

A Novel Methyl-CpG Binding Protein 2 (MeCP2) Variant in an Indian Girl with Rett Syndrome

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ABSTRACT

Rett syndrome is an X-linked dominant disorder that is primarily seen in females and is linked to mutations in the gene coding for Methyl-CpG Binding Protein 2 (MeCP2). It is a neurodevelopmental disorder characterised by impairments in language, repetitive movements, early-onset seizures, delayed growth, autistic features, intellectual disability and abnormal Electroencephalograms (EEG). Author's reported a case of three year six months old Indian girl who was born of a nonconsanguineous marriage presented with stereotypic hand movements, gradual loss of speech, inability to walk independently and frequent episodes of seizure. Genetic testing for analysis of MeCP2 mutations was performed and a novel *de novo* missense variant (c.361G>A, p.Asp121Asn) was identified, which was predicted to be disease causing on the basis of *insilico* analysis and clinical findings. The study suggested that a careful evaluation of the pathogenic nature of MeCP2 variants supports clinical diagnosis and aids in genetic counseling and patient management.

Keywords: Epileptiform discharge, Mutations, Neurological syndrome, Seizures, X-linked dominant

CASE REPORT

A three year six months old female child presented to the children's hospital with complaints of breathing difficulties and had seizures since one month. She was born out of a nonconsanguineous marriage, following an uncomplicated pregnancy and full term normal delivery. The mother reported that the child was healthy, cried immediately and fed well. Normal development was observed until the age of 12 months, following which there was a gradual loss of speech, and by the age of three years it was restricted to mere babbling. She was unable to walk independently and could walk slowly with support. Mother was apparently normal and none other family members were affected with similar condition. Stereotypic hand movement included repeated movement of hand towards the mouth, specifically constant biting of middle and index fingers. Skeletal abnormality such as scoliosis was not present; however, she had *genu valgum*. She showed no interest in playing with toys. Head circumference was normal. She used to have disturbed sleep pattern, but it had improved with medication. She had difficulty in breathing.

At the age of three years and three months seizure-like episodes that lasted for one minute were observed once in every 2-3 days. The case was further evaluated and a series of investigations were carried out. MRI and NonContrast CT (NCCT) brain were performed and no abnormalities were identified. Further EEG was done using standard 10-20 international electrode placement systems while patient was awake first, and later asleep. EEG findings were recorded as abnormal in view of: i) epileptiform discharges; ii) very frequent generalised bursts followed by mild electrodecremental response; and iii) generalised paroxysmal fast activity. The patient was put on treatment for seizures and had shown signs of improvement. Rett syndrome was suspected based on clinical presentation and EEG findings and patient was referred to us for genetic evaluation of MeCP2 gene.

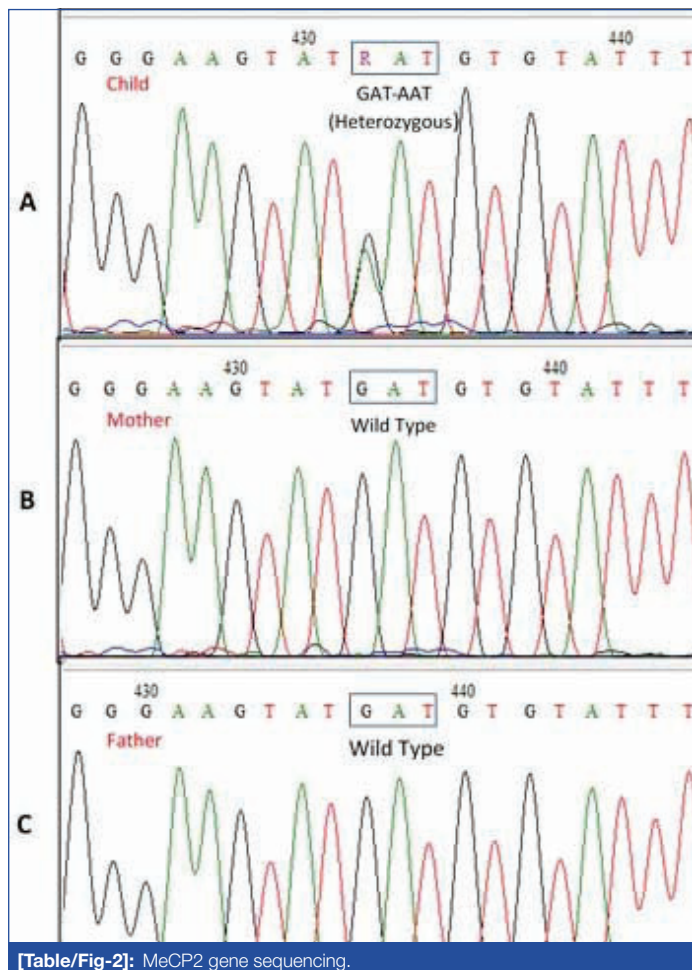
The genomic DNA was extracted from patient's peripheral blood (200 µL) using the Qiagen DNA Mini kit. Exons 1-4 of MeCP2 gene were amplified using primers and method described earlier [1]. The primer sequences and corresponding annealing temperature and amplicon sizes are given in [Table/Fig-1]. The

purified PCR products were sequenced directly using the BigDye[®] Terminator version 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) on Applied Biosystems 3500 Dx Genetic Analyser. The sequences were aligned to the reference sequence available in NCBI database (Genbank accession no.: NM_004992.3) using the Bioedit software. A single base change – c.361G>A, p.Asp121Asn in exon 3 of MeCP2 gene was identified [Table/Fig-2]. This variant was not reported previously in ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>), RettBASE: Rett Syndrome Variation Database (mecp2chw.edu.au/) or 1000 Genome database, nor was it found in the published literature [2]. The *insilico* analysis using PolyPhen-2, MutationTaster and

