CLINICAL REPORT

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GM3 Synthase Deficiency Due to *ST3GAL5* Variants in Two Korean Female Siblings: Masquerading as Rett Syndrome-Like Phenotype

Jin Sook Lee,[1] Yongjin Yoo,[2] Byung Chan Lim,[3] Ki Joong Kim,[3] Junghan Song,[4] Murim Choi,[2] and Jong-Hee Chae[3]*

[1]Department of Pediatrics, Gachon Institute of Genome Medicine and Science, Gachon University Gil Medical Center, Incheon, Korea

[2]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea

[3]Department of Pediatrics, Seoul National University College of Medicine, Pediatric Clinical Neuroscience Center, Seoul National University Children's Hospital, Seoul, Korea

[4]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Gyeonggi-do, Korea

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There have been a few reports of GM3 synthase deficiency since the disease of the ganglioside biosynthetic pathway was first reported in 2004. It is characterized by infantile-onset epilepsy with severe intellectual disability, blindness, cutaneous dyspigmentation, and choreoathetosis. Here we report the cases of two Korean female siblings with *ST3GAL5* variants, who presented with a Rett-like phenotype. They had delayed speech, hand stereotypies with a loss of purposeful hand movements, and choreoathetosis, but no clinical seizures. One of them had microcephaly, while the other had small head circumference less than 10th centile. There were no abnormal laboratory findings with the exception of a high lactate level. *MECP2/CDKL5/FOXG1* genetic tests with an array comparative genomic hybridization revealed no molecular defects. Through whole-exome sequencing of the proband, we found compound heterozygous *ST3GAL5* variants (p.Gly201Arg and p.Cys195Ser), both of which were novel. The siblings were the same compound heterozygotes and their unaffected parents were heterozygous carriers of each variant. Liquid chromatography–mass spectrometry analysis confirmed a low level of GM3 and its downstream metabolites, indicating GM3 synthase deficiency. These cases expanded the clinical and genetic spectrum of the ultra-rare disease, GM3 synthase deficiency with *ST3GAL5* variants.

Key words: ganglioside; GM3 synthase; Rett-like phenotype; *ST3GAL5*; whole-exome sequencing

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INTRODUCTION

The functions of gangliosides in the brain remain unclear; however, they might be involved in the regulation of cell-signaling pathways, especially in cell proliferation, differentiation, migration, adhesion, and apoptosis [Bieberich et al., 2001; Hannun and Obeid, 2002; Yu et al., 2012]. Therefore, deficient gangliosides and accumulated gangliosides can cause dysfunction in the central nervous system [Bieberich et al., 2001; Yu et al., 2012; Schengrund, 2015]. Diseases from the impaired ganglioside catabolism pathway are widely known, such as the lysosomal storage diseases [Kacher and Futerman, 2006]. However, only a few reports of diseases resulting

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*Correspondence to:
Jong-Hee Chae, M.D., Ph.D., Department of Pediatrics, Seoul National University College of Medicine, Seoul Korea, Pediatric Clinical Neuroscience Center, Seoul National University Children's Hospital, 101 Daehakro Jongno-gu, Seoul 03080, Korea.
E-mail: chaeped1@snu.ac.kr

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from disturbance of the ganglioside biosynthesis pathway have been published to date [Simpson et al., 2004; Fragaki et al., 2013; Wang et al., 2013; Boccuto et al., 2014].

GM3 synthase deficiency is a rare neurological disorder that was initially discovered in the Old Order Amish [Simpson et al., 2004], in which mutations of *ST3GAL5* resulted in a complete lack of GM3 and its downstream biosynthetic derivatives. It is characterized clinically by infantile-onset epilepsy, severe intellectual disability, irritability, failure to thrive, blindness, choreoathetosis, and cutaneous dyspigmentation [Simpson et al., 2004; Fragaki et al., 2013; Boccuto et al., 2014].

Here, we report the clinical characteristics of two sisters in detail, both of whom had severe intellectual disability with a Rett-like phenotype, and carried *ST3GAL5* compound heterozygous variants, which were identified by whole-exome sequencing (WES).

