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CASE REPORT

Association of *IMMP2L* deletion with neurodevelopmental disorders: new case report and review of the literature

Ines Ben Abdallah Bouhjar[1]*, Brahim Tabarki[2], Hamoud Alonazi[1], Mishal Alsulami[1], Amal Alhashem[2], Hatem Elghezal[1]

ABSTRACT

Background: The inner mitochondrial membrane peptidase, subunit 2-like, gene in 7q31.1 has been associated with different neurodevelopmental disorders, including autism spectrum disorders, attention-deficit/hyperactivity disorder, and Gilles de la Tourette's syndrome. Since the use of comparative genomic hybridization (CGH) as an essential tool in the diagnostic workup of neurodevelopmental disorders, recurrent copy number variations were identified in the *IMMP2L* gene (MIM 605977) probably caused by breakpoint clustering.

Case Presentation: We report here a 3-year-old girl presenting since early life with complex motor tics, developmental delay, and other various cognitive/behavioral disturbances. CGH analysis showed a 331 Kb de novo pathogenic heterozygous deletion at 7q31.1 into the *IMMP2L* gene, involving exons 1 to 3.

Conclusion: We discuss the functions of the *IMMP2L* gene suggesting that his disruption may act as a high-risk factor for neurodevelopmental disorders and movement disorders.

Keywords: *IMMP2L* gene, deletion 7q31.1, microdeletion, neurodevelopmental disorder.

Background

After the introduction of the array comparative genomic hybridization (CGH) technique in the diagnostic workup of developmental disorder, new recurrent copy number variations (CNVs), and novel microdeletion/microduplication syndromes were identified. Recently, deletions/duplications of the inner mitochondrial membrane peptidase subunit 2-like gene have been associated with different cognitive/behavioral disturbances (1). *IMMP2L* gene (MIM 605977) encodes the inner membrane peptidase (IMP) subunit 2-like protein, a mitochondrial IMP involved in cleaving the space-sorting signals of Cytochrome C1 (*CYC1*) (MIM 123980) and Mitochondrial Glycerol-3-phosphate Dehydrogenase 2 (*GPD2*) (MIM 138430). *IMMP2L* gene is one of the catalytic subunits of the mitochondrial IMP complex in association with the *IMMP1L* gene (MIM 612323). The IMP complex generates mature active proteins in the mitochondrial intermembrane space by proteolytically removing the mitochondrial targeting subunits of nuclear-encoded proteins. Disruption of function by deletions and/or mutations of the *IMMP2L* gene impairs the processing of *CYC1* and *GPD2* signal peptide sequences and this, in turn, increases the superoxide production rate. Interestingly, mitochondrial proteins have been found associated with the appearance of various neurodegenerative and neurodevelopmental disorders (2,3).

The mutations/deletions involving the *IMMP2L* gene in 7q31.1 are related to Autism, Susceptibility TO, 9 (AUTS9, MIM 611015), a subset of complex neurodevelopmental disorders; Attention deficit-hyperactivity disorder (ADHD, MIM 143465) and Gilles de La Tourette's Syndrome (GTS, MIM 137580). GTS (MIM 137580) is a neuropsychiatric disorder characterized by the presence of chronic multiple involuntary motor and/or vocal tics

Correspondence to: Ines Ben Abdallah Bouhjar
*Cytogenetics and Molecular Genetics Laboratory, Central Military Laboratory and Blood Bank, Prince Sultan Military Medical City, Riyadh, Saudi Arabia.
Email: inessebenabdallah@yahoo.fr
Full list of author information is available at the end of the article.
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(4). Tics occurs usually between 2 and 8 years of age. The etiology of La Tourette's syndrome is complex; multiple genes are involved together with environmental factors, but still largely unknown.

In this study, we present the clinical and molecular data of a patient with de novo heterozygote intragenic *IMMP2L* microdeletion. We discuss the effect that 7q31.1 disrupting by deletion is significantly associated with neurodevelopmental disorder susceptibility.

