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Case Report [Olgu Sunumu]

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Genetic approach to the patient and the family: A Rett Syndrome case with p.R270X mutation in *MECP2* gene

[Hasta ve aileye genetik yaklaşım: *MECP2* geninde p.R270X mutasyonu olan Rett Sendromlu olgu]

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ABSTRACT

Rett syndrome is an X-linked dominant neurodevelopmental disorder which is primarily seen in girls. Mutations in the *MECP2* gene are responsible for 80% of affected patients. The most common mutations are found in exons 3, 4 of this gene. Most *MECP2* alterations are de novo and the recurrence risk is low. Approximately 1% of all affected patients are thought to be familial and clinically unaffected carrier mothers have been reported. Here, we present 3 year old girl patient who had all of the diagnostic criteria for typical Rett syndrome. The de novo, heterozygous c.808C>T mutation was detected by sequence analysis of exon 3 in the *MECP2* gene. We report this patient to emphasize the importance of the steps followed in the molecular analysis in Rett syndrome. Hovewer, when the mutation was detected in a patient, the molecular analysis of the mother is extremely important for correct genetic counseling.

Key Words: Rett syndrome, *MECP2* gene, molecular analysis, genetic counseling
Conflict of Interest: Authors do not have any conflisct of interest.

ÖZET

Rett Sendromu, X'e bağlı dominant kalıtılan, esas olarak kızları etkileyen nörogelişimsel bir hastalıktır. Hastaların %80'inden *MECP2* gen mutasyonları sorumludur. En sık mutasyonlar, genin ekzon 3 ve 4'ünde bulunmaktadır. *MECP2* gen değişikliklerinin çoğu de novodur ve tekrar riski düşüktür. Hastaların yaklaşık %1'i ailesel olgulardır ve klinik bulguları olmayan taşıyıcı anneler bildirilmiştir. Biz burada Rett sendromu tanı kriterlerinin hepsini içeren 3 yaşında kız hastayı sunuyoruz. Sekans analizi ile *MECP2* geninin 3. Ekzonunda, de novo heterozigot c.808C>T mutasyonu saptandı. Bu hastayı, Rett sendromu moleküler analizinde izlenen yolun önemini vurgulamak için sunuyoruz. Bununla birlikte, hastada mutasyon saptandığı zaman, annede moleküler analiz yapılması, doğru bir genetik danışma verilebilmesi için oldukça önemlidir.

Anahtar Kelimeler: Rett sendromu, *MECP2* geni, moleküler analiz, genetik danışma
Çıkar Çatışması: Yazarların çıkar çatışması bulunmamaktadır.

Introduction

Rett Syndrome (RTT) (MIM#312750) is a severe neurodevelopmental disorder that primarily affects girls [1]. The incidence of disease is approximately 1 in 10,000 female births [1]. First time, the diagnostic criteria for RTT in female patients have been described in 2002 [2]. Recently, the revised diagnostic criteria have been reported to clarify and simplify the diagnosis of typical RTT [3]. For the diagnosis of typical RTT, history of regression must be present and patient must have all of the main criteria, none of the exclusive criteria (Table I). For the diagnosis of atypical RTT, at least two of the main criteria and five of the eleven supportive criteria must exist in the patient [3].

In 80% of the patients with RTT, mutations are present in the methyl CpG-binding protein 2 gene (*MECP2*) [4]. *MECP2* mutations have also been reported to be related to atypical Angelman syndrome, non-syndromic X-linked MR, autism and male congenital encephalopathy [4,5]. In addition, *MECP2* gene mutation is seen in 2% of nonspecific mental retardation male patients [4]. In atypical RTT, mutations of *MECP2* are present in 20-40% of patients [4]. RTT is an X-linked dominant inherited disease [2]. In mammals, the male is hemizygous for the X chromosome. Some of the mutations are lethal in hemizygous males [6]. For this reason, typical RTT is most commonly seen in girls [4,6]. However, numerical abnormalities of X chromosome and mosaic mutations in *MECP2* gene have been reported in a few male patients with clinical features of RTT [4].

