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Case Report

Three Afghani siblings with a novel homozygous variant and further delineation of the clinical features of *METTL5* related intellectual disability syndrome

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ABSTRACT

Background. *METTL5* gene is one of the members of methyltransferase superfamily and biallelic variants cause intellectual disability syndrome (ID) with microcephaly. This article reports three new cases with *METTL5* related ID syndrome in a consanguineous family.

Case. Afghanistan descent family was affected by a novel homozygous c.362A>G (p.Asp121Gly) *METTL5* gene variant. This variant is predicted to be "pathogenic" by multiple in-silico tools. Patients had dysmorphic and neurodevelopmental features including intellectual disability, microcephaly, poor/absent speech, delayed walking, aggressive behavior, large/posteriorly rotated ears, broad nasal base and short stature, which seem to be the cardinal findings of the designated syndrome.

Conclusions. While the data reported in these individuals indicate characteristic clinical features of *METTL5* related ID syndrome, further investigations and study of additional cases are needed to improve the understanding of disease pathogenesis, and management.

Key words: METTL5, intellectual disability, whole exome sequencing, WES.

Intellectual disability (ID) is a neurodevelopmental condition that affects approximately 1-2% of the population all over the world, characterized by impaired learning and behavioral impairment.[1,2] While genetic factors, congenital metabolism errors and brain malformations are the main factors in ID etiology, approximately 50% of affected cases remain undiagnosed.[3,4] However, in recent years, next generation sequencing (NGS) technologies have improved the rates of diagnosis of rare diseases and the identification of ID related genes. The *METTL5* gene encodes a methyl transferase and plays a key role in the methylation of 18S ribosomal RNA.[5] Recently, biallelic pathogenic *METTL5* variants have been associated with a new ID syndrome (OMIM #618665) which is characterized by moderate to severe ID, developmental delay, microcephaly, various facial dysmorphisms and behavioral abnormalities.[6] To date, only 7 *METTL5* related ID patients have been reported from 3 unrelated consanguineous families, including 3 from Pakistani, 2 from Yemeni and 2 from Iranian descents.[6-8]

Here, we report the clinical and molecular genetic findings of three new *METTL5* related ID cases from a consanguineous Afghanistan family. To the best of our knowledge, this is the first Afghanistan descent family reported in the literature.

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Case Report

Three patients from a consanguineous Afghani healthy couple were referred to our clinic (Fig. 1a). Written informed consent were obtained from parents to undertake genetic investigations and for the publication of any potentially identifiable images or data included in this article.

Patient III-1

He was 11-years-old at the time of examination. He was born by normal and uneventful labor at term. Records on neonatal physical measurements and developmental milestones (head control, sitting and standing) were insufficient. Patient III-1 started to walk at age about 2. He had normal vision and hearing but speech was slurred. Besides, he had learning impairment, temper and aggressive behaviors. On examination, his physical measurements were as follows: Weight (W) 35 kg (10th-25th centiles), height (H) 141 cm (25th-50th centiles) and head circumference (HC) 51 cm (<1st centile). Microcephaly, large and posteriorly rotated ears, broad nasal base, long philtrum and thin upper lip were the dysmorphic features of the patient III-1 (Fig. 2a). Magnetic resonance imaging (MRI) did not reveal any structural brain abnormality.

Patient III-2

He was 8-years-old and was born by normal labor. The labor was complicated with cord entanglement. He started to walk at about 7 years of age. However, the patient III-2 had an ataxic gait and could not climb up and down the stairs without support. III-2 had a febrile seizure at postnatal day 11, and then he had tonic-clonic seizures until the age of 6. III-2 had no speech and vision and hearing were normal. Besides learning impairment, temper and aggressive behavior, he also had self-mutilating behavior. His physical measurements were as follows: W: 23 kg (10th-25th centiles), H: 116 cm (3rd centile) and HC: 49.5 cm (<3rd centile). On physical examination, microcephaly, large and posteriorly rotated ears, broad nasal base, full lips and epicanthal folds were detected (Fig. 2b). MRI did not reveal any structural brain abnormality.

