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LETTER TO THE EDITOR

Autism Spectrum Disorder: FRAXE Mutation, a Rare Etiology

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Abstract Autism spectrum disorder (ASD) is characterized by impaired social interaction and communication, restricted interests and repetitive behaviors. Fragile X E is associated with X-linked non-specific mild intellectual disability (ID) and with behavioral problems. Most of the known genetic causes of ASD are also causes of ID, implying that these two identities share common genetic bases. We present a child with an ASD with a normal range of intelligence quotient, that later evolved to compulsive behavior. FRAXE locus analysis by polymerase chain reaction revealed a complete mutation of the *FMR 2* gene. This report stresses the importance of clinicians being aware of the association between a full mutation of *FMR2* and ASD associated with compulsive behavior despite normal intellectual level.

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Clinical Report

We present a boy who was the first child of healthy, unrelated parents. He was born at 36 gestational weeks by cesarean section. His pre-natal growth was in 5th Percentile in weight (2,680 g), height (47 cm) and head circumference (32.5 cm). The Apgar score was 5 in the first minute and 9 in the fifth. Early postnatal history was normal. Early motor skills were acquired at normal age range, but walking age was delayed until 18 months old. The first parental concerns were evident in the third year of life exhibiting mild language development delay (first words by 24 months of age and phrases at 30) as well as marked behavioral problems characterized by psychomotor agitation and compulsions. Echolalia and verbiage became more obvious with age. In addition, autistic features, such as restricted interests, repetitive behavior and impaired social interaction mainly with other children were also present. When he started pre-school at 3 years old, teachers mentioned that he was isolated, lacked social reciprocity and had major misunderstandings of social cues. He also exhibited various stereotyped behaviors associated with restricted interests. Despite his difficulty to integrate at school, he was good with numbers, sequencing, memory and reasoning. He showed no autonomy in group activities, most of the times looking for an adult to help him. Moreover, this child had an ongoing concern with gas cylinders and electricity. Seizures were never observed.

He was referred at the age of 7 years old to a neurodevelopment outpatient clinic in a tertiary Pediatric

Hospital motivated by behavior problems and academic learning difficulties. No major or minor dysmorphic features were found. Hypopigmented or hyperpigmented macules were excluded as well as other skin abnormalities. Global motor and sensorial neurological examination was normal. General Growth was in the normal range with weight, height and head circumference respectively in the percentiles: 25–50th, 50 and 25th. Normal hearing and vision skills were confirmed. His intelligence quotient (IQ), measured with the *Wechsler Intelligence Scale for Children* (WISC) III (Wechsler 2003) was in the low normal range, with a full scale IQ of 85 (Percentile 16), a verbal IQ of 97 (Percentile 42) and a performance IQ of 78 (Percentile 7). Assessment of ASD was performed using the *Autism Diagnostic Interview Revised* (ADI-R) (Lord et al. 1994), the *Autism Diagnostic Observation Schedule* (ADOS) (Lord et al. 1989) and to determine the severity the *Childhood Autism Rating Scale* (CARS) (Schopler et al. 1980). In ADI-R he exceeded the cutoff in Reciprocal Social Interaction, Communication, Restricted, Repetitive, and Stereotyped Patterns of Behavior and Abnormality of Development; in ADOS—Module 3 he also exceeded the cutoff both in communication and social interaction; in CARS he scored 31 (Scale range: no autism <30; 30 <mild autism <37; severe autism >37). After all, and based on an expert clinical evaluation by an experiment multidisciplinary team he received a diagnosis of autistic disorder based on Diagnostic and Statistical Manual of Mental Disorders, 4th Edition-Text revised (DSM-IV-TR) (American Psychiatric Association 2000) and International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10) criteria (World Health Organization 1992). He started a multidisciplinary approach: special education support, speech therapy and psychology and medical follow-up.

At the follow-up a compulsive behavior characterized by a litany of encyclopedic knowledge was focus of concern. He talked compulsively about a variety of diseases and their specific etiology, always with a monotonous speech. At school, he became good at mathematics and other sciences matters but average in compositions and creativity. Metabolic laboratory tests, along with karyotyping and testing for expansion of the (CGG) trinucleotide repeat in the *FMR1* Gene (Fragile X Syndrome) were conducted as part of the standardized diagnostic procedures but the results were normal. FRAXE locus analysis by polymerase chain reaction revealed a hypermethylation mutation (with an expansion >200 CCG trinucleotide repeats) across this site, in the *FMR2* gene (complete mutation of the *FMR 2* gene). His mother, who was clinically unaffected, was evaluated and she was found to be a carrier of the premutation.