Material and Methods

Clinical report of a patient

The proband was the only child of healthy and nonconsanguineous parents. No family history of neuropsychiatric disorders was reported. The girl was born prematurely by normal delivery after rupture of the membrane at 30 weeks of gestation. At birth, Apgar scores were 9/10, weight was 2,400 g (<3rd percentile), length 45 cm (10th percentile), and cranial circumference (occipital frontal circumference) 32 cm (30th percentile). The neonatal period was complicated by feeding difficulties requiring a nasogastric tube (NGT) for feeding on a thin fluid and puree diet until the age of 8 months. However, mostly she depended on NGT feeding, care of poor sucking, coughing, and vomiting with thin fluid. The patient had unstable chest status, inadequate lip sealing, mild oral spillage, and fair incoordination between sucking, swallowing, and breathing cycles. Subsequently, she developed a global developmental delay. Interestingly, she was also observed to have complex motor tics. Initially, those tics were diagnosed to be seizures, but prolonged electroencephalogram recording was unremarkable. Last examination at 4 years of age was significant for persistent tics and global development delay (weight 9.5 kg, length 91 cm, head circumference 46 cm, all below third percentile). She is hypotonic, microcephalic, and dysmorphic in the form of low-set ears, cleft lip, high and broad nose, short philtrum, small mouth, and short neck. The magnetic resonance imaging brain showed only mild periventricular leukomalacia related to prematurity.

Cytogenetics analysis

Blood samples from the patients and parents were drawn after informed consent.

Chromosomal analysis was performed according to standard procedures. Peripheral blood lymphocytes were cultured in EUROCLONE MEDIA P (Euroclone®, Italy) enriched with 20% fetal calf serum, L-glutamine, antibiotics (penicillin and streptomycin), and antibodies (Phytohemmaglutinine). The cells were cultured for 72 hours in a humidified environment with 5% CO_2 in 37°C incubator until harvest. For the 72-hour culture, the sample was incubated with Colcemid solution (final concentration 0.05 µg/ml) for 45 minutes. After harvesting, the cells were exposed to a hypotonic solution (0.075 mol/l KCl) and fixed with methanol/acetic acid (3:1).

The slides were prepared and stained using the G banding technique on peripheral blood lymphocyte cultures. A minimum of 20 metaphases were analyzed from each sample and karyograms were prepared using the Applied Imaging CytoVision Automated Karyotyping System®. Chromosomal abnormalities have been reported by following the current international standard nomenclature (5).

Comparative genomic hybridization

A 180,000 Agilent Technologies® oligonucleotides array was used according to the manufacturer's instructions "SurePrint G3 Human CGH Microarray kit, 4 × 180 K" with overall median probe spacing of 13 Kb. The patient's DNA and the reference DNA were digested with RsaI and AluI. Digested DNA produced was labeled by random priming with Al5-dUTP or Al3-dUTP. After Columns-purification, the probes were denatured and pre-annealed with 50 µg of human Cot-1 DNA (Invitrogen®, CA, USA). The hybridization was performed at 65°C for 24 hours. After washing, the microarray was then scanned by the agilent microarray scanner (G4900DA). Data analysis was performed by Agilent Feature Extraction® 6.5.018 software. Interpretations of results were carried out with CGH analytics® 5.0 Software with the following parameters: GRCh37/hg19, *z*-score threshold: 2.0, window: 0.2 Mb. A CNV was noted if at least three contiguous oligonucleotides presented an abnormal $\log^2$ ratio (> +0.5 or < -0.5). The results were compared to the data recorded in the database of genomic variants. Web resources included the database of genomic variants: (http://projects.tcag.ca/variation/), University of California Santa Cruz Genome Bioinformatics: (http://genome.ucsc.edu/), Ensembl: (http://www.ensembl.org/index.html), OMIM: (http://www.ncbi.nlm.nih.gov/sites/entrez), and DECIPHER$^{v5.0}$ database: (http://decipher.sanger.ac.uk/).

Result

Chromosomal analysis of the proband and parents showed a normal karyotype in all metaphases.

Array-CGH analysis of the patient DNA showed a de novo heterozygous microdeletion of the long arm of chromosome 7 involving the 7q31.1 as follows: arr[GRCh37]7q31.1(110943144_111274663)x1dn according to "Human Genome Build 37." This deletion was not associated with other anomalies. The size of the deletion in chromosome 7q31.1 was estimated at about 331 Kb (Figure 1). Array-CGH analysis of the parents was normal and the CNV is classified as likely pathogenic.