CLINICAL REPORT

The proband, a 6-month-old girl (II-2), who was referred to our hospital, had normal development until the age of 4 months, but later developed psychomotor regression. She was born after 39 weeks of gestation at 3.16 kg by cesarean section. She was the second baby born to healthy and nonconsanguineous Korean parents, whose first baby had similar clinical features. The patient was reported to be capable of making eye contact at 3 months of age. At the time of her initial evaluation, she was no longer capable of making eye contact and vertical nystagmus was noted. She demonstrated appropriate head control but could not roll over. Her height, weight, and head circumference were 70.6 cm (~90th centile), 8.3 kg (50–75th centile), and 40.5 cm (<10th centile), respectively. She displayed irritability and intermittent jerky movements. She had pale skin without specific nevi or macules. Comprehensive laboratory tests, including metabolic tests, showed no abnormalities, with the exception that the level of lactate was 4.3 mmol/L (normal range, 0.7–2.5) and pyruvate 0.9 mg/dl (normal range, 0.3–0.7). Brain magnetic resonance imaging revealed no abnormalities. Thereafter, at the age of 14 months, she showed hand stereotypies, although she still had purposeful hand movements. At the age of 26 months, she developed an impaired sleep pattern, while breathing pattern was normal. Both expressive and receptive language was poor. She developed no clinical seizures. Electrocardiography was normal. Ophthalmologic evaluation showed no abnormalities in the optic nerves except strabismus, for which she underwent surgery. Auditory brainstem response test was not performed due to sedation failure. Despite active physical therapy and rehabilitation, she did not make gains in development. In the interim, she lost purposeful hand movements and developed intermittent hand stereotypies. At the time of this report, she is 4 years old and cannot sit independently, roll over, or make eye contact. She is nonverbal as well.

Her older sister (II-1) visited our hospital with the proband for developmental delay at an age of 2 years and 5 months. She was born after 38 weeks of gestation at 2.7 kg by cesarean section. There were no perinatal problems. On the first visit, she could control her head, but not roll over. She could maintain sitting and standing without assistance. She had microcephaly (head circumference 44 cm, <3rd centile). She had poor sleep at night and bruxism, with intermittent emotional lability. At that time, the lactate level was 8.9 mmol/L (normal range, 0.7–2.5). At the age of 3 years, she started to show stereotypies such as hand tapping. At the age of 4 years, she displayed severe irritability and still had hand stereotypies with a lack of purposeful hand movement. At the age of 5 years, her height, weight, and head circumference were 101 cm (<3rd centile), 11.9 kg (<3rd centile), and 46 cm (<3rd centile), respectively. She also showed choreoathetotic movements. Ophthalmologic evaluation showed no abnormalities in the optic nerves. She developed no clinical seizures. At her current age of 6 years, she has pepper-like pigmentations on both hands and feet. She can walk a few steps without assistance, but she cannot use her hands and speak any words.

MATERIALS AND METHODS

We performed WES analysis of the proband and the parents. Genetic variants were validated using Sanger sequencing. For the two sisters with *ST3GAL5* variants, plasma GM3, and its downstream metabolites, GD3 gangliosides were measured by the method developed by Huang et al. [2014] with minor modifications (Supplementary Material).

RESULTS

Comprehensive tests, including genetic tests of *MECP2/FOXG1/CDKL5* by direct sequencing and/or multiplex ligation-dependent probe amplification, metabolic tests, and array comparative genomic hybridization, were conducted without overt abnormalities. We performed trio-exome sequencing in the proband (II-2) and her unaffected parents. The runs were in good quality (Supplementary Table SI), and subsequent variant discovery processes revealed novel, compound heterozygous, and missense variants in *ST3GAL5* (NM_003896) (c.584G>C/p.Cys195Ser, and c.601G>A/p.Gly201Arg) as only candidates that fit our criteria. The p.Cys195Ser variant was not found in the 1000 genomes or ExAC databases whereas p.Gly201Arg was found with a very low frequency (minor allele frequency $= 8.3 \times 10^{-6}$), making the expected probability of seeing such compound heterozygous carriers $<1.0 \times 10^{-9}$ (Supplementary Table SII). The mutated amino acid resides are highly conserved among the vertebrate orthologs (Fig. 1C). They were validated with Sanger sequencing in the proband as well as in the other affected sibling (II-1) (Fig. 1A and B). Their unaffected parents were heterozygous carriers of each variant.