Patient III-3

She was 6-years-old and was born by normal and uneventful labor at term. She started to walk at about 3 years of age. III-3 had a febrile seizure history at postnatal day 14. She had no speech. Vision and hearing were normal. Her physical measurements were as follows: W: 16 kg (10th centile), H: 110 cm (3rd centile) and HC: 47 cm (<1st centile). The proband had learning impairment, temper and aggressive behavior, self-mutilating behavior and attention deficit hyperactivity disorder. Other findings included microcephaly, large and posteriorly rotated ears, broad nasal base, long philtrum, thin upper lip, and epicanthal folds (Fig. 2c).MRI did not reveal any structural brain abnormality.

Genetic Analyses

Karyotype and microarray analyses were normal for all 3 children and did not reveal any structural or numerical chromosome abnormalities. Whole exome sequencing (WES) was performed on the genomic DNA of patient III-2 (Fig. 1a). The WES analysis of the patient III-2 revealed homozygosity for a c.362A>G (NM_014168.3, p.Asp121Gly) variant of the *METTL5* gene. The variant was consistent with *METTL5* related ID phenotype and no other *METTL5* variants were identified. In addition, no other alternative homozygous gene variants were identified in other genes related to similar diseases or ID phenotype. Confirmation of the WES result and testing of the genomic DNA of the parents, patient III-1 and III-3 were performed by sanger sequencing (Fig. 1b). While patients III-1 and III-3 were homozygous for c.362A>G variant, mother and father were heterozygous carriers. The c.362A>G variant is located in third exon of the *METTL5* gene, which encodes the S-adenosyl-L-methionine-