Discussion

The use of CGH in the workup of intellectual disability and congenital malformations showed new recurrent CNVs and novel microdeletion/microduplication syndromes. The array-CGH technologies lead to the identification of an increased number of patients carrying CNVs. These rearrangements have a connection between the genomic disturbance and the phenotypic features of patients. Recently, the recurrent CNVs in the 7q31.1 region identified in unrelated children involving only part of the *IMMP2* (MIM 605977) gene were often associated

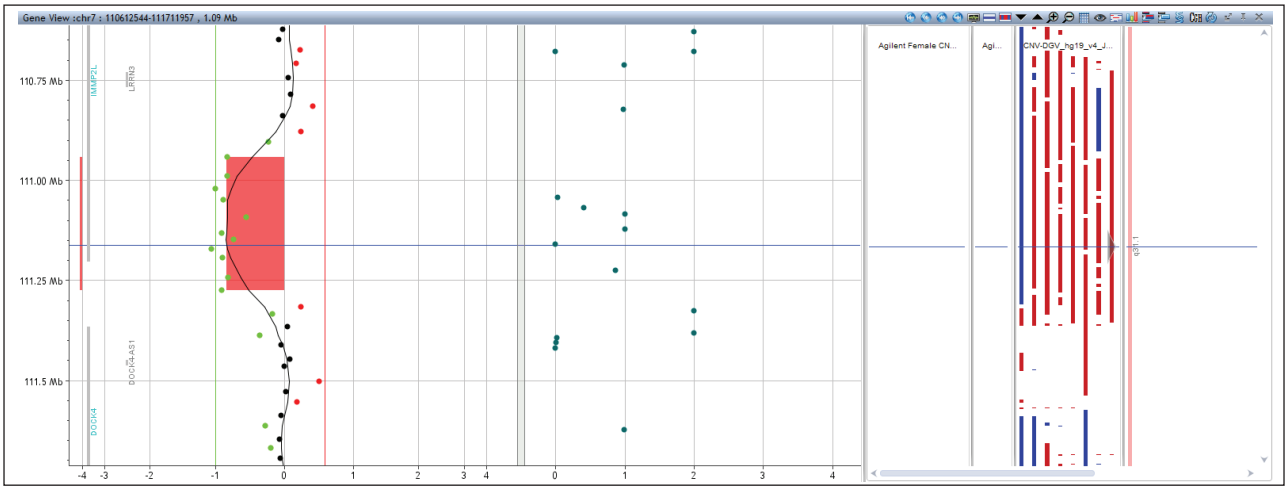

Figure 1. *Result of 180 K agilent micro-array (median probe spacing of 13 Kb) analysis of our patient DNA showing 331 Kb heterozygous deletion in 7q31.1 (chr7:110.943.144 to 111.274.663). The dotted lines represent log2 ratios of (-1) for our patient in the first three exons of the IMMP2L gene.*

with a broader range of phenotypes associated with autism spectrum disorders, ADHD and tics/ GTS (1,6-8).

Mitochondrial dysfunction has been associated with a range of human disorders, including neuropsychiatric disorders, the loss of *IMMP2L* gene (MIM 605977) function may, therefore, be related to the pathogenesis of neuropsychiatric disorders, including AUTS9 (MIM 611015), ADHD (MIM 143465), and GTS (MIM 137580), due to a hyperactive mitochondrion with increased production of superoxide through defective processing of *IMMP2L* substrates, such as *CYC1*and *GPD2*, leading to apoptosis. It is also possible that a defective *IMMP2L* leading to an unbalance in mitochondria function may be a risk factor affecting myelination (8-10). In support of this hypothesis, it has recently been reported that mitochondria dysfunction through oxidative stress, perturbed calcium homeostasis, and release of pro-apoptotic factors could compromise myelinogenesis (11). *IMMP2L* transcripts show high expression in the cerebellum, which is involved in tics/GTS pathogenesis (12). The theory that *IMMP2L* gene alteration may predispose to cognitive/behavioral disturbances is supported by *in vivo* experience using an inactive *IMMP2L* gene (MIM 605977), that increased ischemic brain damage and apoptosis of cerebral neurons (13).

Mitochondria dysregulation at the cellular level and cerebellum involvement at the anatomic level are related to movement disorders such as dystonia and ataxia (14); therefore, it is plausible that an unbalanced mitochondria function through a defective cerebellar *IMMP2L* may contribute to tics formation. However, further studies are necessary to understand the involvement of mitochondria dysfunction due to a deficient *IMMP2L* gene (MIM 605977) function in neuropsychiatric disorders.

