CASE REPORT

Rafiq Syndrome: Old Variant in MAN1B1 Gene and Some New Phenotypic Features

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ABSTRACT

Rafiq syndrome is a congenital disorder of glycosylation type II that develops due to mutations in the Mannosidase Alpha Class 1B Member 1 (MAN1B1) gene encoding α 1,2-mannosidase. In the literature, 45 patients have been reported to date. This study presents a patient with some phenotypic traits that differ from previously reported patients with Rafiq syndrome.Since the patient was not diagnosed despite detailed examinations, whole exome sequencing was performed. The patientss' homozygous c.1000 C>T (p.Arg334Cys) pathogenic variant was detected in the MAN1B1 gene (NM_016219.5), which was consistent with Rafiq syndrome. Our patient's clinical findings were mainly similar to those of previously reported patients. However, our patient had feeding difficulty that started to improve after the fifth month and persistent hyperekplexia .

Feeding difficulty and hyperekplexia concomitant to MAN1B1 gene mutation are reported for the first time. More extensive case series are needed to understand whether these findings are part of the syndrome or incidental comorbid conditions.

Introduction

Congenital disorders of glycosylation (CDG) develop due to hypoglycosylation defects of proteins and lipids along the glycan modification pathway. To date, 165 disorders related to this pathway have been described. Most described disorders are autosomal recessive monogenic disorders that cause multisystem involvement. Disorders of protein glycosylation include N-glycosylation, O-glycosylation, and combined N- and O-glycosylation defects. Disorders of glycan assembly and transfer developing due to defects in the cytosol and endoplasmic reticulum are classified as CDG Type-I, and disorders of glycan assembly and transfer in the Golgi apparatus are classified as CDG Type-II (1).

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Rafiq syndrome is a CDG Type-II developed due to mutations in the Mannosidase Alpha Class 1B Member 1 (MAN1B1) gene encoding α 1,2-mannosidase. It is characterized by intellectual disability, motor delay, hypotonia, and truncal obesity. In addition, phenotypic traits such as facial dysmorphism (hypertelorism, down-slanting palpebral fissures, large and low-set ears, hypoplastic nasolabial folds, thin upper lip), joint hypermobility, and skin laxity have been described in patients (2–4).

The current study presents a patient with a previously described pathogenic variant in the MAN1B1 gene who has some phenotypic traits that differ from previously reported patients.

Case History:

The 36-month-old female patient was admitted to our clinic for the first time when she was 25 months old with complaints of hypotonia, global developmental delay, and dysmorphic facial features. The patient's medical history indicates she was delivered via Cesarean section at full term (40 weeks). At birth, she weighed 3,200 grams and measured 50 centimeters in length, with no complications reported. The patient achieved head control at 15 months and began sitting up at 25 months. However, she has not started crawling or walking and cannot speak meaningful words. Her family history reveals that her parents are cousins. Notably, five cousins on both sides of the family have been diagnosed with metachromatic leukodystrophy, but there are no other significant features reported. The patient was admitted for the first time when she was one week old with complaints of ineffective sucking, inability to swallow the milk, frequent aspiration, startling and tensing up at touch, or loud noises that started in the same period. In the health center she was admitted to, detailed tests were performed due to hypotonia and facial dysmorphism detected in the patient. No pathology was found in the examination to determine congenital hypotonia's etiology. In the department of metabolism where the patient was evaluated, tandem mass, urine organic acid, amino acids in the blood and urine, very-long-chain fatty acids, ammonia, pyruvate, uric acid, lipids, creatinine phosphokinase, liver and kidney function tests were performed. Test results showed no other pathology than a slightly high ammonia level (measured value 190 µmol/dl, normal reference value 31-123 µmol/dl). A protein-loading test was performed on the patient, and the highest ammonia level measured after the loading test was 214 µmol/dl. In the follow-up, ammonia levels returned to normal, and a specific diagnosis could not be made. In the genetics department, the results of mutation and karyotype analyses requested for spinal muscular atrophy were normal. The results of electroencephalography (EEG) and brain magnetic resonance imaging (MRI) performed for the differential diagnosis of hyperekplexia at that time were normal. In electromyography (EMG), no pathology other than a slight decrease in the sensory nerve conduction velocity was detected. During the patient's follow-up, feeding difficulty improved after the fifth month, but other findings remained the same.

