rows 1, 5, 7, 10). Whereas the yeast T203I (human T144I) mutation did not impact yeast cell growth (Figure S1, row 6), substitution of Ala (T203A; Figure 2D, row 10) but not Lys (T203K, Figure S1B, row 8) conferred a slow-growth phenotype.

Overexpression of tRNA\textsuperscript{Met} and eIF2β were previously shown to suppress the slow-growth phenotypes associated with the yeast eIF2γ-I318M (corresponding to human I259M, impaired for Met-tRNA\textsuperscript{Met} binding) and eIF2γ-V281K (human I222T, impaired for eIF2β binding) mutations (Figure 2C, D, rows 5-9), respectively.\textsuperscript{1,5} Intriguingly, overexpression of tRNA\textsuperscript{Met}, but not eIF2α or eIF2β, enhanced the growth of the eIF2γ-I218L and eIF2γ-T203A mutant strains to

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**FIGURE 1**

A. Pedigrees of family 1 and 2 (Fam1, Fam2) and Sanger sequencing chromatograms. WT, wild-type; Mut, mutation carrier. B. Photographs of affected males. C. Western blot of protein cell lysate from the affected male from Fam2 (2:II:1) and controls (C1-C3) immunoblotted with the indicated antibodies. D. MRI scans of control (a-d) and patients (e-p). Myelination in the affected male from Fam1 is delayed at 9 months and still incomplete, although progressing at 4.5 years. The corpus callosum is already thin for age at 9 months and does not significantly increase on follow-up. There is progressive atrophy with increasingly wide CSF spaces. Microcephaly is suggested by a relatively small neurocranium in relation to the viscerocranium on midsagittal images, more pronounced on follow-up. In the affected male from Fam2 myelination at 22 months is adequate for age. Atrophy with thin corpus callosum and widened CSF is less pronounced than in the affected male from Fam1, the relation of neuro- and viscerocranium on midsagittal images appears normal. The anterior pituitary appears relatively small, the posterior pituitary (normal bright signal on T1w images) dominates the sella.
| Reference | Protein variant | Age at last examination/age of death and cause of death | Neurological phenotype (severe/moderate/mild) | Stature (SD at last examination) | Weight (BMI/SD, percentile) | Diabetes (age of onset)/other endocrinopathy | Microcephaly (SD at last examination) | Facial features and dysmorphic features | Genital abnormalities | Developmental delay | Behavioral difficulties | Epilepsy (med) (age of onset) | Brain MRI | Neuronal findings | Walking free without support (age) |
|-----------|----------------|-------------------------------------------------|---------------------------------------------|---------------------------------|----------------------------|---------------------------------------------|---------------------------------|---------------------------------|----------------------------|------------------|------------------------|-----------------------------|----------------|------------------|----------------------------|
| 1 This study | p.(Thr144Ile) (Fan 1, singleton) | 9.6 y | Severe | −5.33 | Obese with 3 Y (BMI: 21, >P97) At last examination overweight (BMI: 21, PP2) | Yes (9 y) | −7.45 | Large ears, full cheeks, increase in suprornidal soft tissue, narrow forehead, short philtrum, long eyelashes, thick eyebrows, micrognathia | Microgenitalism | Moderate | Yes (autistic) | Generalized (PH, TPM) (7 m) | WM reduction, thin CC | Axial hypotonia, spastic quadriplegia (9.6 y) |
| 2 p.(Ile159Leu) (Fam2, singleton) | 3 y | Moderate | −0.24 | Obese (BMI: 19.5, >P97) | No/amylose mildly elevated | −3.98 | Large ears, full cheeks, increase in suprornidal soft tissue, epicanthus, thin upper lip and short philtrum | No | Severe | Yes (autistic) | Generalized (VPA, STM, LEV, OXC, LTG) (6 m) | WM reduction, thin CC | Ataxic, spasticity, lower limb, drooling (2 y) |
| 3 Bock et al. | p.(Ile222Thr) (two brothers and maternal uncle) | 34 y | Moderate | −2 to −3 SD, GH low, treatment with GH | No data | No | −3.8 to −4.8 | Oblique, large ears | No | Severe | Yes (autistic) | Generalized (VPA) (5 y) | Thinn CC | Ataxic, spasticity, lower limb, drooling (n.a) |
| 4 | 11 y | Moderate | −2 to −3 | Obese (BMI: 30.5) | No data | No | −3.8 to −4.8 | Long face, large ears | No | Severe | Generalized (VPA, rhGH) | Thinn CC | Ataxic, spasticity, lower limb, drooling (n.a) |
| 5 Adult | Moderate | −2 to −3 | Obese (BMI: 30.5) | No data | No | −3.8 to −4.8 | No | Microgenitalism | Moderate to severe | No | No | n.a | Ataxic, spasticity, lower limb (n.a) |