Discussion

The rare fragile sites which are located in the Xq27.3-q28 region of the human X chromosome are indistinguishable from each other on conventional cytogenetic techniques but readily separated by molecular genetics (Sutherland and Baker 1992, 2000). FRAXA was the first locus found to be related to ID. In the first years after discovering the fragile sites at Xq27/28, all individuals were cytogenetically positive but some of them were negative for the CGG expansions associated with FRAXA expression and did not exhibit hypermethylation or transcriptional silencing of *FMR1*. Then, it was discovered that some of these individuals shown to express fragility at sites more distal in Xq28, designated FRAXE and FRAXF (Gécz et al. 1997; Hirst et al. 1993; Knight et al. 1993, 1994; Sutherland and Baker 1992). Mulley et al. were the first to provide data suggesting an etiologic relationship between FRAXE and non-specific X-linked mental impairment (Russo et al. 1998).

The *FMR2* gene was cloned just in 1996, from within a submicroscopic deletion (Gécz et al. 1996; Gu et al. 1996). After its discovery and its characterization it was possible to test genotype/phenotype relationships, trying to clarify the role of *FMR2* gene in males with intellectual disability (Gécz 2000). *FMR2* encodes a large protein of 1,311 amino acids, and is a member of a gene family encoding proline-serine-rich proteins that have properties of nuclear transcription factors (AF4, LAF4, FMR2, and AF5q31). The proteins associated with this family localize to the cell nucleus. FMR2 protein is localized in neurons of the neocortex, Purkinje cells of the cerebellum and the granule cell layer of the hippocampus, structures involved with cognitive function, consistent with the learning deficits seen in FRAXE individuals (Miller et al. 2000). FMR2 protein has a role as transcriptional activator with a positive action on RNA elongation. FMR2 localizes to nuclear speckles, subnuclear structures considered as storage/modification sites of pre-mRNA splicing factors, and modulates alternative splicing via the interaction with the G-quadruplex RNA-forming structure. Furthermore, overexpression of FMR2 interferes with the organization and/or biogenesis of nuclear speckles (Bensaid et al. 2009; Hillman and Gécz 2001; Melko et al. 2011; Melko and Bardoni 2010).

Nevertheless, there are still many unanswered questions regarding the function of FMR2 protein in processes underlying its association with mild non-specific ID. Non-specific and non-syndromal ID is considered when a lower GIQ (<70) is present in physically normal individuals (Gécz 2000). After Fragile X syndrome, *FMR2* is individually the most commonly identified gene responsible for non-specific X linked ID and to date the only one associated with mild (IQ = 50–70) to borderline (IQ = 70–85)

ID (Gécz 2000; Russo et al. 1998). However, several FRAXE full mutations have been diagnosed in males with IQ within the normal range (Flynn et al. 1993; Hillman and Gécz 2001). Nigro et al. described a FRAXE mutation in a subject with ID and in his phenotypically normal twin brother (Nigro et al. 2000). These differences in the intellectual level may be related to the extent of the deletion within the *FMR2* (Gécz et al. 1996).

Furthermore, recent literature suggests that functional redundancy exists among the AFF family members of genes (AFF1/AF4, AFF2/FMR2, AFF3/LAF4 and AFF4/AF5q31) in the regulation of splicing and transcription: it is possible that other members of the AFF family compensate for the loss of AFF2/FMR2 activity which might explain the relatively mild to borderline phenotype observed in some FRAXE patients (Melko et al. 2011).

After the molecular characterization of the FRAXE fragile site and its possible implications in learning difficulties a number of special education need and development or language delayed group of children were evaluated for the presence of FRAXE expansions (Allingham-Hawkins and Ray 1995; Chan and Wong 1998; Gécz 2000; Knight et al. 1996; Milà et al. 1997; Murray et al. 1996; Youings et al. 2000). Putting all the data together, from a large sample of higher than 13,000 individuals with mental retardation/ID, only seven FRAXE full expansions were identified. Using these studies, the frequency of FRAXE in ID populations varied between 0 (none individual founded in some groups) and 0.6 % (number of individuals tested in each group between 300 and 737). This suggests that FRAXE is not a common etiological factor among ID male patients. They defended the hypothesis that FRAXE was either very rare or a benign fragile site not associated with any clinical phenotype (Allingham-Hawkins and Ray 1995). However, it is important to refer that most of these studies tested the individuals for FRAXE CCG expansions and not to *FMR 2* gene mutation (Gécz 2000). As a consequence, the prevalence of FMR2 associated ID is probably higher than the estimated FRAXE prevalence.