In the first examination at our clinic, her height and weight values were within the normal range. The patient had global developmental delay, hypotonia, persistent agitation, crying spells, excessive startling, and tensing up triggered by sounds or tactile stimuli. In addition, broad eyebrows, down-slanting palpebral fissures, short neck and philtrum, wide nose, small chin, low-set ears, joint laxity and hypermobility, and cutis

laxa were detected. The patient could neither respond to her name nor follow basic directions. Since the patient could not be diagnosed despite the examinations performed up until then, whole exome analysis (WES) was requested. The results of repeated brain MRI and EEG were normal in the patient.

When the patient was re-evaluated at 35 months old, it was discovered that she had begun crawling at 34 months. All other observations were unchanged.

Written informed consent for publishing the case report was obtained from the patient's parents.

Genetic analysis:

Twist Human Comprehensive Exome NGS Panel was used to evaluate the patient's molecular genetics. Family screening was also done using Sanger sequencing. For this study, Genomic DNA was extracted from peripheral blood samples according to the manufacturers' protocols (Magpurix Blood DNA Extraction Kit, Zinexts Life Science Corp., New Taipei, Taiwan). Sequencing libraries were prepared using Library Preparation EF 2.0 with Enzymatic Fragmentation (Twist Bioscience, San Francisco, CA, USA) according to the manufacturer's instructions, and exome hybridization was achieved following the Twist Human Core Exome Multiplex Hybridization Protocol (Twist Bioscience, San Francisco, CA, USA). All libraries were validated and quantified with the Agilent® DNA 7500 Assay and a Thermo Fisher Scientific® Qubit® dsDNA Broad Range Quantitation Assay. Average fragment length should be between 375 bp and 425 bp. Human Comprehensive Exome NGS was performed on an Illumina MiSeq NGS System (Illumina, Inc., San Diego, CA, USA) using the MiSeq Reagent Nano Kit v3 (Illumina, Inc., San Diego, CA, USA). FASTQ sequencing files were collected and transferred to "SEQ" variant analysis software (Genomize, Istanbul, Turkey). The data received had an average read depth of more than 20× coverage in more than 95% of the targeted regions. Variants were filtered by pathogenicity, frequency, inheritance pattern, and clinical phenotype. The patient was carrying a homozygous c.1000 C>T (p.Arg334Cys) variant according to the NM_016219.5 transcript in the MAN1B1 gene. This variant was classified as pathogenic in the ClinVar database (ClinVAR rs387906886). Sanger sequencing was performed on the proband and parents. After the segregation analysis, parents carried heterozygous c.1000 C>T variant (Figure 1). The results were consistent with Rafiq Syndrome with an autosomal recessive inheritance pattern (OMIM# 614202).

Discussion

CDG represent a diverse group of conditions that vary both clinically and genetically. This study introduces a patient who, for the first time, exhibits a MAN1B1 gene mutation characterized by neonatal onset of hyperekplexia and significant feeding difficulties, which are more pronounced than hypotonia.

The MAN1B1 gene encodes the class I alpha-1,2-mannosidase enzyme, which plays a part in N-glycan biosynthesis and is a member of the glycosyl hydrolase family 47. This enzyme is involved in converting Man9GlcNAc glycan to Man8GlcNAc isomer B. N-glycan trimming to Man5-6GlcNAc2 is required in the degradation pathway in the endoplasmic reticulum (2, 5).