| 6 Moortgat et al. | p.(Ile259Met) (two brothers) | 35 y/17 y (severe respiratory distress) | Severe | −8.7 | GH low, no treatment | Normal at 15 y (BMI: 38.8, P23) | −8 | Large ears | Micropanis, delayed puberty (testosterone inj) | Severe | Yes (autistic) | Generalized (LTG + LEV) (9 m) | WM reduction, thin CC | Axial hypotonia, spastic quadriplegia (n.a) |
| 7 | 18 y | Severe | −9 | GH low, no treatment | Underweight at 18 y (BMI: 17.3, P2) | No/hypoglycemia during a functional insulin test | −8.5 | Large ears | Delayed puberty | Severe | No | No | WM reduction, thin CC | Axial hypotonia, spastic quadriplegia (n.a) |
| 8 p.(Ile465Serfs*4) (singleton) | 1 y/1 y (multi-systemic failure) | Severe | −4.5 | No data | No/hypoglycemia | −7 | Micrognathia | Microgenitalis | Severe | No | Generalized (10m) | Thinn CC | Generalized hypotonia, no visual contact (n.a) |
| 9 Skopkova et al., Stanik et al. | p.(Ile465Serfs*6) Family 1, index patient, (2 affected: index patient and maternal uncle) | 3 y | Severe | −5 | Obese at 5 y (BMI: 19.8, P × 97) | Yes (10m) | −8.4 | Large ears, full cheeks, downturned corners of mouth, epiphora, long eyelashes and thick eyebrows, tapered fingers, talipes | Micrognathia, cryptorchidism | Severe | Yes (no social interaction) | Partial complex epileptic seizures, well controlled (VGB + PH + TPM) (4 m) | Myelination delay, cerebral atrophy | Centr al hypotonia, peripheral hypotonia, reacts only to strong stimuli (n.a) |
| Reference | Protein variant | Age at last examination/age of death and cause of death | Neurological phenotype (severe/moderate/mild) | Stature (SD at last examination) | Weight (BMI kg/m², percentile) | Diabetes age of onset/other endocrinopathy | Neurological findings | Neurological phenotype | Developmental delay | Behavioral difficulties | Epilepsy (med) (age of onset) | Brain MRI | Neurological findings | Walking free without support (age) |
|-----------|----------------|-------------------------------------------------------|---------------------------------------------|---------------------------------|--------------------------------|--------------------------------------------|----------------------|------------------------|---------------------|----------------------|--------------------------|-----------|----------------------|-----------------------------|
| 10        | p.(Ile465Serfs*4) | 3 y Severe                                             | −4.7                                        | Obese at 3 y (BMI: 19.1, P97)   | Yes (10 m)                     | n.a                                        | Large ears, full cheeks plus other features similar to patient 9 | Hypogenitalism        | Severe               | Yes (no social interaction) | Seizures, therapy-resistant (6 m) | Myelinization delay | Hypotonia, no voluntary movements | No |
| 11        | Stokerova et al, Steinhüller et al | 2 y/2 y (refractory seizures)                          | Severe                                      | Obese at 2 y (BMI: 19.2, P97)   | Yes (6 m)                      | −8.2                                       | Large ears, full cheeks, narrow forehead, facial telangiectasias, downturned corners of mouth, edematous hands and feet, tapered fingers, bilateral talipes | Hypogenitalism        | Severe               | n.a                      | Seizures, therapy-resistant (Gm) | n.a | n.a | No |
| 12        | Stokerova et al | 4.7 y Moderate to severe                              | −3.2                                        | Obese as infant (BMI P97th)     | normal at the 4.7 y (BMI: 14.2, P10) | −3.4                                       | Not mentioned | Cryptorchidism/ hypopituitarism | Moderate to severe | n.a | n.a | Central hypotonia, peripheral hypotonia and spasticity | No |
| 13        | Gregory et al | p.(Pro432Ser) Family 4, singleton                      | 14.6 y                                     | Mild                            | −2.05, GH low, rGH therapy since age 2 y | Normal at 14.6 y BMI: +1.48 SD | No | Hypoglycemic/ hypopituitarism | Mild | Yes | Hypoglycemic seizures at 2 y | small AP | No | Yes |
| 14        | 14.6 y         | Mild                                                   | −2.07, GH low, rGH therapy since age 2 y    | Normal at 14.6 y BMI: +0.57 SD  | No | Hypoglycemic/ hypopituitarism | Mild | Yes | Hypoglycemic seizures at 2 y | WM reduction, small AP, thal-CC | No | Yes |
| 15        | 8.8 y          | Mild                                                    | −0.3, GH low, rGH therapy since age 1.8 y   | Normal at 8.8 y (weight: −0.2 SD) | No | Hypoglycemic/ hypopituitarism | Mild | Yes | No | No | WM reduction, small AP, thal-CC | No | Yes |

