A Rare Missense Mutation, c.910A>G (p.K304E), of the MECP2 Gene in a Thai Girl with Classical Rett Syndrome

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ABSTRACT

Rett syndrome is a severe X-linked dominant neurological disorder affecting girls almost exclusively. According to RettBASE, several common and rare mutations have been reported. Some rare variants have no clinical description and have not been categorized as polymorphism or mutation. Here, we report a rare missense, c.910A>G (p.K304E), in a 10-year-old Thai girl with classical RTT based on clinical diagnosis. Only normal A alleles were found in her parents and 500 control chromosomes. This variant has been previously reported in 2 cases with unknown clinical features and uncertain mutation/polymorphism status. Thus we confirmed that c.910A>G (p.K304E) is likely to be a rare mutation causing RTT.

Keywords: Rett syndrome, MECP2, mutation, missense, Thai

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CASE REPORT

Rett syndrome (RTT, OMIM 312750) is a severe X-linked dominant neurological disorder almost exclusively affecting girls. RTT is one of the leading causes of mental retardation and developmental regression in girls. Patients with classical RTT show an apparently normal psychomotor development during the first 6-18 months of life. Thereafter, they enter a short period of developmental stagnation followed by a rapid regression in language and motor development. Purposeful hand use is often lost and replaced by repetitive, stereotypic movements.1 RTT is caused by mutations in a gene encoding the methyl-CpG binding protein 2 (MECP2).2 The MECP2 gene is located in Xq28 and contains 4 exons. There are two isoforms of the MeCP2 protein known as the MeCP2_e1 and MeCP2_e2 forms. The MeCP2_e1 contains 498 amino acids encompassing exons 1, 3 and 4. The MeCP2_e2 contains 486 amino acids encompassing parts of exons 2, 3 and 4.3,4 Numerous mutations (missense, nonsense, frameshift, deletion and duplication) have been reported and most common mutations are presented in exon 4. Some rare variants have no clinical description and have not been categorized as polymorphism or mutation (RettBASE: http://mecep2.chw.edu.au/cgi-bin/mecep2). In this study we report a rare mutation (c.910A>G or p.K304E) of the MECP2 gene in a 10-year-old Thai girl with classical RTT.

The patient was born at term by normal labor and had no prenatal complications as well as during birth. Her body sizes were birth weight 2300 g (< 3rd centile), length 45 cm (< 3rd centile), and occipito-frontal circumference (OFC) 34 cm (50th-75th centile). Although her weight and length were low she had no remarkable problems. At the age of 8 months she was hospitalized for 2 days with febrile convulsion. She was not placed on the long term treatment with antiepileptic drug before the age of 5 years since she had not had a recurrent seizure. Development during 1-18 months of age was normal (i.e. at ~ the age of 17-18 months she walked by herself and could speak some meaningful words), but after that her development slowed down and then became regressive (i.e. several months later she was exhibiting the abnormal behavior of always holding one hand with the other while she walked, and still could speak only 1-2 words). She developed unclassified abnormal hand movements at the age of 22 months and also had poor eye contact. Her clinical features did not much change from 2-5 years. At the age of 5 years, her parents became concerned that she might have seizure since she occasionally had abnormal
hand movements. An EEG was performed at that time and showed epileptiform discharge. Although she had never had another episode of seizure since the age of 8 months, she had been constantly on the antiepileptic drug, carbamazepine, and has taken it regularly. At the age of 5 years and 7 months, she developed teeth grinding and stereotypic hand movements of claspings, mouthing and wringing. Her OFC was 46 cm (< 3rd centile) and her height and weight were 104 cm and 14 kg, respectively (< 3rd centile). At the age of 8 years 8 months, she walked independently but developed truncal ataxia. She had severe stereotypic hand movements and could not speak any meaningful words. At the age of 10 years, her OFC was 47 cm (< 3rd centile) and she developed kyphosis, although she could walk independently. Her truncal ataxia was still present, however she had better hand function and diminished stereotypic hand movements of claspings, mouthing and wringing. She was able to maintain some eye contact, but could not socialize. Taking these findings all together, she has fulfilled the diagnosis of classical RTT.