Initially, in 1996, Boghosian-sell et al. (15) reported a GTS family with partial or full expression of the disease, which was shown to segregate with an balanced t(7;18) (q22-q31;q22.3) translocation using somatic cell-hybrid analysis, the 7q breakpoint could be positioned between D7S515 and D7S522 and the rearrangement of these loci are associated with disruption of 7q31. The cDNA of the human *IMMP2L* gene (MIM 605977) was cloned, and analysis of the complete 1,522-bp transcript revealed that it encompassed six exons spanning 860 kb of genomic DNA. In a screening study of 188 unrelated GTS's syndrome patients, Bertelsen et al. (8), reported seven patients with intragenic *IMMP2L* microdeletion suggesting that this cryptic rearrangement is a susceptible factor for GTS and the *IMMP2L* gene (MIM 605977) alterations could be considered as predisposing high factors for neurodevelopmental disorder, putatively with incomplete penetrance as reported in the database of genomic variants. The partial deletions of the *IMMP2L* gene are mostly phenotypically neutral and, recently, Vasilyev et al. (16) explained that de novo differentially methylated regions were shown to be significantly enriched in patients with partial *IMMP2L* gene deletion with neurodevelopmental disorders compared to control groups. These differentially methylated regions inside the CNVs may be one of the mechanisms explaining the incomplete penetrance of CNVs associated with neurodevelopmental disorders.

Our reported case is detected by array-CGH. The microdeletion identified affect exclusively the first three exons (exons 1, 2, and 3) but does not involve the third intron of the *IMMP2L* gene (MIM 605977) containing the *LRRN3* gene (MIM 619748) which encodes a neuronal leucine-rich protein, nested within its large third intron, highly expressed in fetal brain and probably playing a relevant role in a target recognition, axonal pathway finding, and cell differentiation during neural development (17). Therefore, the largest deletion in the *IMMP2L* region involving the *LRRN3* gene (MIM 619748) could cause behavioral/cognitive disturbance in addition to the GTS features (18). Nevertheless, the deletion identified in our patient does not involve the *LRRN3* gene (MIM 619748).

Recent publications reported patients with exon 1, 2, and 3 deletions are represented in Figure 2 and Table 1 by Baldan et al. (1) (case 1), Gimelli et al. (18) (patients