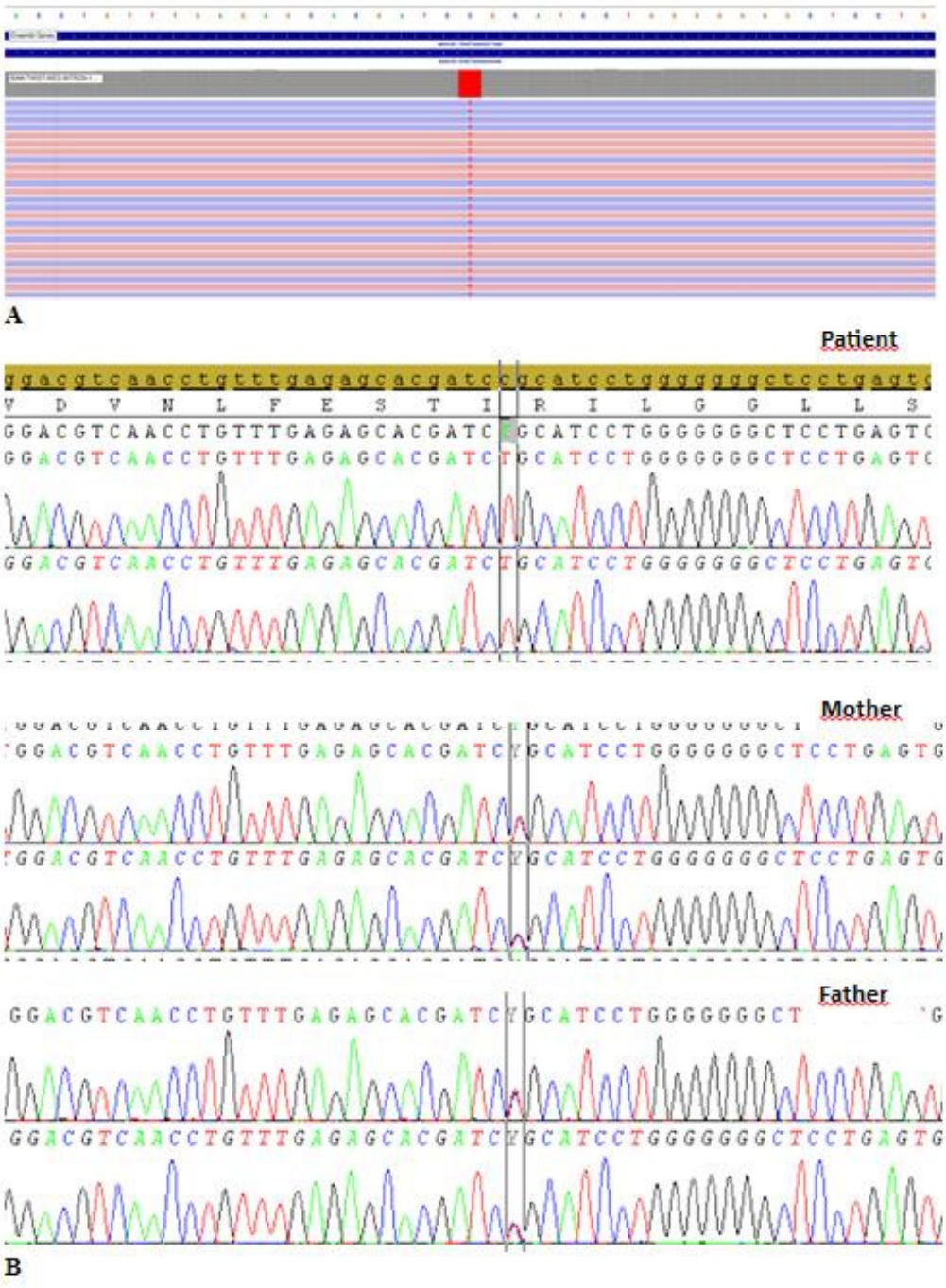

Figure 1. A: Integrative genomics viewer (IGV) images of next-generation sequencing data of MAN1B1 c.1000C>T variant in patient, B: Sanger results of patient, mother and father

