LETTER TO JMG

MECP2 gene nucleotide changes and their pathogenicity in males: proceed with caution

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Rett syndrome (RTT) is one of the most frequent neurodevelopmental disorders affecting almost exclusively females. It is characterised by normal development until 6-18 months followed by a more or less rapid decline of acquired functions mainly of the higher brain functions, such as communicative speech and purposeful hand use. Mutations in the *MECP2* gene have been described in RTT patients world wide.[1–4] Along with classical RTT females, several RTT variants, like the preserved speech variant, congenital RTT, and a patient with an Angelman syndrome-like phenotype, have been reported to carry mutations in the *MECP2* gene.[4–8] RTT has been considered an X linked dominant disease lethal in males. This assumption was supported by the small number of affected RTT males and by a lethal knock out mouse model.[9] Recently, however, two independent groups have shown that nullizygous male and heterozygous female knock out mice are viable.[10 11] This finding strongly questions the hypothesis of male lethality of the RTT trait. Our recently published findings about a very high percentage of paternal origin of the mutation in the *MECP2* gene in sporadic cases of RTT provide a simple explanation for the scarcity of affected males.[12] Meanwhile, there has been a series of reports describing mutations in the *MECP2* gene of males. The affected males can be divided into two groups according to the type of their mutation. The first group carries mutations in the *MECP2* gene either already described in RTT females or mutations of unquestionable pathological value (frameshift mutations or nonsense mutations).[5–16] All these patients are characterised by early onset of the disease and a severe encephalopathy. A subgroup are males with a 47,XXY karyotype who present with the typical RTT symptoms. The second group includes patients carrying mutations inherited from their mother, which have never been found in Rett females.[17 18] These patients always have mental retardation of different degrees and possibly further symptoms.

METHODS AND RESULTS

We investigated a group of 30 male patients with a clinically heterogenous phenotype ranging from non-specific mental retardation to a severe neonatal encephalopathy. We found the c.1282 G>A (G428S) nucleotide change in a boy with a severe encephalopathy, the c.1030 C>T (R344W) variation in a male with a Rett-like phenotype, and the c.590 C>T (T197M) variation in a male with congenital encephalopathy, microcephaly, and severe developmental delay. Furthermore, by screening our female Rett patients, we found the c.1196 C>T (P399L) in a patient of German origin and the c.1126 C>T (P376S) variation in a girl of Korean origin, both nucleotide changes having been detected in their corresponding healthy father. Except for the c.590 C>T (T197M) variation, located between the MBD and TRD domains of *MECP2*, all amino acid changes affect the C-terminal segment of the MeCP2 protein downstream from the MBD and TRD.

DISCUSSION

Imessaoudene *et al*[17] described a boy with a non-progressive encephalopathy of neonatal onset who carries the c.1282 G>A (G428S) nucleotide change. His mother and two of her sisters who are healthy are also carriers of this variation. The analysis of the grandmother's DNA did not show the presence of the mutation; the grandfather's DNA was not available. The authors carried out a linkage analysis and stated, without presenting the complete linkage data, that germinal mosaicism in the grandfather is the "most likely hypothesis". We detected the same c.1282 G>A nucleotide change in a boy with a very severe encephalopathy and untreatable seizures who died at 18 months of age (table 1). To rule out the possibility of a non-pathogenic, rare genetic variant, we investigated the DNA of his parents and grandparents. The analysis showed the healthy mother and her healthy father to be carriers of the

Table 1 Clinical findings in two males with the same *MECP2* variation

Clinical data	Imessaoudene *et al*[17] (G428S)	Our case (G428S)
Occurrence	Isolated	Isolated
Pregnancy/delivery	Normal	Normal
Developmental delay	+	+
Severe mental retardation	+	+
Acquired microcephaly	-	+
Seizures	-	Frequent and resistant to treatment (presented first at age of 12 months)
Neurological findings	Restless, uncoordinated movements	Periodic breathing, dorsal extension of the hands
	Hypotonia, hyperlaxity, mild distal muscular atrophy	Neurogenic muscular atrophy
Purposeful hand skills	+	Never acquired
Loss of skills	Retained	-
Electroencephalogram	Bradyarrhythmia (absence of paroxysmal anomalies)	Generalised slow waves
Brain imaging (MRI)	Normal	Marked brain atrophy
Survival	Still alive aged 3 years	Died of respiratory failure at 18 months

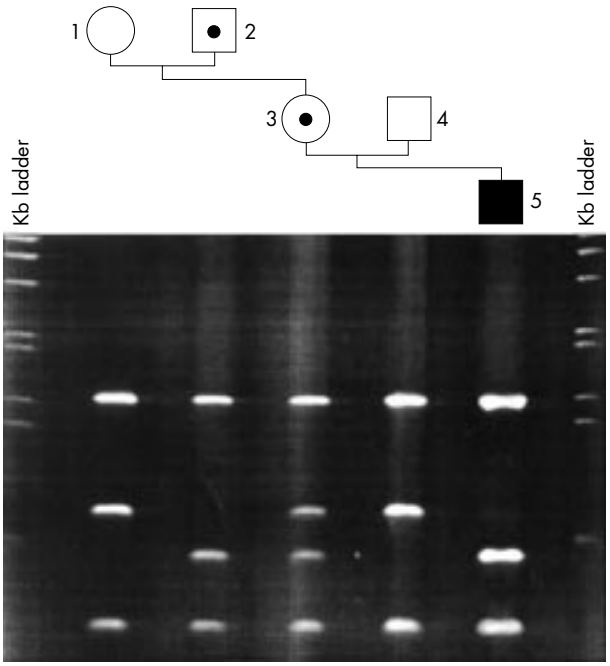

Figure 1 The genotype of the parents and maternal grandparents of our male patient with the c.1282 G>A variation. The mutation creates a restriction site for the *Alu*I endonuclease. We exploited this for genotyping the family. The index patient (5), his mother (3), and his maternal grandfather (2) carry the same variation.