**Abbreviations:** AP, anterior pituitary; CC, corpus callosum; GH, growth hormone; LEV, levetiracetam; LTG, lamotrigine; n.a., not available; OXC, oxcarbazepine; P, percentile; PG, pituitary gland; STM, sultiam; TPM, topiramate; VGB, vigabatrine; VPA, valproate; WM, white matter.

*a* The following definitions are used to categorize the neurological phenotype: severe: patients with spastic quadriplegia and severe/moderate ID, moderate: patients with ataxia and severe/moderate ID, mild: patients with learning difficulties and normal neurological examination.

*b* The following definitions are used to categorize weight status: Underweight—BMI <5th percentile for age and sex; normal weight—BMI between the 5th and <85th percentile for age and sex; Overweight—BMI between >85th and 95th percentile for age and sex; Severe obesity—severe (class II) obesity is defined as BMI ≥120% of the 95th percentile values or a BMI ≥35 kg/m².

*c* Three other male family members were reported with a similar phenotype including neonatal hypoglycemia, severe microcephaly, developmental delay, micropenis, short stature, epileptic seizures and early death. They were unavailable for genetic testing.
near WT levels (Figure 2C,D, rows 10-13), suggesting that these G domain mutations might directly or indirectly affect Met-tRNA_{Met} binding to eIF2. As GTP and Met-tRNA_{Met} binding to eIF2 is thermodynamically coupled such that increasing the levels of either binding partner will enhance ternary complex formation,\textsuperscript{13} and based on the location of the T144I and I159L mutations in critical elements of the G domain, we propose that the new MEHMO mutations impair eIF2 function by weakening GTP binding.

To more directly test the impact of the I218L and T203A mutations on eIF2 function, we used reporter assays to assess translational

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**FIGURE 2**

A. Overview of the EIF2S3 variants from this study (bold and underlined) and from literature, along with changes and positions in the corresponding human (Hs) and yeast (Sc) eIF2\textgamma proteins. B. Ribbon and sphere representation of *Saccharomyces cerevisiae* (Sc) eIF2\textgamma from the structure of the translation preinitiation complex (PDB code 3JAP) using PyMOL software (Schrödinger). Components are colored as follows: eIF2\textgamma, cyan ribbons; tRNA\textsubscript{Met}, green spheres; Met, gray spheres; eIF2\textbeta helix \textalpha_1, gray ribbon; GDPCP (GTP), black sticks. The G domain and domains II and III of eIF2\textgamma are labeled, and the sites of new MEHMO mutations T144 (ScT203) and I159 (ScI218) are depicted as spheres and colored red and yellow, respectively. Residues in the Sw2 element are colored red, and the NKxD motif that specifies guanine nucleotide binding is colored sand. Sites of previously identified MEHMO mutations are labeled. C and D, Growth assay of yeast strains expressing the indicated WT or mutant form of eIF2\textgamma and co-transformed with empty vector or high copy-number plasmids containing the yeast eIF2\textalpha, eIF2\textbeta, or tRNA\textsubscript{Met} genes. E and F, GCN4-lacZ reporter (E) or his4(UUG)-lacZ and HIS4(AUG)-lacZ reporters (F) were transformed into yeast strains expressing the indicated WT or mutant forms of eIF2\textgamma with or without tRNA\textsubscript{Met} overexpression. Statistically significant differences in \beta-galactosidase activities are indicated for strains expressing mutant vs WT eIF2\textgamma (*) or for strains overexpressing tRNA\textsubscript{Met} vs empty vector (#) and were calculated using ANOVA followed by a post-hoc Tukey’s test (P < .05).
control of the GCN4 mRNA and start codon selection stringency. Regulated reinitiation at upstream open reading frames in the GCN4 mRNA results in elevated expression of GCN4 under conditions that lower eIF2 ternary complex levels.\textsuperscript{1,3,5,14} Like the eIF2γ-I318M mutation that impairs Met-tRNA\textsubscript{Met} binding to eIF2,\textsuperscript{5} the I218L and T203A mutations increased GCN4-lacZ expression 24- and 22-fold, respectively (Figure 2E). Moreover, increased expression of tRNA\textsubscript{Met} dampened GCN4-lacZ expression in all three mutant strains by more than 65% (Figure 2E). These data support the idea that the I218L and T203A mutations reduce eIF2 ternary complex levels, perhaps by indirectly impairing Met-tRNA\textsubscript{Met} binding.