Here, we report a three-year-old girl patient with typical RTT who has de novo heterozygous c. 808C>T (p.R270X) mutation in *MECP2* gene.

Case Report and Methods

A 3-year-old female patient was referred to us due to moderate developmental delay, severe growth retardation. She is the first child of the healthy, non-consanguineous parents. The patient was born at term after an uneventful pregnancy with cesarean section. The mother and father were 21 and 27 years old, respectively, at the time of the infant's birth. The prenatal and perinatal history was considered normal. Her birth weight was 3600 g (50-75 centile), birth length 50 cm (50 centile), and head circumference was 35 cm (50 centile). The patient held up her head at 4 months, later she could not be held up to the head properly. She sat with support at age 10 months, and without support at 15 months. She never crawled or walked. She could not talk. There was no epilepsy in her history.

At the age of 3, her height was 97 cm (50-75 centile), weight was 12.5 kg (10-25 centile) and head circumference was 45 cm (<3rd centile). On physical examination, she was hypotonic and had stereotypic hand movements. During examination the patient constantly was putting her hand into her mouth, twisting and clapping her hands and gnashing her teeth (Fig. 1). Eye contact was absent and visual fixation was poor. Deep tendon reflexes were normal. The complete blood count, liver enzymes, electrolytes, renal function tests, thyroid function tests, creatine kinase, lactic acid, pyruvic acid, ammonia and tandem mass were normal. Abdominal ultrasonography, cranial MRI, spine X-rays and electrocardiograms were evaluated as normal. In interictal electroencephalography, we observed sharp wave activity at the right central region of the brain. On ophthalmologic examination, eye fundoscopy was normal. The hearing test revealed normal. Her karyotype was 46,XX. The patient's phenotype is evaluated compatible with typical RETT syndrome (Table I).

We isolated genomic DNA from blood lymphocytes of the patient by standard procedures. Three coding exons (2, 3 and 4) of the *MECP2* gene were amplified using previously reported primers [4]. The polymerase chain reaction products were purified and sequenced on an ABI PRISM 3130 automated DNA sequencer (Applied Biosystems). The heterozygous c.808C>T (p.R270X) mutation was showed in exon 4 (Fig. 2). This mutation was not identified in the DNA sample from her mother, suggesting that this mutation occurred de novo (Fig. 2). Genetic counseling was given to the parents of the patient.

Discussion

Rett Syndrome was originally described in the 1960's by Andreas Rett [7]. The previous diagnostic criteria for typical RTT had eight necessary criteria, five exclusion

Fig. 1: Appearance of the patient.

Table 1. The Revised diagnostic criteria for RTT. Modified from Neul JL et al: Ann Neurol 2010;68:944-950.

Revised diagnostic criteria for RTT 2010 Consider diagnosis when postnatal deceleration of head growth observed		
Required for typical RTT	1 A period of regression followed by recovery or stabilization 2 All main criteria and all exclusion criteria 3 Supportive criteria are not required, although often present in typical RTT	
Required for atypical RTT	1 A period of regression followed by recovery or stabilization 2 At least 2 out of the 4 main criteria 3 5 out of 11 supportive criteria	
Main Criteria	1 Partial or complete loss of acquired purposeful hand skills 2 Partial or complete loss of acquired spoken language 3 Gait abnormalities: Impaired (dyspraxic) or absence of ability 4 Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing and washing/rubbing automatisms	
Exclusion Criteria for typical RTT	1 Brain injury secondary to trauma (peri- or postnatally), neurometabolic disease, or severe infection that causes neurological problems 2 Grossly abnormal psychomotor development in first 6 months of life	
Supportive Criteria for atypical RTT	1 Breathing disturbances when awake 2 Bruxism when awake 3 Impaired sleep pattern 4 Abnormal muscle tone 5 Peripheral vasomotor disturbances	6 Scoliosis/kyphosis 7 Growth retardation 8 Small cold hands and feet 9 Inappropriate laughing/screaming spells 10 Diminished response to pain 11 Intense eye communication "eye pointing"

criteria, and eight supportive criteria. However, there was no requirement for any of the supportive criteria for the diagnosis of typical RTT [3]. Recently, the revised diagnostic criteria for RTT have been published in 2010 (Table I) and the supportive criteria have been entirely eliminated from the diagnostic criteria for typical RTT [3].

