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Characterization of Clinical and Neurocognitive Features in a Family with a Novel *OGT* Gene Missense Mutation [c. 1193G>A/ (p. Ala319Thr)]

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Abstract

X-Linked Intellectual Disability (XLID) is an extremely heterogeneous disorder for which many of the causative genes are still unknown. So far, more than one hundred genes of the X chromosome have been found to alter in males manifesting intellectual disability. *OGT* (O-linked N-acetyl-Glucosamine-Transferase) gene is well known to be involved in endocrine alterations by the resistance of insulin in muscles and adipocytes and therefore the initiation of diabetes. It is reported to be involved also in cancer, brain development, and neurodegenerative diseases. However, its implication in chromosome X-Linked Intellectual Disability (XLID) has not been pinpointed up until now. In this study, we consider a family of three brothers having a non-syndromic intellectual disability and developmental delay while developing a genetic diagnosis. In the present study, clinical investigations, and medical exams were performed according to the French bioethics law. We performed X-exome sequencing in two patients. Sanger sequencing was accomplished to confirm novel mutations. X-chromosome inactivation was executed in the mother. Affected boys had a severe intellectual disability and mild dysmorphic features. The heterozygous mother had mild cognitive impairment. Her X-chromosome inactivation pattern was not skewed. We identified a novel missense mutation (c. 1193G>A) in the *OGT* gene. This mutation was inherited by the affected males, and it segregated with the abnormal phenotype. It was predicted to be damaging by SIFT (score 0). The mother was heterozygous and the only normal son was not mutated. The pathological phenotype of our patients might be linked to the new missense mutation, however, more similar clinical cases and functional studies are required to conclude the correlation between the genotype and the phenotype.

Keywords: Intellectual disability; *OGT* gene; X exome sequencing; Sanger sequencing; X inactivation; X linked intellectual disability

Introduction

Intellectual disability is a serious and lifelong problem that places heavy demands on society and on the public health services. It is defined by an intellectual quotient (IQ) <70 [1]. A proportion of 3% of the population has an intellectual disability [2] and 10% of intellectual disability in boys is caused by X-linked genes [3]. X-Linked Intellectual Disability (XLID) involves almost 100 genes identified to date and more than 200 syndromes [4,5]. The O-Linked N-acetyl-Glucosamine transferase gene (*OGT*) locates on the Xq13.1 band; it has been reported to regulate proteins involved in chromatin remodeling [6] and target a wide range of intracellular proteins which protect cells from the damaging effects of metabolic stress [7]. Alteration of the *OGT* gene has been linked to diabetes, cancer and neurodegenerative syndromes, including autism and Alzheimer's disease [8,9]. Despite these investigations, no study has examined the possibility of *OGT* being directly involved in intellectual disability. Here we report a novel missense mutation within the *OGT* gene c.1193G>A (Genbank accession number: NM_181672) in a family of three brothers with intellectual disability, and a symptomatic heterozygous mother whose only healthy second son is not mutated in *OGT* gene (Figure 1). This mutation is predicted to be deleterious by SIFT. It was absent from public databases of control individuals (Exome Variant Server, 1000 genomes, dbSNP135, and ExaC) and in > in-house 200 X-exomes of index patients from other XLID families. In this study, we are discussing the pathogenicity of this mutation, we discuss also whether *OGT* could be the cause of the phenotype and therefore, whether *OGT* could be a candidate as new X-Linked intellectual disability gene (XLID).

Patients and Methods

The French family included three affected brothers with severe Intellectual Disability (ID) and one unaffected brother. All family members have been clinically evaluated in the medical genetics center of the Parisian university Necker hospital where they have been followed since 2007. Cognitive assessment was achieved in patient (II-1) and (II-3) using the Wechsler Intelligence Scale Child version four (WISC-IV). Skeletal radiography and brain Magnetic Resonance Imaging (MRI) was performed in patient (II-3). Informed consent for genetic studies was obtained from parents, according to the French bioethics law.