Not only cognitive, but also morphological and behavioral manifestations of FRAXE are highly variable. There isn't any consistent dysmorphology in individuals with FRAXE (Melko and Bardoni 2010), although some dysmorphic features are mentioned in the literature. In our case no major or minor dysmorphic features were found. General growth was in the normal range and normal hearing and vision skills were confirmed.

The characteristics features of the FRAXE phenotype are ID, learning difficulties and behavioral problems, among other neurodevelopmental disorders (Bensaid et al. 2009; Gécz 2000; Knight et al. 1996; Murray et al. 1996; Youings et al. 2000). These comprise aggression, impulsivity, agitation, attention deficit, hyperkinesia and hyperactivity. In addition the *FMR 2* gene was speculated to play a role in other phenotypes such as autism (Barnicoat et al. 1997; Holden et al. 1996; Stettner et al. 2011).

Our patient exhibited a typical case of an ASD, with restricted interests, repetitive behavior and impaired social interaction which got better with an intensive therapeutic approach. However, a clinical profile characterized by a marked compulsive behavior with mule speech, encyclopedic knowledge about diseases and their pathophysiology as well as a specific interest with gas and electricity equipments deteriorated his social relationships. Specific learning difficulties became worse in spite of normal IQ.

In some cases described, pronounced delays in language development and behavioral problems including autistic features seem to be the most prominent symptoms (Gécz et al. 1996; Stettner et al. 2011). Some presented isolated excessive hand flapping or other minor stereotypic behavior as the only behavioral symptom, just isolated speech delay and no ID whereas others showed a prominent global development delay (Barnicoat et al. 1997; Gécz et al. 1996; Gedeon et al. 1995; Stettner et al. 2011).

Milá et al. described a case whose most notable traits were personality disorders and psychotic behavior, besides the ID (Murray et al. 1996). As in our case, as he grew, the clinical characteristics of neuropsychiatric disorder became more severe. He showed recurrent and persistent thoughts regarding specific subjects, such as religion and science. This specific trait was previously associated with a *FMR2* mutation in the literature. Wang et al. and other authors described a patient with a full FRAXE mutation who presented with obsessive–compulsive disorder (Barnicoat et al. 1997; Wang et al. 2003). Further analysis of the family members revealed another member with a full FRAXE mutation who had the clinical phenotype of speech impairment (Wang et al. 2003).

According to the recent literature, ASD, as well as other disorders of cognition and language, may result from subtle deviations in the expression levels or function of one or a number of synaptic proteins. Since the synapse is composed of an interconnected network of proteins, each one intimately dependent on the function of the others, any disturbance in one or more of these proteins is likely to change its structure and function. These small changes can have a significant influence on the ability of the synapse to remodel in response to experience and lead to impairments in the brain's ability to interact and learn from the environment. Therefore, ASD are the result of variants in the expression levels of synaptic proteins, leading to alterations in synaptic connectivity and network function, ultimately manifesting as neurodevelopmental delays, failure to acquire and apply new skills and social and communication issues (Cook et al. 2013).

Accumulating evidence suggests that in FRAXA, the loss of a single protein involved in translation control affects multiple stages of brain development and leads to debilitating consequences in human cognition. Even small deviations in the level or functions of any synaptic proteins can have effects on the ability of the synapse to transmit and remember information, resulting in inability to properly remodel or prune neuronal connections in response to experience (Cook et al. 2013).

Thus, FRAXE mutations can manifest in a wide variety of clinical neurodevelopmental/neuropsychiatric diagnoses crossing categorical boundaries.

This is the only FRAXE positive case in our outpatient clinic of autism, where the FRAXE locus analysis by polymerase chain reaction was systematically requested in 516 children with autism and only this case was positive ($\sim$0.2 %). Otherwise the FRAXA locus analysis was requested in 934 cases and 22 were positive (2.4 %). This shows that in our experience FRAXA is twelve times more prevalent. This is consistent with other studies, in which FRAXA was ten to nineteen times more prevalent than FRAXE (Knight et al. 1996; Milà et al. 1997; Youings et al. 2000).

Routine FRAXE screening is not indicated in children with ID, although follow up testing may be useful in selected unknown etiology and FRAXA negative subjects with ID (Brown 1996; Holinski-Feder et al. 1996).

In FRAXE as well as in many other genetic disorders, patients have been shown to exhibit autistic symptoms spanning from mild to severe, associated or not with ID. However, there are still many unanswered questions about the relation between the genotype and the phenotype of children with FRAXE. More studies need to be done in order to characterize the population of FRAXE individuals.

