Case Report

Presentation of a recurrent FMR1 missense mutation (R138Q) in an affected female

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Abstract. Fragile X syndrome (FXS) is the most common single gene cause of intellectual disability (ID). Due to X-linked inheritance and variable X inactivation, males are affected at over twice the rate of females. Common features include developmental delay, ID, characteristic physical features, and behavioral challenges. The diagnosis is most commonly made secondary to a full mutation (>200 CGG repeats) in *FMR1* leading to abnormal gene methylation. While deletions and point mutations have infrequently been reported, missense variants are exceptionally rare. To date, only three missense mutations have been both associated with a FXS phenotype and supported by functional studies. One mutation, R138Q, has been reported previously in two unrelated males. Here we report this recurrent R138Q mutation in a female with mild ID and possible seizures. This report suggests missense mutations may be underdiagnosed as the gene is seldom sequenced in the setting of normal repeat analysis.

Key words: FMR1 gene, missense mutation, partial fragile X syndrome, R138Q

1. Background

Fragile X syndrome or FXS (MIM# 300624) was initially described in 1943 as a mental defect showing sex-linkage later to be called Martin-Bell syndrome in reference to its discoverers [16]. However, identification of the molecular etiology was not described until 1991 when the Fragile X Mental Retardation-1 (*FMR1*) gene was reported [22, 32, 33].

FXS is now recognized as the most common single gene cause of autism spectrum disorder and inherited intellectual disability (ID) [10]. Accounting for 1–2% of diagnosed ID, FXS affects approximately 1 in 5000 males and 1 in 8000–9000 females [3, 6, 25]. Other possible features of FXS include developmental delays, hyperactivity, long face, large and prominent ears, seizures, anxiety, and sensory difficulties. An X-linked condition, males are known to be more severely affected than females. For individuals with full mutations, the diagnosis of ID (IQ < 70) is made in 85% of males and 25–30% of females [10, 14].

The presence of >200 CGG repeats (full mutation) and subsequent hypermethylation of the *FMR1* gene is the most common cause of FXS, accounting for an estimated 99% of cases [19]. Full or partial gene deletions comprise the next largest percentage of cases of FXS [2, 5, 11]. Point mutations are

Table 1
Pathogenic *FMR1* missense mutations previously reported in the literature

	De Boulle et al., 1993	Collins et al., 2010	Myrick et al., 2014	Sitzmann et al., 2018	Present case
Mutation	c.1100T > A, I304N	c.413G > A, R138Q	c.797G > A, G266Q	c.413G > A, R138Q	c.413G > A, R138Q
Sex	M	M	M	M	F
Age	27	~7	16	10	10
Ethnicity	Caucasian	Caucasian	Caucasian	Caucasian	Hispanic
Developmental delay	Yes	Yes	Yes	Yes	Yes
Autism spectrum disorder	NR	No	Yes	Yes	No
Intellectual disability	Profound	Mild to moderate	Severe	Moderate	Mild
Intelligence Quotient (IQ)	<20	42	NR	42	NR
Seizures	Focal	Partial, intractable	No	Partial complex	Possible multifocal
Dysmorphic features	Yes	No	Yes	Yes	No
Maternal inheritance	No	Yes	Yes	Yes	No
Maternal symptoms	n/a	Learning difficulty, anxiety	None	NR	n/a
Reported diagnosis	FXS	Partial FXS	FXS	FXS	Partial FXS

NR = not reported.

found in yet a smaller subset of patients, the frequency of which has not been well established [4, 12, 15, 30].