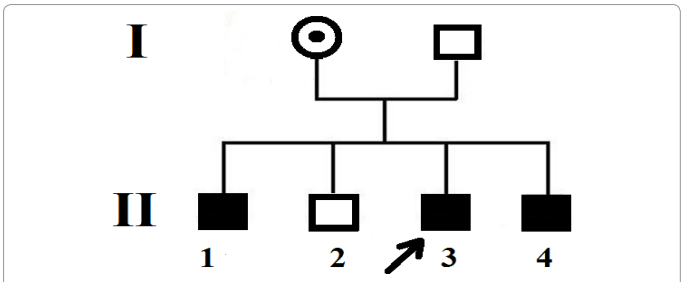

Figure 1: Pedigree of the family. Black symbols indicate intellectual disability associated with the novel mutation c. 1263G>A identified in all affected patients. The mother was a carrier and symptomatic. The arrow shows the index case. A circle with a centred black dot represents symptomatic carrier female.

DNA was extracted from peripheral blood using the standard procedure of phenol chloroform method [10]. Purity and concentration were assessed by NanoDrop ND-1000 Spectrophotometer V3-7

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(Thermo Fisher Scientific, Wilmington, DE, USA). DNA from patients (II-3) and (II-4) was included in a next generation sequencing project for XLID patients in our institute using SOLiD 5500 sequencer (Life Technologies, Grand Island, NY, USA). Five micrograms of DNA were enriched by micro droplet PCR procedure (Raindance technology, Billerica, MA, USA) to target 11,575 exons. Sorting and calling of SNP/InDel were performed using SAMTOOL and GATK software's. Novelty was assessed by filtering the variants against a set of polymorphisms that are available in public databases such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 genomes. Only non-synonymous variants or changes affecting splice sites were analyzed. All sequence variants were prioritized by scoring phylogenetic conservation and functional impact (SIFT and Polyphen-2). Candidate variants were selected and confirmed by Sanger sequencing, using the 3500XL Genetic analyzer and the Big Dye cycle sequencing Kit of Applied Biosystem technology. Forward (5'GCATTACCAGCCATTAGGC3') and reverse (5'CTGCTTTCCCTCTACTATCATGC3') primers were designed to amplify exon 8 of the *OGT* gene.

X-chromosome inactivation study was carried out in the mother, according to the method of methylation-sensitive PCR and fragment-length analysis of androgen-receptor CAG repeat polymorphism [11]. Karyotype, microarray and Fragile X testing were performed in affected boys prior to exome sequencing.

Results

Case report

The mother: This 40-year-old female had been adopted during childhood. Family history is unknown. Language was delayed. She had no dysmorphism. She attended school until the age of 16. She has performed odd jobs since then. She married a non-relative husband. Three of her four children had developmental delay and ID.

Patient (II-1): This male patient was born at term, after a normal pregnancy. Birth weight was 3.7 kg (+0.7SD), birth height was 50 cm (mean), and birth head circumference was 37 cm (+1.63SD). Apgar score was 10 over 10 minutes. Early psychomotor development was delayed. Walking started after the age of two. At the age of 9 he was still incontinent. He could make a few short sentences. He could eat without help, but needed help for the other daily tasks. He had very poor social interactions. He was restless and aggressive. He had severe myopia. At the age of 9, height was 123.5 cm (-1.33SD), weight was 23 kg (-2SD), Head circumference was 53 cm (mean). IQ was 40. Facial dysmorphic features included a long narrow face, a high forehead with frontal hair upsweep, a high nasal bridge, and a long philtrum. Fingers and toes were long and thin. The nails of the 5th toes were hypoplasic.

Patient (II-2): This 12-year-old male had normal psychomotor development and no dysmorphic features. He followed his schooling suitably.