Mutations in yeast eIF2 can also reduce the stringency of translation start site selection and enable ribosomes to initiate translation at near-cognate non-AUG codons.\textsuperscript{1,3,5,14} Whereas, cells expressing WT eIF2γ displayed a high level of start site selection stringency with expression from a UUG-initiated HIS4-lacZ reporter at ~1% the level observed for the paired AUG-initiated reporter (Figure 2F), the I318M, I218L, and T203A mutations increased the UUG/AUG initiation ratio by ~4- to 9-fold (Figure 2F). Thus, in addition to lowering ternary complex levels, the I218L and T203A mutations decrease the fidelity of translation start site selection, perhaps due to premature release of eIF2 from Met-tRNA,\textsubscript{Met} and the scanning ribosome at the near-cognate UUG codon. The inability of tRNA,\textsubscript{Met} overexpression to suppress near-cognate initiation in the mutant strains (Figure 2F) is consistent with the notion that the mutations cause premature release of eIF2 but not tRNA,\textsubscript{Met} from the scanning ribosome.

4 | DISCUSSION

We report on novel EIF2S3 pathogenic missense variants. While the affected male from Fam1 presented with severe MEHMO, the affected male from Fam2 has a comparatively milder phenotype.

Our studies in yeast revealed that the corresponding variants of the affected males, p.(Ile218Leu) and p.(Thr203Ala), severely impaired growth, elevated GCN4 expression, and relaxed the stringency of translation start site selection, comparable to previous results seen for other MEHMO variants and thus consistent with the novel variants at these positions being pathogenic in the patients.

We also compared clinical findings with phenotypes of previously published patients\textsuperscript{1-4,7,8} (Table 1). While the number of patients with a pathogenic variant in EIF2S3 is still small, severely affected males presented with all clinical features of MEHMO and less affected males exhibited only a subset of these features. All patients have small stature. Seven patients have low-growth hormone level and growth hormone therapy was performed in five patients. Seven of 12 patients presented with obesity. Ten patients showed glucose dysregulation with four patients having non-autoimmune diabetes and six having hypoglycemia. Patients from one family with the p.(Pro432Ser) mutation have a unique pancreatic phenotype with fluctuation between hyperinsulinemic hypoglycemia and hyperglycemia, supporting a critical role for EIF2S3 in human hypothalamo-pituitary development and function, and glucose regulation. Children with the severe phenotype and classical MEHMO have some facial features with long eyelashes, full cheeks, increase in supraorbital soft tissue, and micrognathia. Ten of 15 patients showed hypogonadism and two were reported as having delayed puberty. Affected males with the mild neurological phenotype have normal neurological examination findings, slight learning problems and attend a normal school. Patients with classical MEHMO showed a severe movement disorder with spastic quadriaparesis and severe to moderate developmental delay starting at birth. Several of these have a complete lack of expressive speech, little interest in social communication and autistic behavior patterns. Some patients with severe to moderate developmental delay have an ataxic movement disorder, were able to walk freely without support and therefore have a rather moderate neurological phenotype. Ten of 15 patients had seizures. Patients with the mild neurological phenotype had occasional seizures due to hypoglycemia. Epilepsy was often resistant to treatment and started in infancy. MRI findings in our patients consisted of the non-specific combination of myelination delay and atrophy as well as a relatively small anterior pituitary gland in one patient, consistent with the few reported cases with brain imaging findings. Based on the reported cases, atrophy with thin corpus callosum and a variably widened CSF space is the most common finding. Myelination delay with secondary white matter changes was reported in one patient and a small anterior pituitary with a normal posterior pituitary in the three boys with the mild neurological phenotype. Clinical features of patients with ID-hypogonadism/hypogonadism syndromes can be similar to those of patients with MEHMO. We therefore propose including EIF2S3 mutation search in the differential diagnosis of such unsolved cases.