To date, over 120 missense variations of unknown significance have been reported in the *FMR1* gene. However, only three missense mutations have been reported to be associated with a FXS-related phenotype and supported by functional studies (Table 1). The first mutation, I304N, was reported in 1993 in a male with a severe presentation of FXS [7]. The mutation was de novo and multiple functional studies have supported its pathogenicity [8, 27, 34]. The second mutation, R138Q, was initially reported in 2010 in a male with a diagnosis of partial FXS secondary to intellectual disability and seizures but notably missing the physical stigmata of the condition [4]. In 2014, functional studies of the R138Q mutation revealed impaired presynaptic function of the fragile X mental retardation protein (FMRP) [21]. A second report of the R138Q mutation was published in 2018 in an unrelated male with classic features of FXS including ID, autism, facial dysmorphism, hypermobility, fleshy hands, flat feet, unsteady gait, and seizures [28]. Finally, the third mutation, G266Q, was reported in 2014 in a child with a diagnosis of FXS and autism spectrum disorder [21].

Here we describe the fifth patient and third report of the previously identified R138Q mutation. This is the first detailed clinical report of the presentation of a *FMR1* missense mutation in an affected female.

2. Materials and methods

A gene sequencing panel of 139 genes associated with intellectual disability and autism spectrum disorder was obtained by massively parallel sequencing using the TruSight One kit (v1.0).

3. Case presentation

Our female patient is the second child of healthy, nonconsanguineous, Hispanic parents. She was born full term with a birth weight of 3005 g (31st centile) and birth length of 49.53 cm (58th centile). There were no pre- or post-natal complications. Her newborn course was remarkable for neonatal jaundice requiring phototherapy. The newborn hearing and metabolic screens were normal.

Her medical history is significant for frequent falling and staring spells, initially believed to be behavioral. An EEG was abnormal, demonstrating bilateral independent epileptiform discharges suggestive of a predisposition to multifocal seizures but no seizures observed. Brain MRI and head CT were both unremarkable. At 10 years old, growth parameters revealed weight in the 25th centile, height in the 30th centile, and OFC in the 75th centile. No dysmorphic features were appreciated on exam.

Developmentally she was globally delayed from an early age but demonstrated no regression of skills. She crawled at 10 months and walked at 18 months. She spoke her first words at 12 months, combined words at 2 years old, and now speaks in full sentences. Behaviorally she has difficulty with attention and short term memory. She has received special education services throughout all grades and has a diagnosis of mild intellectual disability.

Family history is significant for a maternal male first cousin once removed with a diagnosis of intellectual disability. The patient's mother has a history of depression and migraines and father is healthy. Neither parent is reported to have a history of intellectual disability or other learning difficulty. There is an older full sister who is healthy and typically developing. There is also an infant maternal half brother who is healthy.

Initial genetics work-up included negative metabolic labs, oligo-SNP chromosomal microarray, and *FMR1* CGG repeat analysis (29 and 37 repeats). Further testing by next generation sequencing panel showed a heterozygous *FMR1* missense mutation in exon 5 at c.413G > A. This mutation causes an arginine to glutamine amino acid change at position 138 (R138Q). The patient's mother was negative for this mutation and testing for father was not able to be pursued however clinical history was unrevealing.

4. Conclusions

We report a third case of the R138Q mutation in the *FMR1* gene. While our patient now represents the fifth patient with a known pathogenic missense mutation, she is the first clinically affected female to be reported. The clinical features shared by all previously reported cases of *FMR1* missense mutations are outlined in Table 1 and include developmental delays (5/5), intellectual disability (5/5, mild to severe), seizures (3/5, excludes our patient despite a predisposition on EEG), and characteristic dysmorphism (3/5). Behavioral phenotypes reported included autism, aggression, ADHD, and poor short term memory, although behavioral problems were not specifically addressed for all cases. The mutations were maternally inherited in 60% of cases. Three reported patients had a diagnosis of FXS (I304N, R138Q, and G266Q mutations) and two patients had a diagnosis of partial FXS (two R138Q mutations).