Patient (III): This male patient was delivered at term, after a normal pregnancy. Birth weight was 3.36 kg mean), birth height was 48 cm (-1SD), and birth head circumference was 34 (-1SD). Apgar score was 10 over 10 minutes. Head holding was acquired after the age of 6 months, sitting at 14 months, and walking at 36 months. At the age of 7 years, weight was 15.7 kg (-2.3SD), height was 110 cm (-2SD) and the head circumference was 51 cm (+1SD). He was severely intellectually impaired with global IQ below 35. Language skills were limited to 10 words; he had problems and difficulties in performing routine daily living tasks. He had stereotypic movements of hands. He had no other abnormal neurological feature. Dysmorphic features included a long narrow face with a high forehead, frontal hair upsweep, mildly downslanting palpebral fissures, flat molar area, a high nasal bridge, a long philtrum, and a small mouth. He was myopic without strabismus (Figure 2a and 2b). His behavior disorders involved hands' stereotype movements and nocturnal awakening. He had a clinodactyly of the fifth finger and a flat foot (Figure 2b and 2c). Furthermore, he did not display any seizure episode. An EEG recording was performed at 7 years old and was normal. Brain MRI, performed at the age of 10 years, and did not reveal any alteration (Figure 3). Metabolic and endocrine analyses were normal. Glycaemia was 0.85 g/L. Lactate 78 mg/L. Insulin fasting was 11 mUI/L. Karyotype and array-CGH did not show any pathogenic chromosomal imbalance.

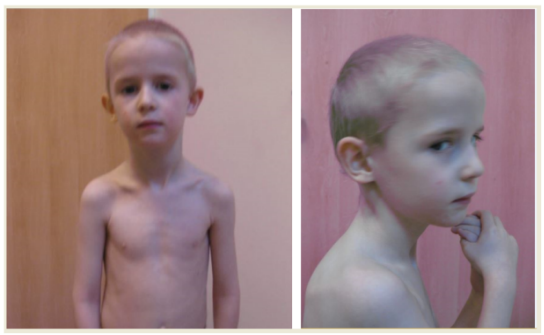

Figure 2: Respectively Facial and profile Photographs of patient II-3. Facial features include long narrow face with a high forehead, frontal hair upsweep, mildly downslanting palpebral fissures, a high nasal bridge, a long philtrum, a small mouth and stereotypic hand movements.

Human	**SVAEAEDCYNTALRLCPTHADSL**
Mutated sequence	**SVAEAEDCYNTTLRLCPTHADSL**
M.Mulatta	**SVAEAEDCYNTALRLCPTHADSL**
F.Catus	**SVAEAEDCYNTALRLCPTHADSL**
M.Musculus	**SVAEAEDCYNTALRLCPTHADSL**
G.Gallus	**SVAEAEECYNTALRLCPTHADSL**
T.Rubripes	**NVSEAEECYNTALRLCPTHADSL**
D.Rerio	**NVSEAEECYNTALRLCPTHADSL**
C.elegans	**SVVEAEQCYNTALELC**
X. Tropicalis	**SVAEAEECYNTALRLCPTHADSL**

Figure 3: The *OGT protein* is highly conserved among all eukaryotes. Alanine in position 319 is well conserved among many species.

Patient (IV): This male patient was born at term of an uneventful pregnancy. Apgar score was 10 over 10 minutes. Birth weight was 3.5 kg (mean), birth length was 53 cm (+1.5SD), and birth head circumference was 35 cm (mean). Early psychomotor development was delayed. Walking was achieved at the age of four. He could repeat a few words. At the age 4, cognitive evaluation showed severe ID (performance IQ=30). He was aggressive and restless He had no obvious dysmorphic features. Karyotype, microarray and X Fragile testing were normal in all the affected males.

Molecular Genetics

Sequencing of X-exome from patients (II-3) and (II-4) identified a single missense mutation (c.1193G>A, Genbank accession number: 181672) that could be considered as potentially pathogenic according

to our filters. This mutation was absent from public databases of control individuals (Exome Variant Server, 1000 genome, dbSNP135, and ExaC) and in > in-house 200 X-exomes of index patients from other XLID families. This mutation was confirmed by Sanger sequencing in the three affected boys as well as in the mother, but was absent in the unaffected brother (Figure 1). This substitution was predicted to be deleterious by SIFT software (score: 0). Predictions with Mutation Taster were in favor of a disease-causing variant (p value: 1). X-chromosome inactivation pattern was not skewed in the mother (55%; 45%). The mutation results in a replacement of an Alanine by a training (p. Ala319Thr).

Discussion

The family that we studied has four children, three of whom have intellectual disability and one is normal. The mother has had a developmental delay. The clinical feature of our index case patient (II-3) is characterized by a long, narrow face, a high forehead with frontal hair upsweep, a high nasal bridge, and a long philtrum. Fingers and toes were long and thin. The nails of the 5th toes were hypoplasic. He has nocturnal awakenings, a limited speech, as well as difficulties in concentration; stereotypic movements and a delay in acquisitions. Both his skeletal radiography and brain imagery by magnetic resonance were normal, there was no corpus callosum dysgenesis. Neither endocrinological nor metabolic alterations were pointed out. Karyotype, microarray and X Fragile testing were normal in the mother and her affected child.