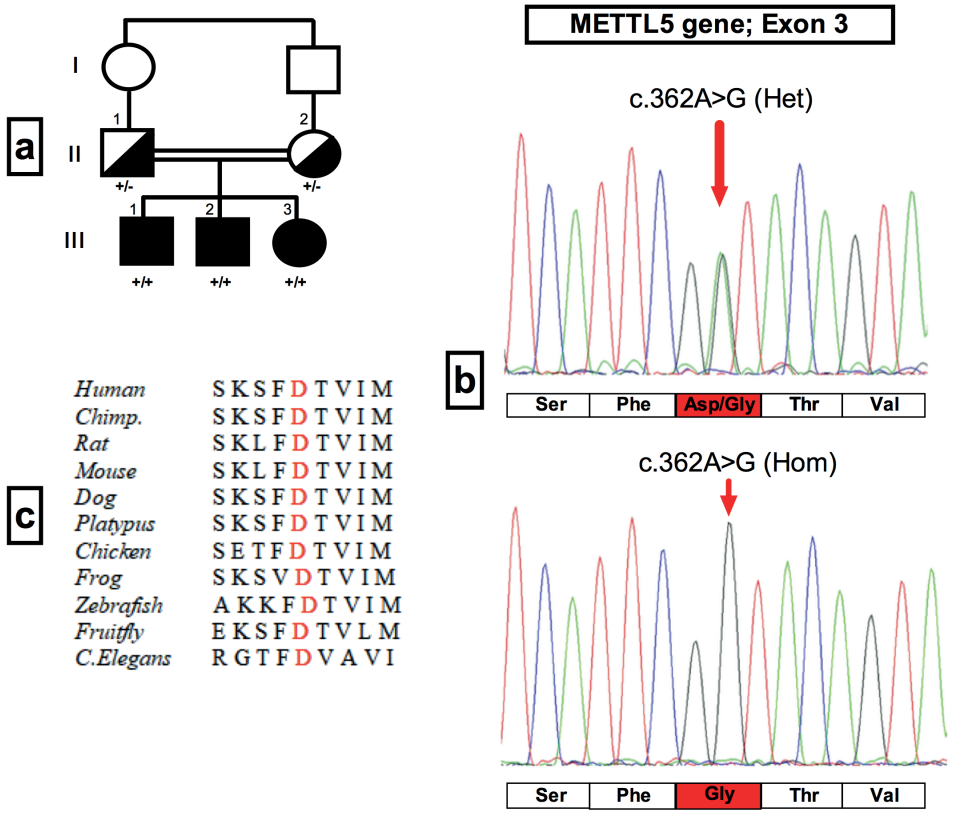

Fig. 1. (a) Pedigree of Afghanistan descent family segregating recessive intellectual disability, microcephaly, poor/absent speech and psychomotor developmental delay. The filled symbols represent affected individuals with homozygous c.362A>G variant in *METTL5* gene. The semi-filled symbols represent heterozygous carrier individuals. [(+/-): Heterozygous; (+/+): Homozygous] **(b)** Sanger sequencing chromatograms depicted *METTL5* c.362G>A homozygous mutation in the affected individuals (lower panel) and carrier status in the parents (upper panel). Amino acid sequences for each codon are also shown. (Het: Heterozygous; Hom: Homozygous) **(c)** Conservation alignment indicating that the affected amino acid of *METTL5* is conserved across different species. (Color figures can be viewed at online version of the manuscript).

dependent methyltransferase domain. Aspartate at position 121 is highly conserved (Fig. 1c). In contrast, the protein change to glycine is found only at a very low frequency in population databases [GnomAD_exome; G=0.000012 (3/246538), ExAC; G=0.000008 (1/120920)]. The p.Asp121Gly variant is predicted as "pathogenic" by multiple in silico tools, including Mutation Taster, Mutation assessor, SIFT, PROVEAN and REVEL. However, using American College of Medical Genetics (ACMG) criteria the variant is classified as a "*variant of uncertain significance (VUS)*" (PP1, PM2, PP3).[9] The variant data has been submitted to ClinVar (https://www-ncbi-nlm-nih-gov.gate2.inist.fr/clinvar/), accession number SUB8342013.

3D Modelling of METTL5 Variant

3D-dimensional structure of human *METTL5* was created on the crystal structure of the human *METTL5-TRMT112* complex, the 18S rRNA m6A1832 methyltransferase at 2.5A resolution.[5] YASARA software was used for modeling and subsequent analysis.[10] The 3D modeling analysis for *METTL5-TRMT112*

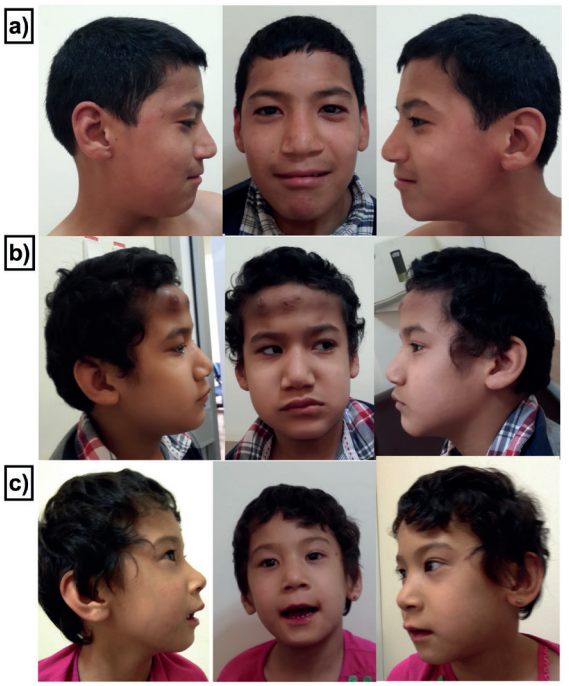

Fig. 2. Facial appearance of affected individuals, patient III-1 **(a)**, patient III-2 **(b)** and patient III-3 **(c)**. All affected individuals show characteristic facial features including microcephaly, large and posteriorly rotated ears and broad nasal base. Long philtrum and thin upper lip (III-1 and 3), full lips (III-2) and epicanthal folds (III-2 and 3) were also noted. Patient III-2 had skin lesions on his forehead caused by self-injurious behaviors. Parental written permission has been obtained to publish the patients' photos. (Color figures can be viewed at online version of the manuscript).