To the best of our knowledge, 45 patients with Rafiq syndrome caused by mutations in the MAN1B1 gene have been reported. In previously reported patients, head and neck anomalies (broad eyebrows, bulbous nose, down-slanting palpebral fissures, highly arched eyebrows, long eyebrows, long face, pointed chin, prominent nose, short neck, short philtrum, sparse eyebrows, sparse lateral eyebrows, thin upper lip vermilion, underdeveloped nasolabial folds, broad nasal bridge, and wide nose), extremity anomalies (clinodactyly of the fifth finger), breast anomalies (inverted nipples), eye abnormalities (hypertelorism, strabismus), anomalies of the

skin and musculoskeletal system (cutis laxa, dolichocephaly, flat occiput, flexion contracture, hypotonia, joint hypermobility, joint laxity, and macrocephaly), nervous system anomalies (overeating, verbal and physical aggression, autistic behavior, cerebellar ataxia, cerebellar hypoplasia, global developmental delay, moderate or severe intellectual disability, motor delay, and seizure), ear anomalies (low-set ears, macrotia) and growth problems (obesity, short stature, and truncal obesity) have been described (6).

The homozygous c.1000 C > T (p.Arg334Cys) pathogenic variant detected in the studied patient was first described in three individuals from the same family by Rafiq et al. In these patients, dysmorphic facial features, varying degrees of developmental delay, speech, and cognitive delays, as well as epilepsy, aggressive behavior, and overeating in one were reported (2). The dysmorphic facial features and many of the clinical symptoms in the studied patient were similar to those of three previously reported patients with the same pathogenic variant. Epilepsy and hyperphagia detected in one of the three patients were not present in the patient. On the contrary, the patient had a history of feeding difficulty that started in the neonatal period and improved after the fifth month. Feeding difficulty is expected in hypotonia, but it was the initial finding at presentation and was prominent in our patient. Cases with ineffective sucking, lack of swallowing reflex, and feeding difficulties requiring nasogastric or parenteral nutrition have been previously reported in disorders such as PMM2-CDG and ALG1-CDG (7). However, to the best of our knowledge, such findings have not been reported in MAN1B1 gene mutations. The reason for the prominent feeding difficulty in the patient may be because the muscle groups involved in swallowing and sucking were affected more and various tissues were affected differently from glycoprotein synthesis. Although the absolute enzyme activity levels of mannosidase I and II in different cell types could not be precisely determined in previous studies, the relative ratios of the two enzymes in different cell types have been shown to vary widely (8).

Hyperekplexia is characterized by an exaggerated startle response triggered by sudden external stimuli such as touch or sound, followed by a brief period of general rigidity. It may develop due to a genetic, idiopathic, or symptomatic cause. It is mainly caused by defects in inhibitory glycinergic neurotransmission. Gene mutations associated with the glycinergic neurotransmission system have been frequently reported in patients with hyperekplexia. The most frequently described genes are GLRA1, SLC6A5, GLRB, GPHN, and ARHGEF9 (9). 61-63% of hereditary hyperekplexia cases are caused by GLRA1, 25% by SLC6A5, and 12-14% by GLRB gene mutations (10). These gene regions were explicitly examined in our WES, and no pathology was detected. In the examined patient, hyperekplexia started in the neonatal period and still continues. The interesting part was that despite having hypotonia in general, she had hyperekplexia attacks. As far as we know, this has not been previously reported in Rafiq syndrome.

In Conclusion

In summary, the feeding difficulties observed at the time of the studied patient's presentation, along with the prominence of hyperekplexia over hypotonia in the context of MAN1B1 gene mutation, have been documented for the first time. Further investigation through a more extensive case series is essential to ascertain

whether these clinical manifestations, specifically hyperekplexia, are integral components of the extensive phenotypic spectrum associated with Rafiq syndrome or if they represent incidental comorbidities.

Acknowledgment

None.

Authors' Contribution

Nezir Özgün, data collection, planning, writing. Merve Saka Güvenç, writing and genetic analysis.

Conflict of Interest

The authors declared no conflict of interest.

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