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Correlation between clinical features and *MECP2* gene mutations in patients with Rett syndrome

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Abstract *Background:* Rett syndrome is a progressive neurodevelopment disorder which mainly affects females and is a common cause of mental retardation. Loss of purposeful hand movements, regression of acquired cognitive and motor skills and autistic features are the main criteria associated with this disorder. Sixty to ninety percent of the cases show *MECP2* gene mutations, which reside on the X chromosome. *MECP2* regulates gene expression in a repressive manner.
The aim of this study is to estimate the incidence of *MECP2* mutations in 32 female Egyptian patients clinically diagnosed with Rett syndrome, and to correlate their clinical features with *MECP2* mutation status.
Patients: 32 female Egyptian patients with a mean age of 36.9 months diagnosed clinically to suffer from Rett syndrome are the cohort of this study.
Methods: Thorough clinical examination, MRI, EEG and testing for *MECP2* gene mutations.
Results: Twenty of the 32 (62.5%) patients showed *MECP2* mutations an incidence which falls within that reported in the literature. Patients with *MECP2 gene* mutations presented with more severe clinical abnormalities.
Conclusions: Mutation screening for *MECP2* is a fast and reliable method to diagnose patients clinically suspected to suffer from Rett syndrome or female patients with atypical Rett syndrome features, mental retardation, developmental delay and other neurological abnormalities who do not fit any specific diagnosis. Also, patients with *MECP2* mutation presented with a more severe phenotype.

1. Introduction

Rett syndrome is a progressive neurodevelopmental disorder and one of the most common causes of mental retardation in females, affecting approximately 1 in every 10,000–15,000 females worldwide.[1] Patients with classic Rett

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syndrome appear to develop normally until 6–18 months of age then gradually lose purposeful hand movements. This phase is followed by regression of the acquired cognitive and motor skills, autistic tendencies and gait apraxia. Eventually the condition becomes stationary. Concurrent manifestations may include episodes of apnea, hypercapnia, and seizures.[2]

The majority of cases result from heterozygous mutations in the *MECP2* gene, the possibility of detecting such mutations in patients with Rett syndrome has changed the face of this unique disorder. *MECP2* gene is located on the long arm of chromosome X (Xq 28) and encodes for the methyl-CpG binding protein 2, which is a ubiquitous transcriptional repressor. This protein functions by selectively binding methyl C-pG islands in the mammalian genome and regulates gene expression, mainly in a repressive manner.[3]

MECP2 protein regulates genes involved in brain function, although the protein is found through-out the body. Within the brain, this protein is vital for the function of neurons. MECP2 has a role in maintaining synapses between the neurons, allowing cell-to-cell communications to take place. The MECP2 protein also participates in processing messenger RNA (mRNA), which serves as genetic blueprints for making proteins. MECP2 controls a process known as alternative splicing, which controls the production of different versions of certain proteins. This process is crucial for normal communication between neurons in the brain.[4]

MECP2 is a member of a family of nuclear protein and is characterized by the presence of a methyl C-pG binding domain (MBD). These proteins are capable of binding specifically to methylated DNA. Methylation is a major modification of the eukaryotic genome essential for the process of mammalian development. Binding of *MECP2* gene to the methylated gene-promoter DNA of certain genes results in transcriptional repression of these genes.[5]

Mutations in *MECP2* may be sporadic or germ line mutations. Alternatively, Rett syndrome may be caused by mutations in *CDKL5* or *FOXG1* genes in 10% of cases. Sporadic cases constitute up to 95% of cases with *MECP2* mutations. The parents in this situation are genotypically normal and the mutated *MECP2* is usually thought to be derived from the normal male copy of the X chromosome. However, the reason behind the sperm to mutate is not yet fully known. On the other hand, germ line mutations are inherited from phenotypical normal females, to her offspring.[6]

The aim of this work is to study the incidence of *MECP2* mutation in a cohort of 32 Egyptian female patients with Rett syndrome and to compare their clinical features with *MECP2* mutation status.

2. Patients and methods

2.1. Patients

This is a retrospective study carried out on a cohort of thirty-two female Egyptian patients diagnosed clinically to suffer from Rett syndrome according to the classic criteria of this syndrome.[7] Their ages ranged from 18 months to 56 months with a mean age of 36.9 months. A written consent was obtained from the patient's guardians.

2.2. Methods

All patients were subjected to a thorough clinical examination. The presence, at time of diagnosis, of psychomotor retardation, stereotyped hand movement, microcephaly and epilepsy were recorded. We also assessed the presence of regression of the acquired milestones in the form of loss of sitting, standing and walking as well as loss of speech. Neuro-imaging in the form of MRI of the brain and electro-encephalographic (EEG) tracing were performed to all cases.

All patients underwent DNA extraction from their peripheral blood leukocytes using the Qiagen QIAampDNAMaxi extraction kit as protocol.

DNA yield and quality were determined using the NanoDrop ND-100 spectrophotometer (Nanodrop Technologies, USA). The protocol of measuring DNA concentration by spectrophotometer is reviewed by Sambrook and Russell.[8]

DNA was PCR amplified using the GeneAmp PCR system 9700 thermocycler (Applied Biosystems, UK) using the following PCR program: 95 °C for 10 min, followed by 40 cycles of 95 °C for 1 min, the optimal annealing temperature for the specific primers, for 1 min and 72 °C for 1 min. On final extension step for 7 min at 72 °C was then performed, followed by a holding step at 4 °C. The specific primers used are listed in Table 1 and it amplifies exons 3 and 4 of *MECP2* gene in 5 overlapping fragments (3.1, 3.2, 4.1, 4.2, and 4.3). This was followed by analysis of the PCR products using agarose gel electrophoresis. Details of protocol for agarose gel electrophoresis are available in Sambrook and Russell.[8]

PCR products were purified using Invetrogen purification kit (Purelink™ Nucleic Acid Purification Rack) (Invetrogen Llt., UK) according to the manufacturer's instruction.

Table 1 Specific DNA primers used to amplify exons 3 and 4 of *MECP2* gene.

Exon	Fragment	Primers	Product size	Tm (°C)
3	3.1F	5′-AAG ATC TGA GTG TAT GAT GGC CTGGG-3′	428 bp	60
	3.1R	5′-TTT GCT TAA GCT TCC GTG TCC AGC-3′		60
	3.2F	5′-AAG AGA AAG AGG GCA AGC ATG AGC-3′	405 bp	60
	3.2R	5′-AAG CAC ACC TGG TCT CAG TGT TCA-3′		60
4	4.1F	5′-CAG TTT GTC AGA GCG TTG TCA CCA CCA T-3′	626 bp	62
	4.1R	5′-TGA CGG AGT ACG GTC TCC TGC ACA ACA GAT-3′		
	4.2F	5′-CAG TTC CTG GGA AGC TCC TTC TCA AGA T-3′	616 bp	62
	4.2R	5′-TGA CTC CTC TGG GCA TCT TCC CTC TTT-3′		
	4.3F	5-CAG TGG GAA AGG ACT GAA GAC CTG TAA G-3″	566 bp	60
	4.3R	5-TGA CCA GTT AAT CGG GAA GCT TTG TCA G-3″		60