The *OGT* gene is highly conserved among all eukaryotes which have been examined [12]. Alanine in position 319 is well conserved among many species (Figure 3). *OGT* has been confirmed to be involved in diabetes by the resistance of insulin in adipose and muscle tissue [13]. It has been found to be more highly expressed in the brain than in other tissues [11-15]. Furthermore, many proteins involved in neuronal communications, synaptic transmission, and synaptic plasticity are O-GlcNAcylated [16], suggesting an important role for this modification in brain function. So far, in terms of brain affection, the *OGT* gene has been cited only in Alzheimer's Disease (AD) [9], which is a neurodegenerative alteration. The main signs of AD are characterized by cognitive decline. The initial signs are mostly episodic memory disorders or behavioral problems. It can sometimes be associated with neurological signs [17,18].

Studies suggest that *OGT* signals a key regulatory modification in the brain, contributing to transcriptional regulation, neuronal communication and neurodegenerative disease [19]. Beyond the involvement of *OGT* in chronic human diseases including diabetes, cardiovascular disease, neurodegenerative disorders, and cancer [13,20,21], O-GlcNAcylation was reported to modulate protein phosphorylation and regulates several cellular signaling and functions, mainly in the brain [15].

X-exome sequencing applied to this XLID family brought out one novel missense mutation within *OGT* gene. The mutation (c.955G>A) was confirmed by Sanger sequencing, it segregated with the pathological phenotype in the three affected boys. The healthy son inherited the non-altered allele. The mother was found heterozygous with an inactivation pattern of (55%-45%). This substitution was predicted to be deleterious by SIFT software (score: 0). Predictions with Mutation Taster were in favor of a disease-causing variant (p value: 1)

Up to now, no intellectual disability involvement of *OGT* gene has been highlighted, we are the first to identify a novel deleterious predicted missense mutation (c.1193G>A) in this gene in a family of three brothers with intellectual disability. The novel mutation in this study segregates with the pathological phenotype in all affected patients. *OGT* gene has been reported to have a direct effect on neuronal development [22]. Although the number of *OGT* gene mutations reported to date has no involvement in Intellectual Disability (ID), in this study, we could suggest that the alteration of *OGT* gene might have an effect on the phenotype of our patients and there could be a genotype, phenotype correlation, however, functional studies with additional cases and large affected families are required to confirm this suggestion. Some limitations of this study involve the size of the family; we could study the transmission of the mutation on several generations, however the family size was limited. There were no similar cases in literature to compare with. Also the gene functional study was not carried out. These data tell us that it is too early to conclude and confirm on the causality of this gene.

Conclusion

The direct effect of *OGT* alteration of brain development has been strongly confirmed nevertheless so far this gene has not been attested to be related to mental disability. We are the first to report on a novel missense mutation within *OGT*, segregating with intellectual disability in a family of three affected brothers without endocrine anomalies. The phenotype of our patients could be linked to the new missense mutation of the *OGT* gene nevertheless, our single case cannot be generalized and despite evidence of *OGT* gene effect on neuronal physiology, and brain development, more studies with additional cases are warranted, to shed light on the cognitive role of the *OGT* gene.