The normal psychomotor development for the first 6 months which is one of the diagnostic criteria in 2002 was excluded from the diagnostic criteria for typical RTT in 2010. They suggested that some alterations in initial development can be present in typical RTT patients [3]. The grossly abnormal psychomotor development in first 6 months of life was added in the exclusion criteria for typical RTT. However, these authors eliminated postnatal deceleration in head growth from the necessary criteria because this feature in not found in all individuals with typical RTT [3]. Four main criteria which were absolutely required for the diagnosis of typical RTT have been reported (Table I) [3]. Mutations in the *MECP2* gene are not synonymous with RTT. *MECP2* mutations are neither necessary nor sufficient to make the diagnosis of RTT according to the diagnostic criteria of 2002 and the new diagnostic criteria of 2010. As a result of this, RTT remains a clinical diagnosis [3]. Our

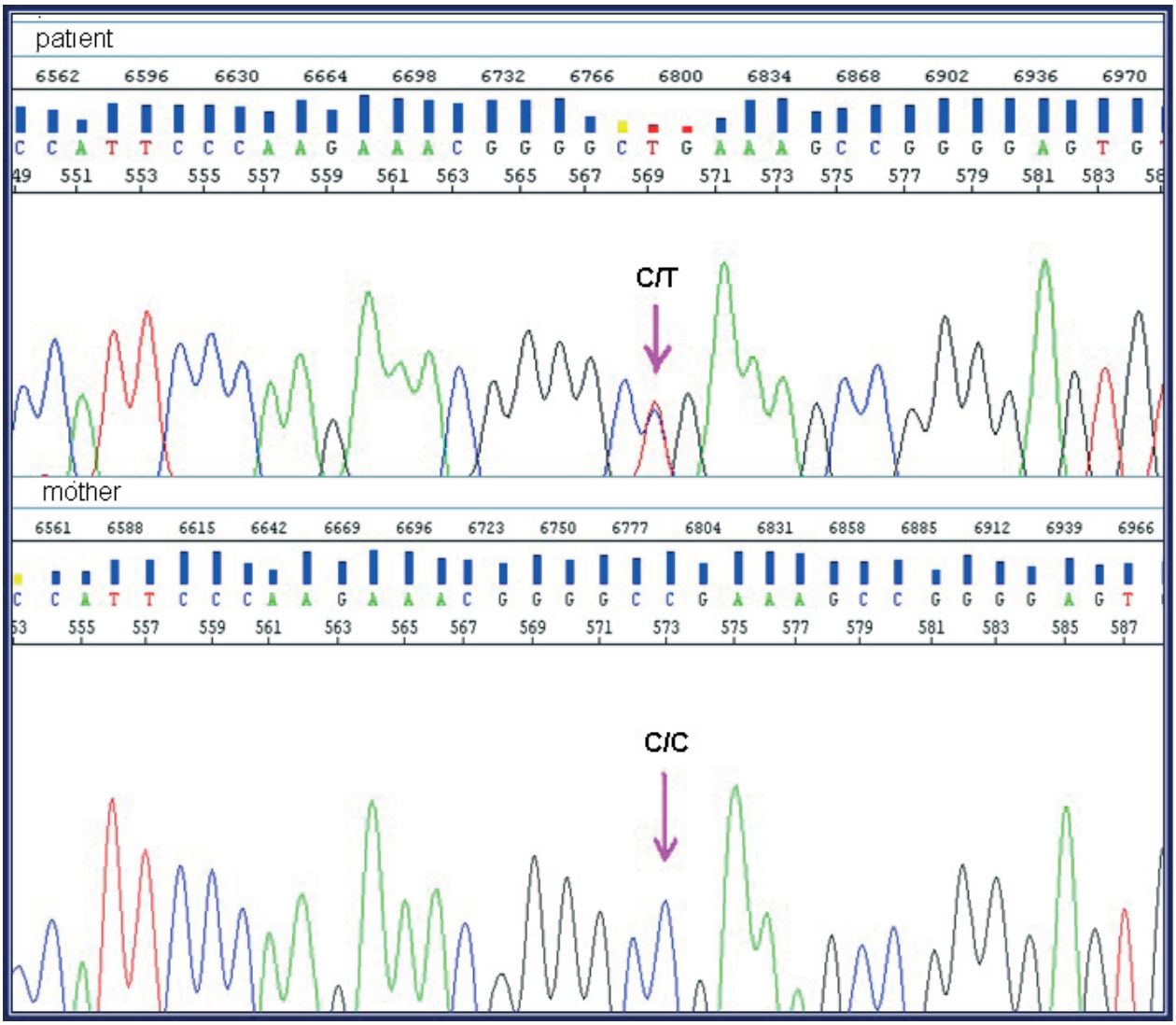

Fig. 2: Chromatogram of the heterozygous change, c.808C>T, in the patient (upper trace). Mother is wild type for 808.position of cDNA (bottom trace).

patient fulfilled the diagnostic criteria. Therefore the patient was evaluated as typical RTT.

Seizures have been reported in 50-80% of RTT patients [8]. In 2010, Glaze at al., revealed 602 RTT patients and reported that the frequency of seizures was reported as 53% in patients with p.R270X mutation in the *MECP2* gene [8]. Our 3-year-old patient with p.R270X mutation had no seizure history.

MECP2 gene, located on the long arm of X chromosome (Xq28) includes 4 exons. More than 600 pathogenic *MECP2* mutations have been reported [5]. In about 70% of RTT patients mutation is in the form of nucleotide transition from cytosine to thymine (C > T) [5]. The most common eight mutations in the *MECP2* gene are 473C>T (12.2%), 502C>T (11.9%), 763C>T (10.7%), 808C>T (9.6%), 880C>T (8.2%), 916C>T (6.4%), 397C>T (5.4%), 316C>T (4.8%) [5]. Li et al., evaluated the correlation between *MECP2* genotype and phenotype in 126 RTT patients and demonstrated that phenotypes of the RTT patients with nonsense mutations in the region of methyl-CpG-binding domain were more severe than those with missense mutations in the same region [9].