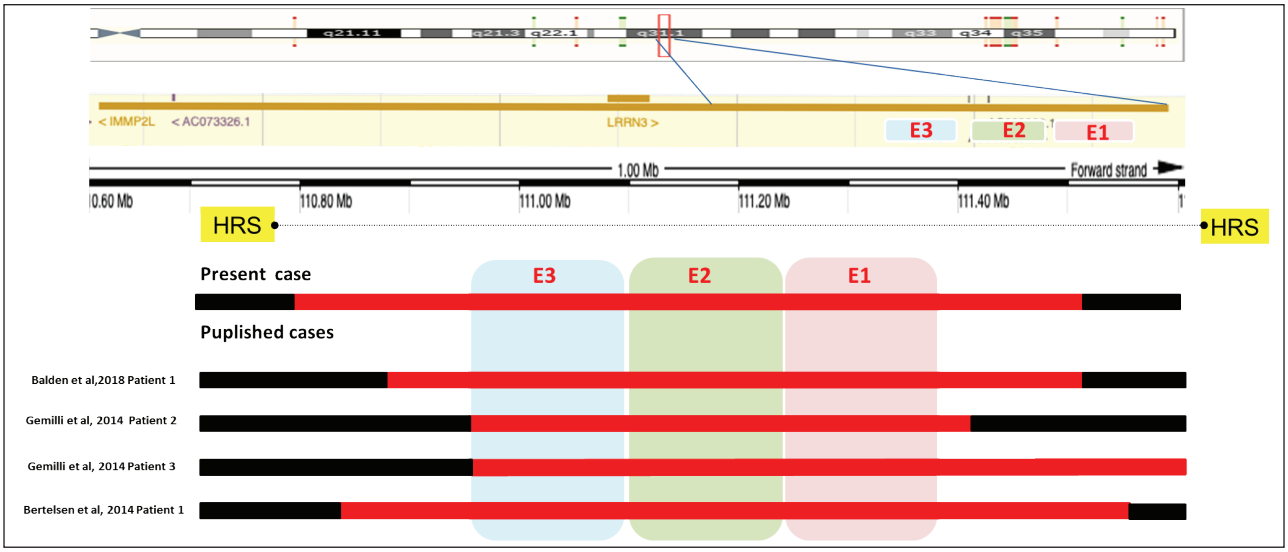

Figure 2. *Schematic illustration of the long arm of chromosome 7 and summary of the molecular findings in patients previously reported with 7q13.3 microdeletions in this region. The genomic distances (in base pairs from the 7p telomere) shown at the top of the figure were according to ensemble genome browser 59 (hg19) (http://www.ensembl.org/Homo_sapiens). For each patient, a normal copy number is illustrated as a black line and the deleted segment as a red line. One critical region was delineated within this segment probably between two homologous repetitive sequences (represented in yellow box): the exon 1 (E1), the exon 2 (E2), and the exon 3 (E3) seems to be responsible for the neurodevelopmental disorder and represent the smallest region of overlap between the patient 1 reported by Balden et al. (1) (chr7:111019752-111274663), the patient 2 reported by Gimelli et al. (18) (chr7:111066736-111201968), the patient 3 reported by Gimelli et al. (18) (chr7:111066736-111316651), and the patient 1 reported by Bertelsen et al. (8) (chr7:110959798-111290945). Note that the LRRN3 gene involved in the occurrence of behavioral/cognitive disturbance in addition to the neurodevelopmental disorder features, was retained in all the patients of this series.*

Table 1. *Comparison of clinical features of patients with IMMP2L in Exon 1, 2, and 3 microdeletions.*

Patients	Age/sex	First abnormal base	Last abnormal base	Size in Kb	Exon	Inheritance	Clinical details
Our case	1 year/F	110943144	111274663	331	1, 2, and 3	De novo	Feeding difficulty, psychomotor delay, microcephaly, hypotonia, speech delay, dysmorphic features with low set ears, cleft lip, high and broad nose, short philtrum, small mouth, and she had a short neck
Balden et al. (1) case 1	5 years/M	111019752	111274663	255	1, 2, and 3	maternal	Developmental delay, language delay, lightly clubfooted, ASD, no signs of dysmorphism
Gimelli et al. (17) patient 2	5 years/M	111066736	111201968	153	1, 2, and 3	paternal	Psychomotor delay, language delay, Hetero-aggressive, hyper-phagia, epilepsy, paratonia, some autistic symptoms, brachydactyly, flat feet, minor facial dysmorphism, small hand, and feet
Gimelli et al. (17) patient 3	9 years/M	111066736	111316651	250	1, 2, and 3	paternal	Psychomotor delay, language delay, epilepsy, hypotonic, scoliosis, dysmorphic features with arched palate and large central incisors
Bertelsen et al. (8) patient 1	NA/M	110959798	111290945	331	1, 2, and 3	paternal	Dyslexia, temper, neuropsychiat-ric features, Tourette's syndrome, ADHD.

2 and 3), and Bertelsen et al. (8) (patient 1), who represented the smallest region of overlap between these patients (Figure 2, Table 1). This recurrent rearrangement is predominately caused by nonallelic homologous recombination (NAHR), this region is probably flanked by highly homologous repetitive sequences as low copy repeat, Alu sequences, and long interspersed nuclear elements (LINEs). Due to their high degree of sequence

homology, these segmental duplications provide substrates for NAHR. Misalignment and subsequent recombination between two homologous repetitive sequences on the same region causes deletions and duplications. The rearrangement size tends to correlate with the distance between these sequences (19). Our case and all the reported patients in Table 1 showed major clinical features of psychomotor delay, language delay, epilepsy, hypotonia, autistic symptoms, and dysmorphic features. These data suggest that partial deletions of the *IMMP2L* gene (MIM 605977) may act as risk factors for neurological diseases.

In our opinion, this case may add to the emerging theme that the same genomic variants, in association with distinct genetic backgrounds, and environmental conditions, may contribute to different features and phenotypes in patients with intragenic *IMMP2L* microdeletion. This present case suggests that intragenic deletion of the *IMMP2L* gene (MIM 605977) may be a genetic risk factor in neurodevelopmental disorder pathogenesis, investigation of further and larger chronic neurodevelopmental disorder cohorts is necessary to understand the role of this gene in the pathogenesis of GTS-like phenotype at earlier age and other associated neurodevelopmental disorders.

