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CASE REPORT

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Identification of a mutation in *TNRC18* in a patient with clinical features of Fazio-Londe disease

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Key Clinical Message

Fazio-Londe disease and Brown-Vialetto-Van Laere syndrome are rare related neurological disorders. Although *SLC52A3* and *SLC52A2* that encode riboflavin transporters are their only known causative genes, many patients without mutations in these genes have been reported. Clinical and genetic data of a patient with features suggestive of Fazio-Londe disease are presented. Neurological examination revealed significant involvement of cranial nerves and weakness in the lower extremities. Pontobulbar presentations were prominent. EDX study suggested motor neuronopathy. Hearing was normal. She was diagnosed with FL disease. Response to riboflavin supplementation was not favorable. The patient's pedigree suggested recessive inheritance. *SLC52A3* and *SLC52A2* were screened and mutations were not observed. Results of exome sequencing and segregation analysis suggested that a mutation in *TNRC18* is a candidate cause of disease in the patient. The three dimensional structure of the TNRC18 protein was predicted and it was noted that its two conserved domains (BAH and Tudor) interact and that the valine residue affected by the mutation is positioned close to both domains. A mutation in *TNRC18* is cautiously reported as the possible cause of FL disease in the patient. The finding warrants further inquiries on *TNRC18* about which little is presently known.

KEYWORDS

Brown-Vialetto-Van Laere syndrome, Fazio-Londe disease, *SLC52A2*, *SLC52A3*, *TNRC18*

1 | INTRODUCTION

Fazio-Londe (FL) disease (OMIM; 211,500) and Brown-Vialetto-Van Laere syndrome (BVVL; OMIM 211530) are related progressive neurological disorders characterized by pontobulbar palsy due to involvement of lower motor cranial nerves.[1,2] Upper motor neuron defects may appear with disease progression. Other possible manifestations include limb weakness and atrophy, speech defects, ophthalmoplegia, optic atrophy, sensory symptoms, sensory signs, and respiratory insufficiencies. Age at onset ranges from infancy to early adulthood. Survival time ranges from a few years to several decades; respiratory compromise is the most common cause of death. Although FL

and BVVL are both rare diseases, fewer cases of FL disease have been described. FL is clinically distinguished from BVVL by presence of bilateral sensorineural hearing loss only in the latter.[1,3,4] Both diseases have some similarities with juvenile amyotrophic lateral sclerosis (ALS).[5]

SLC52A3 (also known as RFVT3) and *SLC52A2* (also known as RFVT2) have been identified as BVVL causative genes.[2,6,7] Whereas only mutations in *SLC52A3* have been reported in FL disease patients, this may be because fewer FL disease patients have been genetically screened.[2,8] *SLC52A3* and *SLC52A2*, respectively, encode solute carrier 52, riboflavin transporter, members 3 and 2. Notably, BVVL and FL disease patients without mutations in these genes have been reported.[3,9,10] In fact, in screenings of relatively large cohorts, mutations in *SLC52A3*, and *SLC52A2* were not observed in two thirds or more of the patients.[3,11] These findings are consistent with the presence of genetic heterogeneity for BVVL and FL, and, in fact, other candidate disease causing genes have been suggested.[3,9,10] Patients with mutations in *SLC52A3* and *SLC52A2* generally benefit from riboflavin supplementation, while those without mutations in these genes do not respond favorably.[3,9–11]

Here, we present clinical data and results of genetic analysis on a patient with features suggestive of FL disease in whom mutations in *SLC52A3* and *SLC52A2* were not found. Results of exome sequencing and segregation analysis were consistent with the possibility that a mutation in *TNRC18* may be a candidate cause of disease in the patient.

2 | PATIENT, METHODS, AND RESULTS

The research was performed in accordance with the Declaration of Helsinki and with approval of the ethics board of the University of Tehran. All participants of the study consented to participate after being informed of the nature of the research.

The clinical features of the patient who was an Iranian female are presented in Table 1 (Figure 1A). She herself and family members reported that she was normal and quite active until onset of disease at the age of 19 years. The first complaint reported by the patient was asymmetric hand weakness, however history and thorough neurological examination revealed symptoms and signs of significant involvement of cranial nerves including dysarthria, dysphagia, facial weakness, mastication problem, masseter muscle atrophy, bilateral symmetrical ptosis, ophthalmoplegia, and also weakness in the lower extremities. Although all the early manifestations, particularly those involving the bulbar region, further deteriorated in the following years, sensory function, hearing, and mental status remained normal throughout the disease course. Electrodiagnostic (EDX) study was consistent with motor neuronopathy in cranial, cervical, thoracic and

TABLE 1 Clinical features of patient II-4 with mutation in *TNRC18*.