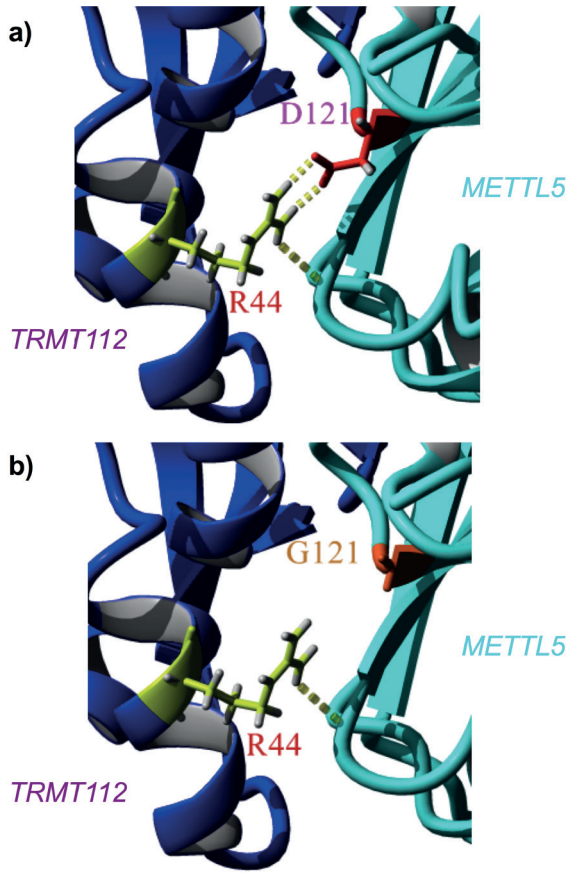

Fig. 3. 3D modeling of the *METTL5-TRMT112* complex. (a) D121 of *METTL5* (red) is able to form salt-bridges with R44 of *TRMT112* (yellow) and contribute the interactions of proteins. (b) c.362A>G variant causes D121 to be replaced by glycine (orange) and disturbs the formation of salt-bridges.

complex revealed that wild-type residue (D121) is located in S-adenosyl-L-methionine-dependent methyltransferase domain and is involved in formation of a salt-bridge with R44 of *TRMT112* (Fig. 3a) c.362A>G variant causes the D121 to be replaced by glycine. This substitution disturbs the salt bridge between *METTL5-TRMT112* complex (Fig. 3b).

Discussion

Intellectual disability related disorders show extensive clinical and/or genetic heterogeneity. To date, more than two thousand genes related to ID have been identified and the application of NGS technologies such as WES continues to reveal new genes. The *METTL5* gene has been postulated as a candidate gene for ID in two different clinical studies.[7,8] In 2019, a detailed functional and clinical study in seven patients with intellectual disability, microcephaly, poor/absent speech and aggressive behavior phenotypes identified *METLL5* as a causative gene for autosomal recessive ID.[6] Here, we report three new *METTL5* related ID syndrome cases in a consanguineous family of Afghanistan descent with a novel homozygous missense variant. The clinical and genetic findings of this case and previous cases are compared in Table I.

METTL5 is one of the members of methyltransferase superfamily and act as m^6A-

Table I. Clinical manifestations of *METTL5* gene related cases.

