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First report of an unusual novel double mutation affecting the transcription repression domain of MeCP2 and causing a severe phenotype of Rett syndrome: Molecular analyses and computational investigation

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ABSTRACT

Rett syndrome is an X-linked neurodevelopmental disorder that develops a profound intellectual and motor disability and affects 1 from 10 000 to 15 000 live female births. This disease is characterized by a period of apparently normal development until 6–18 months of age when motor and communication abilities regress which is caused by mutations occurred in the X-linked *MECP2* gene, encoding the methyl-CpG binding protein 2.

This research study reports a molecular analysis via an exhaustive gene sequencing which reveals an unusual novel double mutation (c.695 G > T; c.880C > T) located in a highly conserved region in *MECP2* gene affecting the transcription repression domain (TRD) of MeCP2 protein and leading for the first time to a severe phenotype of Rett syndrome.

Moreover, a computational investigation of *MECP2* mutations demonstrates that the novel mutation c.695 G > T is highly deleterious which affects the MeCP2 protein showing also an adverse impact on *MECP2* gene expression and resulting in an affected folding and decreased stability of *MECP2* structures. Thus, the altered TRD domain engenders a disrupted process of *MECP2* functions.

Therefore, this is the first study which highlights a novel double mutation among the transcription repression domain (TRD) of MeCP2 protein in Rett patient with a severe clinical phenotype in North Africa region.

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1. Introduction

Rett syndrome (RTT; OMIM 312750), first described by the Austrian pediatrician Rett, is one of the leading causes of intellectual disability and developmental regression in girls [1]. Its prevalence is estimated to be from 1:10 000 to 1:15 000 females worldwide with most cases being sporadic [2]. Patients with classical Rett syndrome 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. Additional symptoms were also described in Rett syndrome including acquired microcephaly, gait ataxia/apraxia, seizures, and episodic apnea and/or hyperpnea [3,4].

Rett syndrome is a X-linked dominant disorder caused mainly by de novo mutations at methyl-CpG-binding protein 2 (*MECP2*) gene on chromosome Xq28 [5]. This gene codes for the prototype of proteins family sharing a methyl-binding domain, MeCP2 protein, whose expression predominates in brain, fibroblast, and lymphoblast cells [6]. MeCP2 binds to methylated cytosines in DNA to

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either activate or repress transcription [7] containing two crucial functional domains: the methyl-binding domain (MBD), which allows specific binding to DNA at methylated CpG islands and a transcription repression domain (TRD), an important recruitment platform being in charge of recruiting proteins that mediated transcription repression [8,9]. This domain (TRD) which modulates gene transcription is responsible for the interaction with co-repressors Sin3A and for the recruitment of histone desacetylases (HDACs) in Rett syndrome [10].

MeCP2 acts on epigenetic control of gene transcription, through DNA methylation and remodeling of chromatin [11]. Besides its role in epigenetic control of transcription, it is recognized that MeCP2 acts on the regulation of splicing [12], and is apparently capable of repressing transcription through binding to unmethylated DNA [13]. It is known that *MECP2* gene expression in the central nervous system is greater during the period of neuronal maturation [14].

Mutations in *MECP2* were first reported by Amir et al., 1999 [5]. 80% of classical Rett syndrome patients are caused by mutations in *MECP2* coding exons [15]. Individual nucleotide changes which lead to pathogenic mutations have been described in the Rett base (RettBASE; mecp2.chw.edu.au). These mutations include a range of missense, nonsense, frameshift, and in-frame insertions or deletions, as well as large deletions. Almost 50% of Rett patients are induced by one of 8 common C > T transitions at CpG dinucleotides [16,17]. There are about 10% of cases due to C-terminal deletions and about 6% with complex chromosomal rearrangements [18].

Here, we described a patient affected with Rett syndrome and presenting a novel double mutation in the exon 4 of the *MECP2* gene: a new mutation (c.695G > T; p.G232V) and the common c.880 C > T transition (p.R294X). Computational investigation reveals that the combination of identified mutations has a damaging impact on different *MECP2* structures decreasing their stability and hence altering their functions.