Primer	Sequence	Annealing Temperature	Amplicon Size (bp)
Exon 1-F	5'-GCACTCGGTGCATCTGTGGACAGAG-3'	65°C	671
Exon 1-R	5'-CATCCGCCACGCCGTGTCGTCGCCAC-3'		
Exon 2-F	5'-TGTGTTTATCTTCAAATGT-3'	57°C	418
Exon 2-R	5'-GTTATGTCCTTAGTCTTTGG-3'		
Exon 3-F	5'-CTGGGGCCTTGCATGTGGTG-3'	58°C	626
Exon 3-R	5'-GGTCATTTCAAGCACACCTG-3'		
Exon 4a1-F	5'-CGAGTGAGTGGCTTTGGTGA-3'	60°C	305
Exon 4a1-R	5'-ACAGATCGGATAGAAGACTC-3'		
Exon 4a2-F	5'-CCACCCAGGTCATGGTGATC-3'	58°C	384
Exon 4a2-R	5'-TGAGTGGTGGTGGTGGTGGT-3'		
Exon 4b-F	5'-GCAGGAGACCGTACTCCCCATC-3'	66°C	366
Exon 4b-R	5'-GCTCTCCCTCCCTCCGTTGT-3'		
Exon 4c/d-F	5'-GCAGGAGACCGTACTCCCCATC-3'	63°C	375
Exon 4c/d-R	5'-GCTCTCCCTCCCTCCGTTGT-3'		
Exon 4e-F	5'-GGAGAAGATGCCACAGAGAG-3'	60°C	455
Exon 4e-R	5'-CGGTAAGAAAAACATCCCCA-3'		

[Table/Fig-1]: MeCP2: Primer sequences, Annealing temperatures and Amplicon sizes.

Sifting Intolerant From Tolerant (SIFT) predicted this novel variant to be damaging or pathogenic. In order to establish *de novo* nature of the variant, parental blood samples were analysed for presence of c.361G>A variant. Sequencing of exon 3 of MeCP2 gene showed absence of c.361G>A variant in both the parents [Table/Fig-2]. Informed consent was obtained from the parents for genetic testing. The parents were counseled for the condition and patient was asked to meet the referring doctor for further management. The report was discussed with the referring doctor and the importance of regular medical care including medication for associated symptoms such as seizures, muscle stiffness, heart and gastrointestinal issues etc. Also, need for physical, occupational, nutritional, speech and language therapy was discussed with the parents.



[Table/Fig-2]: MeCP2 gene sequencing.

DISCUSSION

Rett syndrome is an X-linked dominant neurodevelopmental disorder with an incidence rate of 1 in 10000–15000. It is one of the most common causes of intellectual disability in females and was originally described in the 1960's by Andreas Rett [3,4]. Clinical diagnosis of Rtt rests on a careful exploration of clinical symptoms and specific pattern of symptom progression. The age of disease onset vary from later infancy with global developmental delays, gradual speech and communication impairments, progressive microcephaly, seizures and autistic behaviors. There is significant overlap in phenotypic features as well as disease severity which sometimes mask underlying diagnosis. Mutations in the gene encoding MeCP2 are present in 95–97% of patients with typical Rtt but only in 50–70% of atypical cases [5]. Majority (95%) of the Rtt cases are sporadic, show presence of *de novo* mutations in the MeCP2 gene that is not inherited from either parent and are mostly detected in females since affected male fetuses have lethal phenotype [6,7]. Over the last few years mutations in Cyclin Dependant Kinase Like 5 (CDKL5) and Forkhead Box Protein G1 (FOXP1) genes

have also been documented to be associated with Rtt [5]. It is important that Rtt should be diagnosed and differentiated from other disorders with overlapping phenotype such as cerebral palsy and autism, as the patient management and outcomes differ significantly [8].

In the present case, the major clinical features comprised of stereotypic hand movements, gradual loss of speech, inability to walk independently and frequent episodes of seizure seizures. A missense variant (c.361G>A, p.Asp121Asn) was identified, which was not reported previously in any of the databases, however, *insilico* analysis predicted it to be damaging. It was not identified in the parents and segregation analysis confirmed *de novo* nature of the variant. In view of the clinical history provided and parental analysis, this *de novo* missense variant (c.361G>A, p.Asp121Asn) was classified as a 'Likely Pathogenic' (Class 4) mutation as per American College of Medical Genetics and Genomics (ACMG) variant classification guidelines, thereby confirming diagnosis of Rtt in the patient [9]. Parents were counseled regarding the implication of *de novo* mutation and disease risk to other family members.

Though close to 1000 different mutations in MeCP2 gene are recorded in the RETT database, there are certain mutations such as T158M, R168X, R255X, R270X and R306C found at higher frequencies [10–14]. Novel mutations comprising of missense, small insertions and deletions as well as large and complex rearrangements are being regularly identified [10,12,14]. Genetic evaluation of suspected Rtt cases thus involves complete MeCP2 gene sequencing, followed by analysis of large rearrangements in MeCP2 and testing of other associated genes if indicated. Clinical evaluation and genetic testing followed by counseling is important as early identification and timely intervention can help both the patient and their families.

CONCLUSION(S)

Diagnosis of Rtt is challenging due to diverse phenotypic variation. Careful evaluation of novel genetic variants along with genetic studies on parents to find *de novo* nature of the variant is important to establish genetic diagnosis.

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