	Richard et al., 2019; Riazuddin et al., 2017			Richard et al., 2019		Hu et al., 2019		Present Study		
	Family PKMR43M			F47949		M8600616				
	II-1	II-2	II-3	III-2	III-3	III-3	III-5	III-1	III-2	III-3
	F	M	M	M	M	M	F	M	M	F
	c.344_345delGA (p.Arg115Asnfs*19)			c.571_572delAA (p.Lys191Valfs*10)		c.182G>A (p.Gly61Asp)		c.362A>G (p.Asp121Gly)		
	Clinical Findings									**Total**
Dysmorphic features										
Short stature	+	+	+	+	+	-	-	-	+	+	7/10 (70%)
Long philtrum	-	-	-	-	-	+	+	+	-	+	4/10 (40%)
Large ears	+	-	+	-	-	+	-	+	+	+	6/10 (60%)
Posteriorly rotated ears	-	-	-	+	+	-	-	+	+	+	5/10 (50%)
Strabismus	-	-	-	-	-	+	+	-	-	-	2/10 (20%)
Broad nasal base	-	+	-	+	+	-	-	+	+	+	6/10 (60%)
Narrow nasal base	-	-	-	-	-	+	+	-	-	-	2/10 (20%)
Overhanging nasal tip	-	+	+	-	-	-	-	-	-	-	2/10 (20%)
Thin upper lip	-	-	-	-	-	+	+	+	-	+	4/10 (40%)
Abnormal dentition	-	-	+	-	-	N/A	N/A	-	-	-	1/8 (12.5%)
Neurodevelopmental features										
Hypotonia	-	+	-	-	-	N/A	N/A	-	-	-	1/8 (12.4%)
Intellectual disability	+	+	+	+	+	+	+	+	+	+	10/10 (100%)
Microcephaly	+	+	+	+	+	+	+	+	+	+	10/10 (100%)
Seizure	-	-	-	-	-	-	+	-	+	+	3/10 (30%)
Delayed walking	+	+	+	+	+	-	-	+	+	+	8/10 (80%)
Unbalanced gait	-	-	-	N/A	N/A	-	+	-	+	-	2/8 (25%)
Poor or absent speech	+	+	+	+	+	+	N/A	+	+	+	9/9 (100%)
Spasticity	-	-	-	+	+	N/A	N/A	-	+	-	3/8 (37.5%)
Aggressive behavior	-	+	+	-	-	+	+	+	+	+	7/10 (70%)
Self-mutilating behavior	-	-	-	+	+	-	-	-	+	+	4/10 (40%)
ADHD	+	+	+	-	-	-	-	-	-	+	4/10 (40%)
Additional findings	-	-	-	Fetal tachycardia, hydrops fetalis	ASD, PS	-	-	Brachydactyly, hypoplastic nails	Full lips, epicanthus, mild SNHL	Epicanthus	

(+), the feature is present; (-), the feature is absent; F: female, M: male, N/A: not available, ADHD: attention deficit hyperactivity disorder, ASD: atrial septal defect, PS: pulmonary stenosis, SNHL: sensorineural hearing loss

methyltransferase that methylates the 18S rRNA gene.[6,11] *METTL5* is expressed in the human brain from the embryonic period to adulthood. Taken together, defects in *METTL5* functions are likely to lead to neurodevelopmental disorders due to impairment in the epigenetic processes. Recently, Ignatova et al.[11] revealed that *METTL5* knock-out (KO) mice had craniofacial and brain abnormalities. It was also postulated that pre/post synaptic effects and altered translation capacity due to defects in rRNA modification seem to be the cause of abnormal development. In addition, Richard et al.[6] defined an autosomal recessive ID and microcephaly syndrome in humans which is caused by the defects in *METTL5* gene (OMIM #618665). *In vitro* studies in COS7 cells confirmed that biallelic truncating variants (c.344_345delGA and c.571_572delAA) in *METTL5* gene decrease the expression and stability of METTL5, but the missense variant (c.182G>A) does not.

In this study, a novel biallelic c.362A>G missense variant was detected in a consanguineous Afghanistan descent family with three affected individuals. Clinical features of these cases were similar to the ID phenotypes reported by Richard et al. (Table I).[6] The homozygous c.362A>G *METTL5* variant was predicted as "pathogenic" by multiple *in silico* tools, although by ACMG criteria, it was classified as a VUS (PP1, PM2, PP3). PP1 is supporting evidence for the pathogenicity and indicates that the variant is co-segregating with disease in multiple affected family members. PM2 is considered as a moderate piece of evidence for pathogenicity and indicates that the variant is absent from the control population or is extremely low in frequency for recessive diseases. PP3 is also supporting evidence for the pathogenicity and reflects multiple lines of computational evidence supporting a deleterious effect on the gene or gene product. The lack of functional studies supporting the deleterious effects of the variant is the most important reason why the variant was identified as a VUS. Therefore, we observed the functional effect by revealing the effects of the c.362A>G variant on the 3D structure of the *METTL5* protein. *TRMT112* is a methyltransferase activator and *METTL5* must form a heterodimeric complex with *TRMT112* to gain metabolic stability in cells.[5] The interaction between the *METTL5-TRMT112* complex is provided by eight hydrogen bonds and two salt-bridges. Salt bridges are combination of two non-covalent interactions: hydrogen bonding and ionic bonding and forms bonds between oppositely charged residues of proteins that are close enough to each other.[12] Although non-covalent interactions are known to be relatively weak interactions, they contribute to protein structure and to the specificity of interaction of proteins with other biomolecules. Wild-type D121 residue of METTL5 is a negatively charged amino acid and forms a salt bridge with positively charged R44 residue of TRMT112. c.362A>G variant causes the wild-type residue (D121) to be replaced by uncharged glycine. It is most likely that this change would impair the metabolic stability of METTL5 by disrupting the interaction between METTL5-TRMT112 complex (Fig. 3b).