In 2006, Williamson at al., evaluated the clinical and molecular approaches to diagnosis of RTT and they suggested that PCR-based screening analysis of exon 3 and exon 4 is the first step for genetic diagnosis of RTT because of the majority of mutations are seen in these regions (Fig. 1) [5]. If no mutation is identified in these exons, further screening analysis of exons 1 and 2 is recommended [5]. Deletions in the *MECP2* gene have been reported in approximately 10% of affected patients. Large deletions can be missed by PCR-based screening strategies. Southern analysis, quantitative PCR of genomic DNA and Multiplex Ligation Probe Amplification (MLPA) are recommended for detection of large deletions [3,5]. Mutations of *CDKL5* and *NTNG1* gene have been reported in some patients with clinical features that overlap significantly with RETT syndrome [4,5].

Molecular analysis of *CDKL5* gene is suggested in patients who had seizures or infantile spasms in the first 6 months of life [5]. In the first step, we performed the sequence analysis of exons 3 and 4 in the *MECP2* gene in our patient. Heterozygote c.808C>T (p.R270X) mutation was detected in exon 4.

The majority of mutations (>%99) in the *MECP2* gene are de novo and the recurrence risk is low [5]. Familial cases are usually explained by germline mosaicism or skewing of X-inactivation towards the wild-type *MECP2* allele in the carrier mothers [10]. If the mutation is found in the mother, preimplantation or prenatal diagnosis must be advised in all subsequent pregnancies [4]. If the mutation is de novo (no mutation in patient's mother), although germinal mosaicism cannot be excluded, the recurrence risk for siblings of proband is low, estimated to be less than one in 300 empirically [5]. The prenatal screening should be discussed with families.

As a result, we would like to emphasize that the steps followed in the molecular analysis in patients with typical and atypical Rett syndrome are important for genetic diagnosis. However, when the mutation is detected in a patient, the molecular genetic analysis of patient's mother is extremely important for genetic counseling.

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