This report stresses the importance of clinicians being aware of the association between a full mutation of *FMR2,* ASD and compulsive behavior in a child without ID and also the importance of a careful neuropsychiatric examination of children with FRAXE.

References

Allingham-Hawkins, D., & Ray, P. (1995). FRAXE expansion is not a common etiological factor among developmentally delayed males. *American Journal of Human Genetics, 56*, 72–76.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders* (4th ed, text rev.).

Barnicoat, A., Wang, Q., Turk, J., Green, E., Mathew, C., Flynn, G., et al. (1997). Clinical, cytogenetic, and molecular analysis of three families with FRAXE. *Journal of Medical Genetics, 34*, 13–17.

Bensaid, M., Melko, M., Bechara, E., Davidovic, L., Berretta, A., Catania, M., et al. (2009). FRAXE-associated mental retardation protein (FMR2) is an RNA-binding protein with high affinity for G-quartet RNA forming structure. *Nucleic Acids Research, 37*(4), 1269–1279.

Brown, W. (1996). The FRAXE syndrome: Is it time for routine screening? *American Journal of Human Genetics, 58*, 903–905.

Chan, S. Y., & Wong, V. (1998). DNA diagnosis of FRAXA and FRAXE in Chinese children with neurodevelopmental disorders and fragile X syndrome. *Clinical Genetics, 53*(3), 179–183.

Cook, D., Nuro, E., & Murai, K. K. (2013). Increasing our understanding of human cognition through the study of fragile X syndrome. *Developmental Neurobiology, 31*, 1–72.

Flynn, G., Hirst, M., Knight, S., Macpherson, J., Barber, J., Flannery, A., et al. (1993). Identification of the FRAXE fragile site in two families ascertained for X linked mental retardation. *Journal of Medical Genetics, 30*, 97–100.

Gécz, J. (2000). The *FMR2* gene, FRAXE and non-specific X-linked mental retardation: Clinical and molecular aspects. *Annals of Human Genetics, 64*, 95–106.

Gécz, J., Gedeon, A. K., Sutherland, G. R., & Mulley, J. C. (1996). Identification of the gene FMR2, associated with FRAXE mental retardation. *Nature Genetics, 13*(1), 105–108.

Gécz, J., Oostra, B., Hockey, A., Carbonell, P., Turner, G., Haan, E., et al. (1997). FMR2 expression in families with FRAXE mental retardation. *Human Molecular Genetics, 6*(3), 435–441.

Gedeon, A., Keinanen, M., Ades, C., Kaariainen, H., Gecz, J., Baker, E., et al. (1995). Overlapping submicroscopic deletions in Xq28 in two unrelated boys with developmental disorders: Identification of a gene near FRAXE. *American Journal of Human Genetics, 56*, 907–914.

Gu, Y., Shen, Y., Gibbs, R. A., & Nelson, D. L. (1996). Identification of FMR2, a novel gene associated with the FRAXE CCG repeat and CpG island. *Nature Genetics, 13*(1), 109–113.

Hillman, M. A., & Gécz, J. (2001). Fragile XE-associated familial mental retardation protein 2 (FMR2) acts as a potent transcription activator. *Journal of Human Genetics, 46*(5), 251–259.

Hirst, M., Barnicoat, A., Flynn, G., Wang, Q., Daker, M., Buckle, V. J., et al. (1993). The identification of a third fragile site, FRAXF in Xq27-28 distal to both FRAXA and FRAXE. *Human Molecular Genetics, 2*, 197–200.

Holden, JJ., Wing, M., Chalifoux, M., Julien-Inalsingh, C., Schutz, C., Robinson, P., et al. (1996). Lack of expansion of triplet repeats in the FMR1, FRAXE, and FRAXF loci in male multiplex families with autism and pervasive developmental disorders. *Am J Med Genet,* Aug; 64(2):399-403.

Holinski-Feder, E., Chahrokh-Zadeh, S., Jedele, K., Meindl, A., Steinbach, P., & Wohrle, D. (1996). FRAXE testing. *American Journal of Human Genetics, 59*(5), 1168–1169.

Knight, S. J., Flannery, A. V., Hirst, M. C., Campbell, L., Christodoulou, Z., Phelps, S. R., et al. (1993). Trinucleotide repeat amplification and hypermethylation of a CpG island in FRAXE mental retardation. *Cell, 74*(1), 127–134.

Knight, S., Ritchie, R., Chakrabarti, L., Cross, G., Taylor, G., Mueller, R., et al. (1996). A study of FRAXE in mentally retarded individuals referred for fragile X syndrome (FRAXA) testing in the United Kingdom. *American Journal of Human Genetics, 58*, 906–913.