Sex	Female
Age at examination	23 yrs
Age at onset	19 yrs
Age of death	25 yrs
Disease duration	6 yrs
Initial presentation	Asymetric hand weakness
Bulbar palsy (CN-9-12)	+ (dysarthria, dysphagia)
CN-5 palsy	Mastication problems and atrophy
Facial weakness (CN-7)	Weakness
Hearing problem (CN-8)	–
Ophthalmoplegia (CN3/4/6 palsy)	+
Ptosis	+ (partial ptosis)
Optic nerve atrophy (CN-2 involvement)	–
Limb weakness	+, more prominent in left upper extremity
Limb muscle atrophy	–
Spasticity	–
DTR	Decreased
Upward plantar reflex	–
Sensory symptoms/ signs	–
Tremor	–
Ataxia	–
Vertigo	–
Tinnitus	–
Epilepsy	–
Mental impairment	–
Psychiatric disorder	–
Autonomic disfunction	–
Respiratory problem	+[a]
Ambulatory status	Needs help for walking
Acylcarnitine profile	Normal
EDX	Compatible with motor neuronopathy
Brain MRI	Normal
Response to riboflavin	–

[a]Progressed rapidly and was the ultimate cause of death.

Abbreviations: CN, cranial nerve; DTR, deep tendon reflex; EMG, electromyography; MRI, magnetic resonance imaging; NCS; nerve conduction studies.