The present findings together with the previous investigations support that genomic rearrangements affecting the *IMMP2L* gene (MIM 605977) may be a predisposing factor involved in ASD, ADHD, and GTS and overlapping neurodevelopmental disorders (18). More investigations of cases with *IMMP2L* gene microdeletions, rearrangements, and functions studies of genes and transcripts are necessary to have a better insight into the role of this gene in neurodevelopmental disease pathogenesis. Proper genetic counseling, the introduction of newborn screening programs, and parenteral diagnosis can play a major role in reducing the burden of such severe disorders (20). This can be accomplished by prenatal genetic testing for monogenetic disorders (PGT-M). PGT and *in vitro* fertilization are options for parents wishing to have future pregnancies (21-23). Although there is no specific management for these cases, patients are treated with supportive treatment.

Conclusion

We discuss the functions of the *IMMP2L* gene suggesting that his disruption may act as a high-risk factor for neurodevelopmental disorders and movement disorders.

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Declaration of conflicting interests
The authors declare that they have no conflict of interest regarding the publication of this case report.

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Consent for publication
Informed consent was obtained for publication.

Ethical approval
Ethical approval is not required at our institution to publish an anonymous case report

Author details
Ines Ben Abdallah Bouhjar[1], Brahim Tabarki[2], Hamoud Alonazi[1], Mishal Alsulami[1], Amal Alhashem[2], Hatem Elghezal[1]
1. Cytogenetics and Molecular Genetics Laboratory, Central Military Laboratory and Blood Bank, Prince Sultan Military Medical City, Riyadh, Saudi Arabia
2. Pediatric Genetics and Metabolic Medicine Department, Prince Sultan Military Medical City, Riyadh, Saudi Arabia

References

1. Baldan F, Gnan C, Franzoni A, Ferino L, Allegri L, Passon N, et al. Genomic deletion involving the IMMP2L gene in two cases of autism spectrum disorder. Cytogenet Genome Res. 2018;154(4):196–200. https://doi.org/10.1159/000489001
2. Lu B, Poirier C, Gaspar T, Gratzke C, Harrison W, Busija D, et al. A mutation in the inner mitochondrial membrane peptidase 2-like gene (Immp2l) affects mitochondrial function and impairs fertility in mice. Biol Reprod. 2008 Apr;78(4):601–10. https://doi.org/10.1095/biolreprod.107.065987
3. Nunnari J, Fox TD, Walter P. A mitochondrial protease with two catalytic subunits of nonoverlapping specificities. Science. 1993 Dec;262(5142):1997–2004. https://doi.org/10.1126/science.8266095
4. Burd L, Kerbeshian J, Wikenheiser M, Fisher W. A prevalence study of Gilles de la Tourette syndrome in North Dakota school-age children. J Am Acad Child Psychiatry. 1986 Jul;25(4):552–3. https://doi.org/10.1016/S0002-7138(10)60016-7
5. McGowan-Jordan J, Hastings RJ, Moore S. ISCN 2020: an international system for human cytogenomic nomenclature. Basel, Switzerland: S. Karger; 2020. https://doi.org/10.1159/isbn.978-3-318-06867-2
6. Patel C, Cooper-Charles L, McMullan DJ, Walker JM, Davison V, Morton J. Translocation breakpoint at 7q31 associated with tics: further evidence for IMMP2L as a candidate gene for Tourette syndrome. Eur J Hum Genet. 2011 Jun;19(6):634–9. https://doi.org/10.1038/ejhg.2010.238
7. Petek E, Schwarzbraun T, Noor A, Patel M, Nakabayashi K, Choufani S, et al. Molecular and genomic studies of IMMP2L and mutation screening in autism and Tourette syndrome. Mol Genet Genomics. 2007 Jan;277(1):71–81. https://doi.org/10.1007/s00438-006-0173-1
8. Bertelsen B, Melchior L, Jensen LR, Groth C, Glenthøj B, Rizzo R, et al. Intragenic deletions affecting two alternative transcripts of the IMMP2L gene in patients with Tourette syndrome. Eur J Hum Genet. 2014 Nov;22(11):1283–9. https://doi.org/10.1038/ejhg.2014.24
9. Rezin GT, Amboni G, Zugno AI, Quevedo J, Streck EL. Mitochondrial dysfunction and psychiatric disorders. Neurochem Res. 2009 Jun;34(6):1021–9. https://doi.org/10.1007/s11064-008-9865-8
10. Tobe RH, Bansal R, Xu D, Hao X, Liu J, Sanchez J, et al. Cerebellar morphology in Tourette syndrome and obsessive-compulsive disorder. Ann Neurol. 2010 Apr;67(4):479–87. https://doi.org/10.1002/ana.21918