Knight, S., Voelckel, M., Hirst, M., Flannery, A., Moncla, A., & Davies, K. (1994). Triplet repeat expansion at the FRAXE locus and X-linked mild mental handicap. *Journal of Human Genetics, 55*, 81–86.

Lord, C., Risi, S., Lambrecht, L., Cook, E., Leventhal, B., Dilavore, P., et al. (1989). Autism diagnostic observation schedule: A standardized observation of communicative and social behaviour. *Journal of Autism and Developmental Disorders, 19*, 185–212.

Lord, C., Rutter, M., & Le Couter, A. (1994). Autism diagnostic interview-revised: A revised version of a diagnostic interview for

caregivers of individuals with possible pervasive developmental disorders. *Journal of Autism and Developmental Disorders, 24*, 659–685.

Melko, M., & Bardoni, B. (2010). The role of G-quadruplex in RNA metabolism: involvement of FMRP and FMR2P. *Biochimie, 92*(8), 919–926.

Melko, M., Douguet, D., Bensaid, M., Zongaro, S., Verheggen, C., Gecz, J., et al. (2011). Functional characterization of the AFF (AF4/FMR2) family of RNA-binding proteins: insights into the molecular pathology of FRAXE intellectual disability. *Human Molecular Genetics, 20*(10), 1873–1885.

Milà, M., Sànchez, A., Badenas, C., Brun, C., Jiménez, D., Villa, M. P., et al. (1997). Screening for FMR1 and FMR2 mutations in 222 individuals from Spanish special schools: identification of a case of FRAXE-associated mental retardation. *Human Genetics, 100*(5–6), 503–507.

Miller, W. L., Skinner, J. A., Foss, G. S., & Davies, K. E. (2000). Localization of the fragile X mental retardation 2 (FMR2) protein in mammalian brain. *European Journal of Neuroscience, 12*(1), 381–384.

Murray, A., Youings, S., Dennis, N., Latsky, L., Linehan, P., McKechnie, N., et al. (1996). Population screening at the FRAXA and FRAXE loci: Molecular analyses of boys with learning difficulties and their mothers. *Human Molecular Genetics, 5*(6), 727–735.

Nigro, C., Faravelli, F., Cavani, S., Perroni, L., Novello, P., Vitali, M., et al. (2000). FRAXE mutation in a mentally retarded subject and in his phenotypically normal twin brother. *European Journal of Human Genetics, 8*, 157–162.

Russo, S., Selicorni, A., Bedeshi, M. F., Natacci, F., Viziello, P., Fortuna, R., et al. (1998). Molecular characterization of FRAXE-positive subjects with mental impairment in two unrelated Italian families. *American Journal of Medical Genetics, 75*(3), 304–308.

Schopler, E., Reichler, R. J., Devellis, R. F., & Daly, K. (1980). Toward objective classification of childhood autism: Childhood Autism Rating Scale. *Journal of Autism and Developmental Disorders, 10*, 91–103.

Stettner, G. M., Shoukier, M., Hoger, C., Brockmann, K., & Auber, B. (2011). Familial intellectual disability and autistic behavior caused by a small FMR2 gene deletion. *American Journal of Medical Genetics Part A, 155A*(8), 2003–2007.

Sutherland, G. R., & Baker, E. (1992). Characterization of a new rare fragile site easily confused with the fragile X. *Human Molecular Genetics, 1*(2), 111–113.

Sutherland, G. R., & Baker, E. (2000). The clinical significance of fragile sites on human chromosomes. *Clinical Genetics, 58*(3), 157–161.

Wang, Q., Gu, Y., Ferguson, J., Chen, Q., Boatwright, Q., Gardiner, J., et al. (2003). Cytogenetic analysis of obsessive-compulsive disorder (OCD): Identification of a FRAXE fragile site. *American Journal of Medical Genetics Part A, 118*(1), 25–28.

Wechsler, D. (2003). Escala de Inteligência de Wechsler para Crianças—terceira edição (WISC-III). Adaptação portuguesa: Simões MR, Rocha A e Ferreira C. Cegoc Lisboa.

World Health Organization. (1992). *ICD-10 classifications of mental and behavioural disorder: Clinical descriptions and diagnostic guidelines*. Geneva: World Health Organization.

Youings, S., Murray, A., Dennis, N., Ennis, S., Lewis, C., McKechnie, N., et al. (2000). FRAXA and FRAXE: The results of a five year survey. *Journal of Medical Genetics, 37*, 415–421.