Although it was classified as a VUS by ACMG criteria, the variant we detected was assumed to be pathogenic for the following reasons; 1) The close resemblance of the clinical findings of patients with other cases reported to date, 2) Cosegregation of the biallelic missense variant with disease in multiple affected family members, 3) Presence of previously described cases with similar clinical findings and missense variants[7], 4) Structural changes in protein interactions revealed by 3D-modeling.

Apart from the variants identified by Richard et al.[6] the CLINVAR database includes 17 more pathogenic or likely pathogenic variants associated with the *METTL5* gene. Sixteen out of 17 are copy number gain/loss variants and gene dosage effect plays a major role in the pathogenesis, and the phenotype is a direct result of the cumulative effect of the imbalance of individual genes located on the deleted/duplicated chromosome region. Remaining 1 out of 17 is a splice site mutation, c.541+1G>C is located in a canonical splice-site and is

predicted to affect mRNA splicing resulting in a significantly altered protein (CLINVAR accession number: VCV000917596.1). These data show that different types of mutations may play a role in the pathogenesis of *METTL5* related ID.

Review of the clinical data showed that intellectual disability, microcephaly and poor/absent speech findings were present in all of the seven previously reported patients as well as in three new patients (Table I). The three new patients we reviewed also had delayed walking, aggressive behavior, large/posteriorly rotated ears and broad nasal base findings which were present in majority of the previously reported patients. Except for patient III-1, patient III-2 and III-3 from our study also had short stature which was present in 70% of the all reported cases. Taken together, intellectual disability, microcephaly, poor/absent speech, delayed walking, aggressive behavior, large/posteriorly rotated ears, broad nasal base and short stature comprise the cardinal findings of this ID syndrome. Besides, long philtrum, thin upper lip, seizure, self-mutilating behavior, attention deficit hyperactivity disorder (ADHD) and spasticity appear as the most common clinical findings after the cardinal findings. Dysmorphic features including narrow nasal base, overhanging nasal tip, strabismus and unbalanced gait as a neurodevelopmental abnormality constituted the less frequently reported clinical findings. Abnormal dentition and hypotonia findings were only reported in one case. The patients in the present study also had some features that were not reported previously. These included full lips, epicanthal folds, brachydactyly, hypoplastic nails and mild sensorineural hearing loss. Whether these findings are related to the c.362A>G *METTL5* missense mutation or to another unknown genetic lesion, remains to be substantiated in future patients with the phenotypic spectrum of this disorder. In addition, Ignatova et al.[11] reported that skull abnormalities or ossicle malformations might be the cause of hearing impairment in *METTL5* knock-out mice. This observation may explain the cause of mild sensorineural hearing loss in patient III-2.

In conclusion, clinical findings of the cases reported here are consistent with a *METTL5* related ID syndrome. To the best of our knowledge, the homozygous missense variant of *METTL5* gene that we found by WES has not been reported before and, these three new cases are the first patients from Afghanistan descent. Although, there is some conflicting data about the role of missense variants in the pathogenesis of the *METTL5* related ID syndrome, the variant found in this study was assumed to be the primary cause of clinical features in the three patients. However, understanding the functional impact of missense variants in *METTL5* functions does require further investigations and thus further examination of any new cases will help to provide a better understanding of the disease pathogenesis.

Ethical approval

Written informed consents were obtained from parents to undertake genetic investigations.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DT, MA; data collection: DT, MA, BÇ, HA; analysis and interpretation of results: DT, BÇ, DSC; draft manuscript preparation: DT, DSC. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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