2. Materials and methods

2.1. Patient

The studied Rett syndrome patient was collected from the "Department of Child and Adolescent Neurology in the National Institute Mongi Ben Hmida of Neurology of Tunis in Tunisia". Informed consent was obtained from patient and her relatives in accordance with the ethics committee of National Institute Mongi Ben Hmida of Neurology (Tunis, Tunisia).

Clinical history interviews, clinical investigation, EEG, and neurological imaging were performed [3].

The proband was native of northern Tunisia and was 4 years old girl, born from a non consanguineous marriage. Pregnancy and delivery were uneventful. In addition, she had one sister who was healthy. The patient showed clinical features fulfilling the main revised diagnostic criteria for typical Rett syndrome. Indeed, she showed a severe intellectual disability, an infantile encephalopathy with an autistic behavior. In addition, this patient developed epilepsy in the age of 12 months. Besides, motor development delay and sleep disorder were detected in the studied patient. Onset of stereotypic hand movements, severe cognitive impairment with absence of language, progressive scoliosis and autistic features were revealed. However, Magnetic resonance imaging (MRI) was normal. Blood samples were obtained from the patient, her parents and her sister after informed consent. In addition, 50 Tunisian healthy individuals from the same ethno-cultural group were tested as controls. These controls should have no personal or family history of neurological disorder.

2.2. Methods

2.2.1. DNA extraction

After getting informed consent from all the participating family's members, total DNA was extracted from peripheral blood using standard phenol-chloroform procedures [19].

2.2.2. Mutational analyses

2.2.2.1. Polymerase chain reaction amplification. The four exons of *MECP2* gene with their flanking exon–intron boundaries were amplified by PCR using the primers described by Amir et al., 1999 [5]. The PCR reaction was carried out in a Perkin–Elmer GeneAmp PCR Sys545tem 9700 thermal cycler in a final volume of 50 μL with 1 × PCR buffer (50 mM KCl, 10 mM Tris HCl, 1.5 mM MgCl2, and 0.1% gelatin), dNTPs (0.25 mM), Taq polymerase (1 U; Fermentas), and primers (1 μM of each one). The conditions for the PCR amplification were as follows: 96 °C for 5 min, 35 cycles (1 min at 94 °C, 1 min of annealing [60°C-68 °C], and 1 min at 72 °C), followed by an extension at 72 °C for 10 min.

2.2.2.2. Sequencing of the MECP2 gene. After PCR amplification, each PCR product was purified using exonuclease before sequencing. Each exon was sequenced on both strands. The regions containing putative variation were amplified and sequenced three times on both strands to exclude that they were PCR artefacts.

Direct sequencing of PCR products was performed with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI PRISM/Biosystems) and the products were resolved on ABI PRISM.

2.2.2.3. Mutation detection. Mutations were identified when a double peak could be seen in the trace, and the nucleotide change was not present in normal control sequences, as determined by the sequence alignment. Both strands were sequenced to confirm the mutations detected.

2.2.3. Computational analyses

The blast homology analyses were performed using the software blast2seq available at the National Center for Biotechnology Information (NCBI) Web site (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq).

2.2.3.1. Pathogenicity prediction. Pathogenicity was explored via several prediction programs.

PolyPhen-2 (**Poly**morphism **Phen**otyping v2): this tool predicts the possible effect of amino acid changes on the structure, stability and function of human proteins using straightforward physical and evolutionary comparative considerations and evaluates the possibility of the missense mutation to be damaging based on a synthesis of all these properties. It determines a score ranging from 0 to 1.000, and classifies the substitution qualitatively, as probably damaging, possibly damaging or benign in agreement with their scores (http://genetics.bwh.harvard.edu/pph2/) [20–23].

SIFT (**S**orting **I**ntolerant **F**rom **T**olerant): this program uses sequence homology to predict if an amino acid substitution may have an impact on his protein function and eventually damages phenotype. The score is the normalized probability that the amino acid substitution is tolerated. SIFT assumes that important positions in a protein sequence have been conserved throughout evolution and therefore substitutions at these positions may affect protein function. The median sequence conservation ranges from 0 to 4.32, ideally the number would be between 2.75 and 3.25. A warning will occur if this is greater than 3.25 because this indicates that the prediction was based on closely related sequences or there were not enough sequences. The SIFT score ranges from 0 to 1. The amino

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