Peripheral blood samples were obtained from the patient, her parents and controls, with consent. The study was approved by a local institutional review board. PCR was performed across the 4 exons, exon-intron boundary, 5' UTR and 3' UTR of the MECP2 gene. The primers of the 5' UTR and exon 1 regions were described in Mnatzakanian et al., and the primers of exons 2, 3, and 4 were described in Amir et al. and Amano et al. The PCR products from the subjects were refined through electrophoresis, then gel-purified and direct sequenced to identify the mutations by an automatic sequencer (ABI 3130 Genetic analyzer, Applied Biosystems, Foster City, CA, USA). The DNA sequencing results were compared with the GenBank database (Access No. AF030876). An A-to-G change at c.910 in exon 4 of MECP2 gene was detected in the patient’s DNA (Fig 1). This variant results in change of a codon for lysine (K) to glutamic acid (E) at protein position 304 of MeCP2_e2 form (K304E). This variant was not found in her parents’ DNA.

To assess the presence of c.910A>G in the general Thai population, PCR-RFLP or DNA sequencing was performed on 500 control alleles. The primers used for PCR-RFLP were 5'-CGAAGCGCCGGACTGG-3' and 5'-GGGCCCCCTTGGGAGCTCTG-3'. 10 µl of PCR product was digested by 0.2 unit TaqI in 20 µl total volume comprised of 1X NEB buffer and 1X BSA (New England Biolabs, Ipswich, MA, USA). Mineral oil was added and the solution incubated at 65°C for 90 min. The digested PCR product was separated in 6% polyacrylamide gel with electrophoresis at 150 volts for 60 minutes. The gel was stained by ethidium bromide and was illustrated by UV transillumination. The undigested PCR product was 328 bps. The homozygous A alleles showed 194 and 134 bps fragments while the heterozygous A/G alleles showed 194, 134 and 101 bps fragments (Fig 2). Only the normal A allele was detected in the parents’ DNA and 500 control chromosomes studied. The alignment of the partial MeCP2 protein in different species shows a conserved lysine at position 304 (Fig 3). This is an amino acid pattern of the protein that is known in genetics, and studies on many other species have indicated that conserved amino acids are essential for protein function.

**DISCUSSION**

The patient showed all the features of classical RTT, with the additional feature of a c.910A>G transition change of the basic amino acid, lysine (K) to the acidic amino acid, glutamic acid (E) at position 304 within the transcriptional repression domain (TRD). According to the RettBASE, this variant has been pre-
Fig 3. Alignment of MeCP2 sequences from different species shows conserved lysine at position 304 (MeCP2_e2 form).

Previously reported in 2 cases with otherwise unknown clinical features and uncertain mutation/polyorphism status. One case was directly submitted to the Rett Base and the other case was a French patient reported by Philippe et al.\textsuperscript{6} Transcriptional repression domain interacts with the transcriptional co-repressor and recruits the co-repressor complexes that mediate repression through deacetylation of core histones, with consequent compaction of DNA into heterochromatin.\textsuperscript{7} This indicates that the p.K304E may affect the ability of the protein to initiate co-repressor complexes and the transcription repression process. This position is also a part of the MeCP2 which is thought to be responsible for the interaction with the CDKL5 (cyclin-dependent kinase-like 5) which is located in Xp22. The MeCP2 and the CDKL5 may utilize the same molecular pathway.\textsuperscript{8} This interaction may account for the MeCP2 phosphorylation, which is believed to be important in the regulation of many other genes. Lysine at position 304 is a highly conserved amino acid across different species (i.e. zebra fish, xenopus and all mammals included in the database), indicating that lysine is a very important amino acid in the MeCP2 protein. Although the precise role of lysine at position 304 of MeCP2 in the interaction with CDKL5 is unknown, variations in this region may affect the MeCP2 and CDKL5 protein interaction which then in turn may alter the level of MeCP2 phosphorylation leading to the abnormal protein function.

Parental analysis by direct sequencing (data not shown) and PCR-RFLP revealed that the mutation was de novo. In addition, only the A allele was found in the 500 control alleles, indicating that c.910A>G is a rare variant. According to the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), c.911A>G (p.K304R) and c.910A>C (p.K304Q) have been reported, but there is no clinical description.\textsuperscript{9} Recently, p.K305E was reported in a Chinese patient with RTT.\textsuperscript{10} Moreover, a few mutations have been reported at close to this position, including p.P302R, p.R306C, and p.R306H (RettBASE), which would indicate that this region is an important domain of the MECP2 gene. These findings, together with the classical Rett features in the patient, indicate that the c.910A>G (p.K304E) is a rare mutation. Another study utilizing protein functional analysis may give a more definite conclusion.

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REFERENCES