The ganglioside levels (mean $\pm$ SD, μg/ml) in the plasma extracts from three healthy individuals were 10.2 ± 4.2 μg/ml for GM3 and 5.9 ± 1.8 μg/ml for GD3 gangliosides. By contrast, the plasma gangliosides from two siblings with *ST3GAL5* variants were barely detected (0.35, 0.66 μg/ml for GM3 and 0.20, 0.22 μg/ml for GD3 gangliosides). Representative multiple reaction monitoring (MRM) chromatograms for major components of GM3 and GD3 ganglioside in the plasma extracts from a healthy control and the proband are shown in Figure 2.

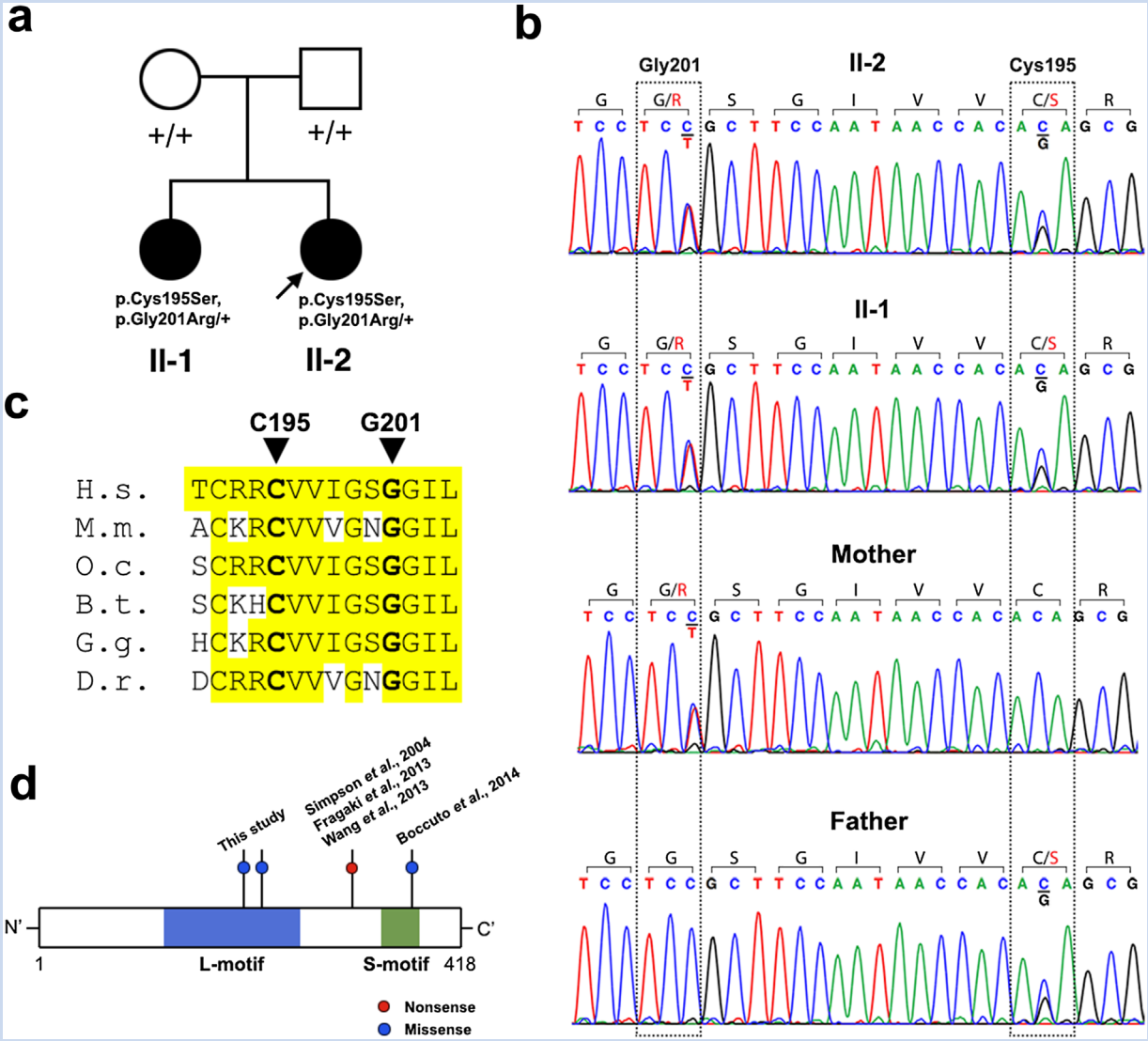

FIG. 1. ***ST3GAL5*** **variants. (a and b) Pedigree and Sanger traces displaying compound heterozygous variants in two affected siblings: c.584G>C (p.Cys195Ser) and c.601G>A (p.Gly201Arg). (c and d) Both compound heterozygous variant was found in the L-motif domain and amino acid conservation of the** ***ST3GAL5*** **Cys195 and Gly201 residues in orthologs from different vertebrate species. H.s. denotes** ***Homo sapiens*** **(human); M.m.,** ***Mus musculus*** **(mouse); O.c.,** ***Oryctolagus cuniculus*** **(rabbit); B.t.,** ***Bos taurus*** **(cow); G.g.,** ***Gallus gallus*** **(chicken); X.t.,** ***Xenopus tropicalis*** **(frog); D.r.,** ***Danio rerio*** **(zebrafish). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].**

DISCUSSION

GM3 synthase deficiency (OMIM#609056) is an extremely rare neurometabolic disorder inherited as an autosomal recessive trait, which was first reported as Amish infantile epilepsy syndrome [Simpson et al., 2004]. *ST3GAL5* encodes a ST3 β-galactoside α-2,3-sialyltransferase 5, glycosyltransferase family member, which synthesizes the ganglioside GM3 by adding sialic acid to lactosylceramide. The molecular defect in the first step of the ganglioside synthesis pathway causes a decrease in the levels of GM3 and other downstream complex gangliosides, which might be important during early and late stages of the nervous system development [Yu et al., 2012]. It remains unclear whether the condition is caused by a lack of GM3 and its downstream metabolites or accumulation of lactosylceramide and its alternative metabolites (o- and globo-series) [Kacher and Futerman, 2006]. This condition resulting from the impaired ganglioside biosynthesis pathway can disturb neurodevelopment, which clinically manifest with severe developmental delay/intellectual disability and epilepsy during infancy [Simpson et al., 2004; Fragaki et al., 2013; Wang et al., 2013; Boccuto et al., 2014]. The disease may also present with irritability during early infancy, failure to thrive, cutaneous dyspigmentation,