In conclusion, this study establishes the link between two novel EIF2S3 missense variants identified in two unrelated affected males with pathogenicity supported by the structural model of eIF2 and impaired eIF2γ translational function in yeast. Further, it strongly supports clinically variable expressivity of MEHMO in patients with deleterious EIF2S3 and eIF2γ changes.

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CONFLICT OF INTEREST

Nothing to declare.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data sharing not applicable.

ORCID

Matias Wagner https://orcid.org/0000-0002-4454-8823

Vera M. Kalscheuer https://orcid.org/0000-0001-6898-3259
REFERENCES

1. Borck G, Shin BS, Stiller B, et al. eIF2γ mutation that disrupts eIF2 complex integrity links intellectual disability to impaired translation initiation. Mol Cell. 2012;48:641-646.

2. Moortgat S, Desir J, Benoit V, et al. Two novel EIF2S3 mutations associated with syndromic intellectual disability with severe microcephaly, growth retardation, and epilepsy. Am J Med Genet A. 2016;170:2927-2933.

3. Skopkova M, Hennig F, Shin BS, et al. EIF2S3 mutations associated with severe X-linked intellectual disability syndrome MEHMO. Hum Mutat. 2017;38:409-425.

4. Gregory LC, Ferreira CB, Young-Baird SK, et al. Impaired EIF2S3 function associated with a novel phenotype of X-linked hypopituitarism with glucose dysregulation. EBioMedicine. 2019;42:470-480.

5. Young-Baird SK, Shin BS, Dever TE. MEHMO syndrome mutation EIF2S3 I259M impairs initiator Met-tRNAiMet binding to eukaryotic translation initiation factor eIF2. J Mol Biol. 2019;47:855-867.

6. Young-Baird SK, Lourenco MB, Elder MK, et al. Suppression of MEHMO syndrome mutation in eIF2 by small molecule ISRIB. Mol Cell. 2020;77:875-886.e7.

7. Stanik J, Skopkova M, Stanikova D, et al. Neonatal hypoglycemia, early-onset diabetes and hypopituitarism due to the mutation in EIF2S3 gene causing MEHMO syndrome. Physiol Res. 2018;67:331-337.

8. Steinmuller R, Steinberger D, Muller U. MEHMO (mental retardation, epileptic seizures, hypogonadism and -genitalism, microcephaly, obesity), a novel syndrome: assignment of disease locus to Xp21.1-p22.13. Eur J Hum Genet. 1998;6:201-206.

9. Hinnebusch AG. The scanning mechanism of eukaryotic translation initiation. Annu Rev Biochem. 2014;83:779-812.

10. Wittinghofer A, Vetter IR. Structure-function relationships of the G domain, a canonical switch motif. Annu Rev Biochem. 2011;80:943-971.

11. Yatime L, Mechulam Y, Blanquet S, Schmitt E. Structural switch of the γ subunit in an archaeal aIF2αγ heterodimer. Structure. 2006;14:119-128.

12. Shin BS, Kim JR, Walker SE, Dong J, Lorsch JR, Dever TE. Initiation factor eIF2γ promotes eIF2-GTP-Met-tRNAiMet ternary complex binding to the 40S ribosome. Nat Struct Mol Biol. 2011;18:1227-1234.

13. Kapp LD, Lorsch JR. GTP-dependent recognition of the methionine moiety on initiator tRNA by translation factor eIF2. J Mol Biol. 2004;335:923-936.

14. Hinnebusch AG. Molecular mechanism of scanning and start codon selection in eukaryotes. Microbiol Mol Biol Rev. 2011;75:434-467, first page of table of contents.

SUPPORTING INFORMATION

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

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