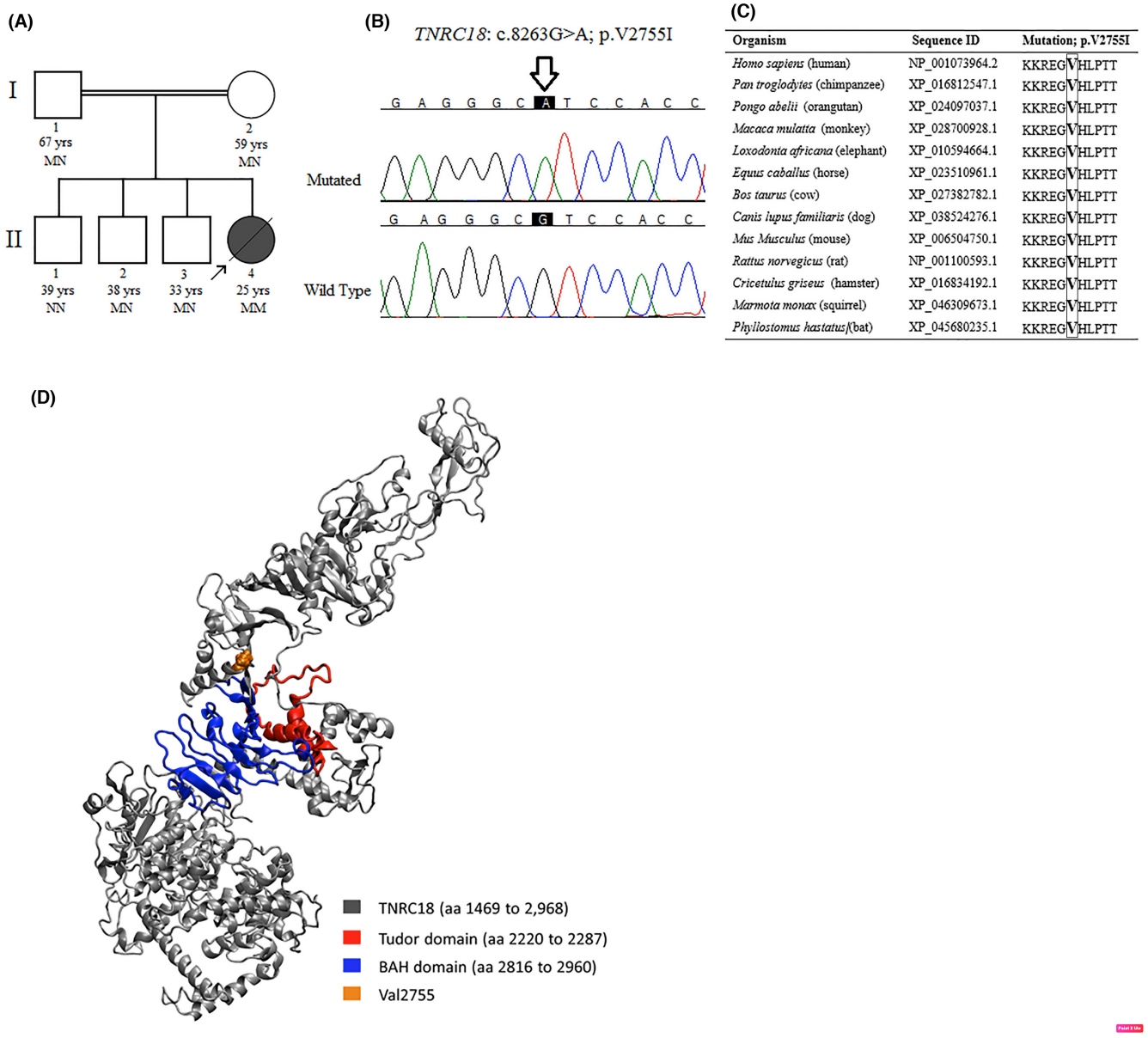

FIGURE 1 Fazio-Londe patient II-4. (A) Pedigree of the patient. Filled circle, affected proband; Unfilled shapes, unaffected individuals. Present age of pedigree members and age at death of II-4, and the genotypes of *TNRC18* for the individuals are shown. NN, MN, and MM, respectively, homozygous normal, heterozygous, and homozygous mutated. (B) Chromatograms of wild type sequence (c.8263G) and mutated sequence (c.8263A) in *TNRC18*. (C) Evolutionary conservation of valine at positions corresponding to p.2755 in the human protein in orthologous proteins of various mammals. (D) The predicted 3D structure of the TNRC18 protein as assessed by the I-TASSER threading method described in references 16 and 17. Only the 1500 amino acids of the carboxyl trminus of TNRC18 (UniProt ID: O15417) were included in the analysis due to limitations of the I-TASSER server. This region includes the Tudor (amino acids 2220–2287) and BAH (amino acids 2816–2960) domains, as well as p.Val2755. The proximity of the Tudor and BAH domains in the 3D structure, and the position of p.2755 with respect to these domains is evident. As expected, replacement of valine by isoleucine at this position did not affect a notable change in the 3D structure of the protein (not shown).

lumbosacral spinal regions. Magnetic resonance images taken at various stages of disease progression were always normal. She was clinically diagnosed with FL disease. This diagnosis was favored to juvenile onset ALS because of the very prominent pontobulbar presentations in the patient. Riboflavin was prescribed at a dosage of 10 mg/kg body weight/day. Her symptoms did not improve in response to riboflavin administration. She died at the age of 25 years after development of severe respiratory infection. Consequences of FL disease likely contributed to her rapid demise.

The patient described (II-4) was born to unaffected consanguineous parents, and she was the only affected individual among four siblings (Figure 1A). The pedigree

was consistent with autosomal recessive inheritance of disease. Genetic analysis was initiated by mutation screening of the exons and adjacent intronic sequences of *SLC52A3* and *SLC52A2* by Sanger sequencing. Candidate disease causing mutations were not observed and analysis was followed by whole exome sequencing. The exome sequencing data were filtered for homozygous or compound heterozygous sequence variations as previously described.[9] Despite consanguinity of the parents, compound heterozygous sequence variations were considered for the sake of stringency. Among the 10 variations that remained after filtering of the data, only mutation c.8263G > A in the homozygous state in *TNRC18* (Trinucleotide Repeat Containing 18 gene) segregated with disease status in the family (Table S1). Both parents and two siblings of the proband were heterozygous carriers of the mutation, and the third sibling was homozygous for the wild type allele (Figure 1B). As evident in the dbSNP database, the c.8263G > A mutation in *TNRC18* has been observed at only very low frequencies (maximum 7 X 10^{-4}, rs374974528, ClinVar accession number VCV002369974.1.) in various screenings (https://www.ncbi.nlm.nih.gov/snp/). The variation has never been reported in the homozygous state. Consistent with being a very rare mutation, it was also not present in the Iranome database that includes whole exome sequence data on 800 Iranians (http://www.iranome.ir/). c.8263G > A in *TNRC18* causes p.Val2755Ile in the encoded protein. Valine to isoleucine mutations are generally considered to be neutral and, consistent with this, various bioinformatics tools predicted that the p.Val2755Ile mutation in the trinucleotide repeat containing 18 protein would not be damaging. Nevertheless, various Val > Ile or Ile > Val mutations that are the putative cause of various diseases have been reported (Table S2). Therefore, although most Val > Ile changes are neutral, some may have detrimental structural/functional effects. It is to be noted that valine at position 2755 in the human TNRC18 protein is evolutionarily conserved among mammals (Figure 1C).