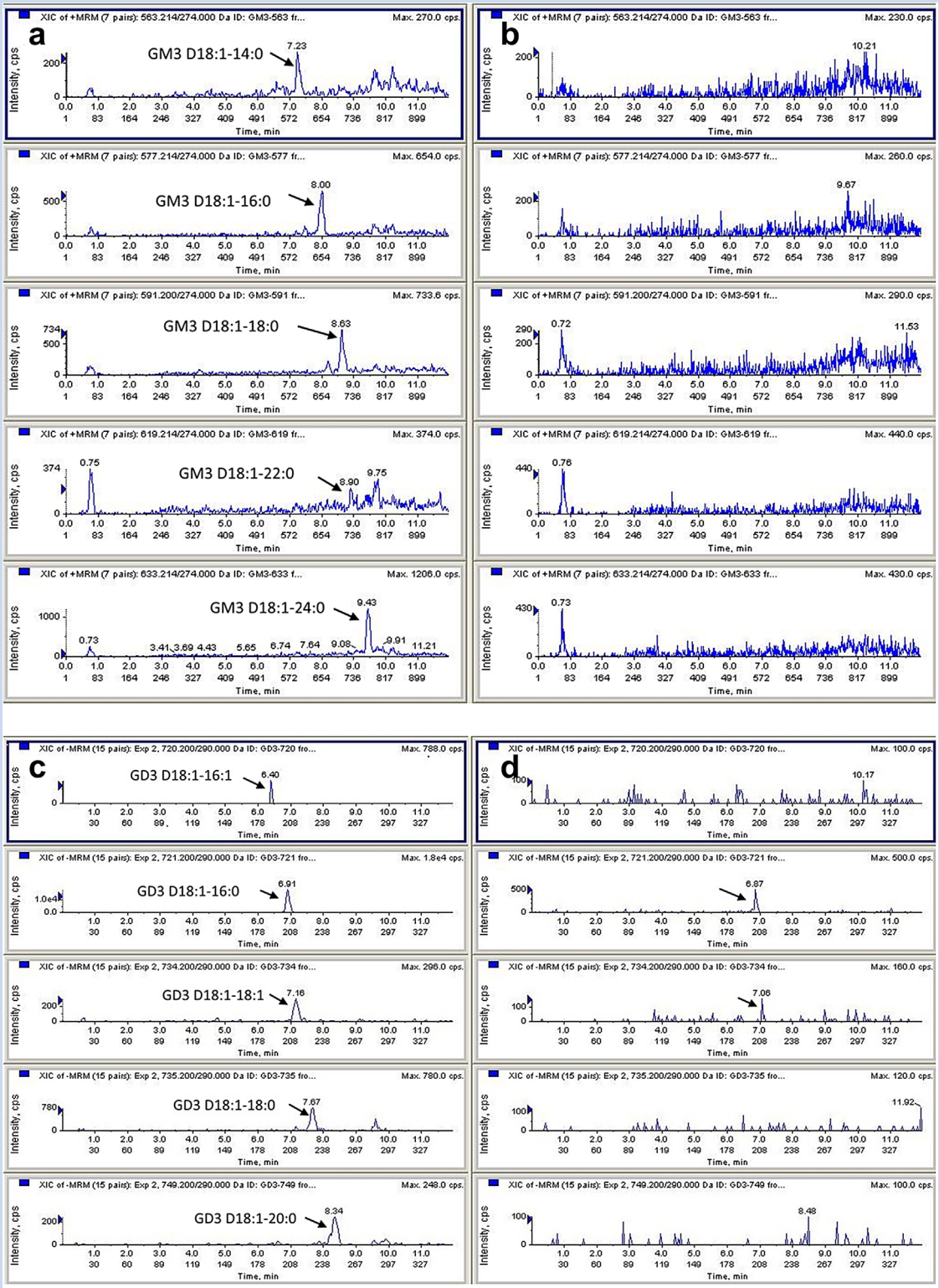

FIG. 2. Representative MRM chromatograms for major components of GM3 (a and b) and GD3 (c and d) ganglioside in the plasma extracts from a healthy control (a and c) and a patient (b and d). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

blindness, and deafness [Simpson et al., 2004; Farukhi et al., 2006; Inokuchi, 2011; Fragaki et al., 2013; Wang et al., 2013; Boccuto et al., 2014].

We initially suspected the two sisters of having a Rett-like phenotype, because although most cases are sporadic, some familial cases of Rett syndrome have been observed [Ravn et al., 2011]. Both sisters had severe psychomotor delay and/or regression, delayed speech, hand stereotypies with a loss of purposeful hand movements, and abnormal choreoathetotic movements. Diagnosis of Rett syndrome is based on clinical criteria [Neul et al., 2010] and most of the cases are caused by *MECP2* mutations. Although *FOXG1* or *CDKL5* mutations account for some of the remainder, there are still some patients with various Rett-like phenotypes, the genetic cause of which remains unclear. The features of the two sisters were suggestive of Rett syndrome, although they did not fulfill the diagnostic criteria completely. Through WES, we identified compound heterozygous variants in *ST3GAL5*, for which the various bioinformatics prediction tools such as Sorting Intolerant From Tolerant (SIFT) and Polyphen-2 were used to suggest the pathogenicity of the variants. We showed the plasma GM3 (a-series) and GD3 (b-series) gangliosides were barely detected and thereby confirmed the biochemical effect of the variants in the affected siblings (Fig. 2), although we did not measure the activity of GM3 synthase in vitro. Increased lactate levels seen in the present study might suggest the secondary mitochondrial dysfunction due to GM3 synthase deficiency [Fragaki et al., 2013]. Yoshikawa et al. reported that ganglioside GM3 is essential for the proper organization and maintenance of stereocilia in auditory hair cells, and therefore GM3 synthase deficiency can result in hearing loss [Inokuchi, 2011; Yoshikawa et al., 2015]. Unfortunately, we could not objectively assess the hearing ability of our patients.