same nucleotide change (fig 1). Maternal X inactivation analysis was not informative. Because of our findings, we described this nucleotide change as a rare genetic variant. However, owing to the rarity of this nucleotide change and it being conserved between *Xenopus*, mouse, and human, Imessaoudene *et al*[17] considered the c.1282 G>A nucleotide change to be a mutation and suggested that the maternal grandfather was mosaic for this mutation. Thus, their conclusions rely on the "assumption" that the grandfather has mosaicism. Unless we hypothesise the presence of mosaicism in the healthy grandfather of our patient, our findings and those of Imessaoudene *et al*[17] conflict with each other. Furthermore, considering the strikingly different phenotypes of both patients carrying the same c.1282 G>A nucleotide change, it is very unlikely that it has been caused by the same nucleotide change. Taking into account all factors (that is, "supposed" presence of a mosaicism, existence of a healthy male carrying the putative c.1282 G>A mutation, and the different phenotypes of two patients carrying this "mutation"), the most probable interpretation is that the c.1282 G>A is a rare genetic variant and that Imessaoudene *et al*[17] overinterpreted their findings based on the false assumption of mosaicism in the most relevant person in their pedigree, the grandfather.

By screening a female with classical RTT, we detected the c.1196 C>T (P399L) variation. Analysis of parental DNA showed the healthy father to be a carrier of the same nucleotide change. We therefore consider this amino acid change also to be a rare genetic variant. This nucleotide change has also recently been suggested by Couvert *et al*[18] to be responsible for the underlying phenotype affecting a male in their group of patients already found to be negative for a CGG expansion in the *FRAXA* gene. The line of evidence provided by Couvert *et al*[18] supporting the pathogenicity of this nucleotide change is based mainly on its rarity and is therefore of a speculative nature. Our findings strongly question their interpretation and in our opinion there is no relationship between this mutation and the underlying phenotype in males, whatever it may be.

Furthermore, we found two further nucleotide changes in two males. The first patient was Turkish and carried the c.1030 C>T (R344W) variation. The second patient was Polish and had the c.590 C>T (T197M) variation. Both amino acid changes were maternally inherited and affect non-conserved amino acid positions. Neither amino acid change has ever been found in our control population or in our Rett female patients (more than 600). Concerning the frequency of the c.590 C>T variation, we screened 109 healthy Polish males without detecting this nucleotide change, which has recently been described as a polymorphism.[5] However, it is not clear from the paper which line of evidence the authors have used. It might be possible to hypothesise about the meaning of the amino acid changes, but we feel that it is opportune to consider these changes as "unclassified" because we do not have any conclusive evidence about their pathological value.

Our report clearly shows the importance of genotyping parental and grandparental DNA to avoid misleading interpretations and suggestions, particularly in the case of *MECP2* mutations in males. Furthermore, in our opinion, it is a good standard not to assume any relationships between novel nucleotide changes and phenotypes until the pathological value of the putative mutations has been assessed and confirmed, particularly in the case of X linked recessive traits. Additionally, the ethnic origin of the patients should be considered in reporting nucleotide changes and a suitable control population should be investigated for the presence of the corresponding variation. To stress this point we report a novel rare genetic variant found in a Rett female of Korean origin, c.1126 C>T, which has never been found in other patients and has never been previously reported. Her healthy father is a carrier of the same nucleotide change. Most probably this is a population restricted polymorphism without any relationship to Rett syndrome.

In this context it is worth considering the pathological value of the recently reported A140V mutation. Briefly, Orrico *et al*[19] reported a familial case of five affected sibs (four males and a female) and their affected mother carrying the A140V mutation. The mutation is inherited in this family in an X linked dominant manner. The same mutation was found by Couvert *et al*[18] in two males with non-specific mental retardation. Both healthy mothers were also carriers of the same mutation. Therefore, the A140V mutation was considered to act in an X linked recessive manner in both cases. In considering this nucleotide change as a real mutation and explaining this different mendelian behaviour, we have to take into account some hypothetical "modifiers", acting either in the family described by Orrico *et al*[19] or in the two unrelated cases described by Couvert *et al.*[18] One important modifier, X inactivation, has been ruled out by both groups. This different behaviour of the A140V mutation leads to questions about its pathogenicity or at least justifies some scepticism about it. The lines of evidence supporting its disease causing role are its low frequency and its conserved position in the amino acid sequence of human, mouse, and *Xenopus*. These features, however, are not sufficient to define an amino acid change as a mutation, as exemplified by the c.1282G>A nucleotide change which is very rare and affects a conserved position but is indeed a genetic variant. The cosegregation of the mutation and the phenotype in the family described by Orrico *et al*[19] is the only valid evidence suggesting this change to be a mutation. Alternatively, it might be possible to interpret the A140V as an indirect marker for a mutation in an adjacent gene, at least in the family described by Orrico *et al.*[19]

In conclusion, we show evidence that some reported *MECP2* mutations are actually merely genetic variants. At the same time, we illustrate the difficulties in finding the true pathogenetic value of novel mutations in the *MECP2* gene in males. We suggest defining novel amino acid changes as "unclassified" until they are definitely confirmed as disease causing mutations. The frequency of the corresponding nucleotide

changes should be assessed in a suitable population and we should bear in mind that neither a very low frequency nor the change of a conserved amino acid are sufficient to define it as a mutation.

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