Information on *TNRC18* and its encoded protein in the literature is sparse. *TNRC18* is a large gene with 36 exons that encodes a correspondingly large protein with 2968 amino acids. A disease causing mutation in the gene has not previously been reported. The TNRC18 protein is predicted to contain two conserved domains consisting of the BAH (bromo-adjacent homology; amino acids 2816–2960) and Tudor (named after extinct European families; amino acids 2220–2286) domains (Conserved Domains Database: https://www.ncbi.nlm.nih.gov/cdd/). Both domains are implicated in protein–protein interactions especially in the context of chromatin binding and regulation of replication and transcription.[12–15] The p.Val2755Ile mutation in *TNRC18* described here is positioned just upstream of the BAH encoding domain in the primary sequence of the gene. As an empirical-based three dimensional (3D) structure for the TNRC18 protein is not available, I-TASSER (https://zhanggroup.org/I-TASSER/) was used to predict its 3D structure.[16,17] I-TASSER is a widely recognized powerful threading (or fold recognition) method that uses multiple threading to identify optimal structural templates for protein fragments, and that subsequently applies assembly simulations for constructing full-length atomic models. The predicted structure obtained favorable confidence values (C-score = 0.37; TM-score = 0.76 ± 0.10).[16] The predicted structure for TNRC18 is presented in Figure 1D. Interestingly, the Tudor and BAH domains are proximal to one another in the predicted structure. P.Val2755 is close to both domains, suggesting it may be involved in interactions of the domains with other proteins in the framework of TNRC18's biological functions. A valine to isoleucine mutation may affect the putative interactions and thus have functional consequences.

3 | DISCUSSION

A mutation in *TNRC18* was here cautiously reported as the possible cause of FL disease in patient II-4 of the pedigree described (Figure 1A). Absence of a positive response to riboflavin administration, absence of candidate causative mutations in *SLC52A3* and *SLC52A2*, results of exome sequencing, segregation of the *TNRC18* mutation with disease status in the family, and reported findings suggesting that *SLC52A3* and *SLC52A2* are not the exclusive FL and BVVL causative genes, together justify cautious consideration of the *TNRC18* mutation as cause of disease in the pedigree. Of course, exome sequencing does not screen the entire genome, and disease associated variation within deep intronic and noncoding regions would not be identified using this sequencing protocol. Although both FL and BVVL are rare disorders, confirmation of the contribution of *TNRC18* to one or both diseases must await finding mutations in the gene in other patients. Finally, the finding being reported warrants further inquiries on the functions of TNRC18. Communication with the scientific community through GeneMatcher resulted in 11 responses in which patients with various neurological clinical anomalies were described (https://genematcher.org/).

AUTHOR CONTRIBUTIONS

Marzieh Khani: recruitment of patient and family members, exome data analysis, mutation screening and segregation analysis in families by Sanger sequencing, and contribution to writing of manuscript; **Marzieh Khani:** and **Shahriar Nafissi:** patient identification and provision of clinical data; **Najmeh Salehi:** Prediction of

three dimensional structure of TNRC18; **Hamidreza Moazzeni:** data analysis; **Hanieh Taheri:** mutation screening and segregation analysis in families by Sanger sequencing; **Elahe Elahi:** design and supervision of the research, and writing the manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The identifed variant in this research was previously reported and is accessible on the dbSNP repository under rs number "rs374974528" and ClinVar under accession number "VCV002369974.1." for the TNRC18 gene mutation.

ETHICS STATEMENT

The research was performed in accordance with the Declaration of Helsinki and with approval of the ethics board of the University of Tehran (ethics approval code no.IR.TUMS.VCR.REC.1397.017). Informed consent for participation was obtained from all subjects and/or their legal guardian(s).

CONSENT

Written informed consent for publication of clinical details and/or clinical images was obtained from the patient, her parents, her siblings, and control individuals. All participants were adults.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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