Sialytransferases have well-conserved sequence motif elements such as L-motif and S-motif, which might be functionally important. The L-motif is thought to be responsible for the donor binding and the S-motif for acceptor as well as donor binding [Datta and Paulson, 1995; Datta et al., 1998]. These compound heterozygous variants were included in the highly conserved L-motif area functioning in binding of the nucleotide sugar donor (Fig. 1C), which might result in the protein instability and impair the disulfide formation important in maintaining an active enzyme conformation [Datta et al., 1998, 2001; Patel and Balaji, 2006; Audry et al., 2011]. As shown in Figure 1C, there have been only two homozygous mutations reported to date: c.862C>T (p.Arg288*) and c.994G>A (p.Glu332Lys). The protein from the nonsense mutation, c.862C>T, was truncated between L- and S-motifs, which has been reported in Old Order Amish patients [Simpson et al., 2004; Fragaki et al., 2013; Wang et al., 2013]. The other mutation, c.994G>A in an African-American family, was within the S-motif, which might result in disturbed function of S-motif, interacting with the lactosylceramide acceptor and a sugar donor [Boccuto et al., 2014]. Compared with the cases with the two mutations reported previously, the two sisters in the present study had neither epilepsy nor blindness. We also did not observe poor feeding, vomiting, or deafness in either of them, although the proband's older sister failed to thrive. They showed intellectual disability with significant language impairment and choreoathetosis. Irritability was observed in both, but not of infantile onset. Skin pigmentation was not noted in the proband's older sister until she was 6 years old. The mild and atypical phenotypic variations of our cases might be affected by the enzymatic changes due to the location and/or type of the variants. Additionally, environmental or additional genetic factors might modify the phenotype, particularly within this family, because clinical severity of the proband seemed more severe than that of her older sister.

There might be still undiagnosed patients with GM3 synthase deficiency. It is challenging to identify these cases without specific neurologic features, because it is a very rare disease, in particular in the non-Amish population [Boccuto et al., 2014]. The cutaneous findings can be useful and offer disease-specific clues for an autosomal recessive neurocutaneous condition, called the salt-and-pepper syndrome. However, it is not always true for all cases and the patient's age should be critically assessed because about half of the patients with GM3 synthase deficiency had no cutaneous features, and this frequency was dependent on increasing age [Wang et al., 2013]. WES in the present study helped to make a diagnosis of this ultra-rare neurologic disease. We propose that *ST3GAL5* should be considered in the causes of atypical Rett syndrome or Rett-like phenotype, together with *STXBP1* and *PTPN4* as reported recently [Romaniello et al., 2015; Williamson et al., 2015]. Additionally, it should be included in the panel of targeted next-generation sequencing for intellectual disability or autistic spectrum disorders. Although no direct molecular and pathogenic link between *MECP2* and *ST3GAL5* has been described, a relationship between Rett syndrome and *ST3GAL5* could be suggested because altered patterns of gangliosides in the brain might be associated with autism [Nordin et al., 1998].

In conclusion, this is the first report to our knowledge showing that patients carrying *ST3GAL5* variants can display a Rett-like phenotype and simultaneously it is the fourth report of novel *ST3GAL5* variants. Additionally, this is the first case of GM3 synthase deficiency in Asian population. These cases broaden the phenotypic and genetic spectrum of GM3 synthase deficiency due to *ST3GAL5* variants. Patients with intellectual disability or furthermore presenting with Rett-like phenotype should be suspected of GM3 synthase deficiency, a disorder of ganglioside biosynthesis.

AUTHORS' CONTRIBUTIONS

J.S.L. and Y.Y. contributed to the data acquisition, prepared the first draft of the manuscript, and edited the manuscript drafts. B.C.L. and K.J.K. made substantial contributions to data acquisition and interpretation and revised the manuscript. M.C. contributed to the sequencing data acquisition and interpretation. J.S. contributed to the biochemical assay and interpretation. J.H.C. designed this study and edited the manuscript drafts until the final draft was produced and mentored J.S.L. and Y.Y. through the process as a correspondence.

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