11. Barateiro A, Vaz AR, Silva SL, Fernandes A, Brites D. ER stress, mitochondrial dysfunction and calpain/JNK activation are involved in oligodendrocyte precursor cell death by unconjugated bilirubin. Neuromolecular Med. 2012 Dec;14(4):285–302. https://doi.org/10.1007/s12017-012-8187-9

12. Bohlhalter S, Goldfine A, Matteson S, Garraux G, Hanakawa T, Kansaku K, et al. Neural correlates of tic generation in Tourette syndrome: an event-related functional MRI study. Brain. 2006 Aug;129(Pt 8):2029–37. https://doi.org/10.1093/brain/awl050

13. Ma Y, Mehta SL, Lu B, Li PA. Deficiency in the inner mitochondrial membrane peptidase 2-like (Immp21) gene increases ischemic brain damage and impairs mitochondrial function. Neurobiol Dis. 2011 Dec;44(3):270–6. https://doi.org/10.1016/j.nbd.2011.06.019

14. Dueñas AM, Goold R, Giunti P. Molecular pathogenesis of spinocerebellar ataxias. Brain. 2006 Jun;129(Pt 6):1357–70. https://doi.org/10.1093/brain/awl081

15. Boghosian-Sell L, Comings DE, Overhauser J. Tourette syndrome in a pedigree with a 7;18 translocation: identification of a YAC spanning the translocation breakpoint at 18q22.3. Am J Hum Genet. 1996 Nov;59(5):999–1005.

16. Vasilyev SA, Skryabin NA, Kashevarova AA, Tolmacheva EN, Savchenko RR, Vasilyeva OY, et al. Differential DNA methylation of the IMMP2L gene in families with maternally inherited 7q31.1 microdeletions is associated with intellectual disability and developmental delay. Cytogenet Genome Res. 2021;161(3–4):105–19. https://doi.org/10.1159/000514491

17. Fukamachi K, Matsuoka Y, Ohno H, Hamaguchi T, Tsuda H. Neuronal leucine-rich repeat protein-3 amplifies MAPK activation by epidermal growth factor through a carboxyl-terminal region containing endocytosis motifs. J Biol Chem. 2002 Nov;277(46):43549–52. https://doi.org/10.1074/jbc.C200502200

18. Gimelli S, Capra V, Di Rocco M, Leoni M, Mirabelli-Badenier M, Schiaffino MC, et al. Interstitial 7q31.1 copy number variations disrupting IMMP2L gene are associated with a wide spectrum of neurodevelopmental disorders. Mol Cytogenet. 2014 Aug;7(1):54. https://doi.org/10.1186/s13039-014-0054-y

19. Gu W, Zhang F, Lupski JR. Mechanisms for human genomic rearrangements. PathoGenetics. 2008 Nov;1(1):4. https://doi.org/10.1186/1755-8417-1-4

20. Alfadhel M, Umair M, Almuzzaini B, Alsaif S, AlMohaimeed SA, Almashary MA, et al. Targeted SLC19A3 gene sequencing of 3000 Saudi newborn: a pilot study toward newborn screening. Ann Clin Transl Neurol. 2019 Oct;6(10):2097–103. https://doi.org/10.1002/acn3.50898

21. Alyafee Y, Al Tuwaijri A, Alam Q, Umair M, Haddad S, Alharbi M, et al. Next generation sequencing based non-invasive prenatal testing (NIPT): first report from Saudi Arabia. Front Genet. 2021 Feb;12:630787. https://doi.org/10.3389/fgene.2021.630787

22. Alyafee Y, Alam Q, Tuwaijri AA, Umair M, Haddad S, Alharbi M, et al. Next-generation sequencing-based pre-implantation genetic testing for aneuploidy (PGT-A): first report from Saudi Arabia. Genes (Basel). 2021 Mar;12(4):461. https://doi.org/10.3390/genes12040461

23. Alyafee Y, Al Tuwaijri A, Umair M, Alharbi M, Haddad S, Ballow M, et al. Non-invasive prenatal testing for autosomal recessive disorders: a new promising approach. Front Genet. 2022 Nov;13:1047474. https://doi.org/10.3389/fgene.2022.1047474