While three of the previously reported males with *FMR1* missense mutations (G266Q and both R138Q) inherited the mutations from their mother, only one of the mothers (R138Q) displayed learning differences and notably did not have a reported diagnosis of ID. This increased incidence of disease in males is clearly explained by the X-linked inheritance of the *FMR1* gene. Furthermore, due to differences in X-inactivation patterns, only 25–30% of females with full CGG repeat expansions are

diagnosed with ID [10, 13, 14]. While we may expect a similar ratio of affected and unaffected females with FXS-related phenotypes secondary to missense mutations, future studies of females with *FMR1* missense mutations will be necessary to demonstrate this effect.

Sequencing of the *FMR1* gene has thus far revealed over 120 missense variations yet there are only four prior reports of individuals presenting with a FXS-related phenotype with mutations supported by functional studies. [31]. Of those previously reported mutations, all lie within the N terminus of the *FMR1* gene, a region integrally important for protein-protein interactions along with RNA binding and domain dimerization [1, 24, 26]. The G266E and I304N mutations are both found in the RNA binding domains of FMRP leading to abnormal RNA binding and polyribosome association [21, 34]. The R138Q partial loss-of-function missense mutation is located within the aminoterminal domain of FMRP and is associated with only abnormal presynaptic FMRP function and preserved translation regulation of FMRP [20, 21]. This dysfunction lies in contrast to that observed in FXS due to complete loss of FMRP which impairs function in both the pre-synaptic and post-synaptic compartments. In the future, variants within the N-terminus of the *FMR1* gene should be considered carefully for possible pathogenic effect and the impact of these mutations on protein function may differ markedly based on their location within this region.

Many have explored possible explanations for the seemingly low prevalence of *FMR1* missense mutations compared to that observed in other genes. One such possibility is that some individuals with *FMR1* missense mutations may display a milder or atypical phenotype compared to individuals with FXS due to a full repeat expansion. Currently global developmental delays and intellectual disability are estimated to affect between 1–3% of children in the general population [17, 23]. Perhaps individuals with *FMR1* point mutations would not meet criteria for such labels and instead may only display learning disability or mild cognitive differences that do not otherwise suggest genetics work-up.

Largely considered first-line testing for individuals with developmental delays, chromosomal microarray analysis and *FMR1* CGG-repeat analysis is only diagnostic in about 15–20% and 2% of cases, respectively [9, 18, 29]. Gene sequencing, on the other hand, is often not pursued due to high cost and limited resources. Therefore, *FMR1* gene sequencing has not previously been established as the standard of care for individuals with developmental deficits but otherwise normal CGG repeat size. Further studies assessing *FMR1* point mutations will be necessary to determine a more precise phenotype distinguishing it from FXS secondary to a full repeat expansion.

Identifying *FMR1* point mutations in individuals has major importance for both genetic counseling and consideration of treatment strategies or clinical trials. Beyond counseling for a new diagnosis in a proband, addressing the subjects of Fragile X-associated tremor/ataxia syndrome (FXTAS) and Fragile X-associated primary ovarian insufficiency (FXPOI) may need to be considered as inevitably families will encounter this information in the FXS literature available to them. While there is currently no evidence to suggest families with *FMR1* missense mutations are at higher risk for FXTAS or FXPOI, to our knowledge there have also been no reports specifically studying this association.

In conclusion, while rarely reported, a precise prevalence of missense mutations in the *FMR1* gene remains unknown. It appears that *FMR1* missense mutations can be associated with a spectrum of phenotypes depending on the particular variant. It has also been suggested that there is a milder or atypical phenotype associated with these mutations explaining the seemingly low frequency of such reports. This new case lends further evidence to the pathogenicity of the recurrent R138Q missense mutation and demonstrates its presentation in an affected female. This report supports the use of *FMR1* sequencing in the work-up of an individual with otherwise unexplained ID. Increasing the utilization of sequencing in the diagnostic work-up will further elucidate the incidence of pathogenic missense variants and allow for increased phenotypic delineation of individuals with CGG repeat expansions versus *FMR1* missense mutations.

Acknowledgments

We would like to thank our patient's family for their generous contribution of information to the medical literature.

Conflicts of interest

The authors declare no conflicts.

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