References

1. Aicardi J (1998) The etiology of developmental delay. Semin Pediatr Neurol 5: 15-20.
2. Chelly J, Khelfaoui M, Francis F, Chérif B, Bienvenu T (2006) Genetics and pathophysiology of mental retardation. Eur J Hum Genet 14: 701-713.
3. Ropers HH (2010) Genetics of early onset cognitive impairment. Annu Rev Genomics Hum Genet 11: 161-187.
4. Bouazzi H, Lesca G, Trujillo C, Alwasiyah MK, Munnich A (2015) Nonsyndromic X-linked intellectual deficiency in three brothers with a novel MED12 missense mutation [c.5922G>T (p.Glu1974His)]. Clin Case Rep 3: 604-609.
5. des Portes V (2013) X-linked mental deficiency. Handb Clin Neurol 111: 297-306.
6. Howerton CL, Morgan CP, Fischer DB, Bale TL (2013) O-GlcNAc transferase (*OGT*) as a placental biomarker of maternal stress and reprogramming of CNS gene transcription in development. Proc Natl Acad Sci U S A 110: 5169-5174.
7. Bullen JW, Balsbaugh JL, Chanda D, Shabanowitz J, Hunt DF, et al. (2014) Cross-talk between two essential nutrient-sensitive enzymes: O-GlcNAc transferase (*OGT*) and AMP-activated protein kinase (AMPK). J Biol Chem 289: 10592-10606.
8. Liu F, Shi J, Tanimukai H, Gu J, Gu J, et al. (2009) Reduced O-GlcNAcylation links lower brain glucose metabolism and tau pathology in Alzheimer's disease. Brain 132: 1820-1832.
9. Lazarus MB, Nam Y, Jiang J, Sliz P, Walker S (2011) Structure of human O-GlcNAc transferase and its complex with a peptide substrate. Nature 469: 564-567.
10. KIRBY KS (1956) A new method for the isolation of ribonucleic acids from mammalian tissues. Biochem J 1957: 495-504.
11. Kubota T, Nonoyama S, Tonoki H, Masuno M, Imaizumi K, et al. (1999) A new assay for the analysis of X-chromosome inactivation based on methylation-specific PCR. Hum Genet 104: 49-55.
12. Kreppel LK, Blomberg MA, Hart GW (1997) Dynamic glycosylation of nuclear and cytosolic proteins. Cloning and characterization of a unique O-GlcNAc transferase with multiple tetratricopeptide repeats. J Biol Chem 272: 9308-9315.
13. Yang X, Ongusaha PP, Miles PD, Havstad JC, Zhang F, et al. (2008) Phosphoinositide signalling links O-GlcNAc transferase to insulin resistance. Nature 451: 964-969.

14. Vosseller K, Trinidad JC, Chalkley RJ, Specht CG, Thalhammer A, et al. (2006) O-linked N-acetylglucosamine proteomics of postsynaptic density preparations using lectin weak affinity chromatography and mass spectrometry. Mol Cell Proteomics 5: 923-934.

15. Liu Y, Li X, Yu Y, Shi J, Liang Z, et al. (2012) Developmental regulation of protein O-GlcNAcylation, O-GlcNAc transferase, and O-GlcNAcase in mammalian brain. PLoS One 7: e43724.

16. Zeidan Q, Hart GW (2010) The intersections between O-GlcNAcylation and phosphorylation: implications for multiple signaling pathways. J Cell Sci 123: 13-22.

17. Blennow K, Zetterberg H (2015) The past and the future of Alzheimer's disease CSF biomarkers-a journey toward validated biochemical tests covering the whole spectrum of molecular events. Front Neurosci 9: 345.

18. Lozano L, Lara-Lemus R, Alvarado-Vásquez N (2014) The mitochondrial O-linked N-acetylglucosamine transferase (m*OGT*) in the diabetic patient could be the initial trigger to develop Alzheimer diseasel. Experimental Gerontology Journal 198-120.

19. Rexach JE, Clark PM, Hsieh-Wilson LC (2008) Chemical approaches to understanding O-GlcNAc glycosylation in the brain. Nat Chem Biol 4: 97-106.

20. Laczy B, Fülöp N, Onay-Besikci A, Des Rosiers C, Chatham JC (2011) Acute regulation of cardiac metabolism by the hexosamine biosynthesis pathway and protein O-GlcNAcylation. PLoS One 6: e18417.

21. Caldwell SA, Jackson SR, Shahriari KS, Lynch TP, Sethi G, et al. (2010) Nutrient sensor O-GlcNAc transferase regulates breast cancer tumorigenesis through targeting of the oncogenic transcription factor FoxM1. Oncogene 29: 2831-2842.

22. O'Donnel N, Zachara NE, Hart GW, Marth JD (2004) *Ogt*-dependent X-chromosome-linked protein glycosylation is a requisite modification in somatic cell function and embryo viability. Mol Cell Biol 24: 1680-1690.