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Brief Communication

Mutation Analysis of 16S rRNA in Patients With Rett Syndrome

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Rett syndrome (RTT) is a progressive neurodevelopmental disorder that affects one in 10,000-15,000 females. RTT is mainly sporadic; familial cases have an estimated frequency of less than 1%. Before the recent identification of de novo dominant mutations in the X-linked MECP2 gene, many other hypotheses had been proposed to explain the particular pattern of inheritance and the phenotypic expression of the disease. The involvement of mitochondrial DNA had been investigated because of the structural and functional mitochondrial abnormalities evident in the patients. In 1997 the finding of mutations at 16S rRNA in several affected RTT females and their mothers was reported, suggesting that mitochondrial DNA might play a key role in the pathogenesis of RTT. To investigate the relevance of such mutations, we used the same methodologic approach to analyze RTT mitochondrial DNA in our series. No 16S rRNA alterations were evident in 27 Spanish patients with classic RTT.

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Introduction

Rett syndrome (RTT) is one of the most common causes of mental retardation in females, with a prevalence of one in 10,000-15,000 [1]. Before the recent identification of de novo dominant mutations in the X-linked MECP2 gene [2], many other hypotheses had been proposed to explain the particular pattern of inheritance and the phenotypic expression of the disease. Several investigators had studied the involvement of mitochondrial DNA (mtDNA) because of the structural and functional mitochondrial abnormalities evident in the patients [3]. In 1997, Tang et al. [4] reported the finding of mutations at 16S rRNA in several affected RTT patients and their mothers, suggesting that mtDNA might play a key role in the pathogenesis of RTT. In its classic form the disease is characterized by neurodevelopmental arrest and regression after a 1-year period of normal growth and development. Loss of acquired motor skills and speech, stereotypical hand movements, microcephaly, autism, seizures, and hyperventilation crisis are typical signs of RTT [5,6]. Clinical variation allows definition of several atypical forms of the disease. RTT almost always affects females, and it is sporadic in more than 99% of cases. The pattern of inheritance of the disorder has been widely discussed, but the lack of familial cases has hampered classic linkage analyses. The most commonly accepted hypothesis assumed de novo–dominant mutations at an X-linked gene that was subjected to lyonization. Autosomal-dominant inheritance with sex-limited expression, dynamic mutations, metabolic interference, and mtDNA mutations had also been proposed.

Recently, Amir et al. [2] identified mutations within the coding region of the MECP2 gene in both sporadic and familial cases of RTT. MECP2 maps to Xq28 and undergoes X inactivation. The gene encodes methyl-CpG–binding protein 2, a ubiquitously expressed protein that probably plays a crucial epigenetic role during development.

Before the identification of MECP2 gene, Tang et al. [4] published a mutational analysis of mtDNA in children with RTT. The maternal inheritance pattern observed in several reported familial cases and the morphologic and functional mitochondrial abnormalities observed in some patients with RTT led the investigators to hypothesize that mtDNA mutations might play an important role in the pathogenesis of the disease. They analyzed 15 patients with RTT and 14 of their mothers and identified a mutation in 16S rRNA (2,835C→T) in seven affected females and six mothers. Single-stranded conformational polymorphism analyses revealed migration abnormalities in six more patients and five mothers within the same region. These findings suggested that point mutations in 16S rRNA might be related to the disease. We report evidence that such 16S rRNA changes are not present in the Spanish patients with RTT that we have studied.

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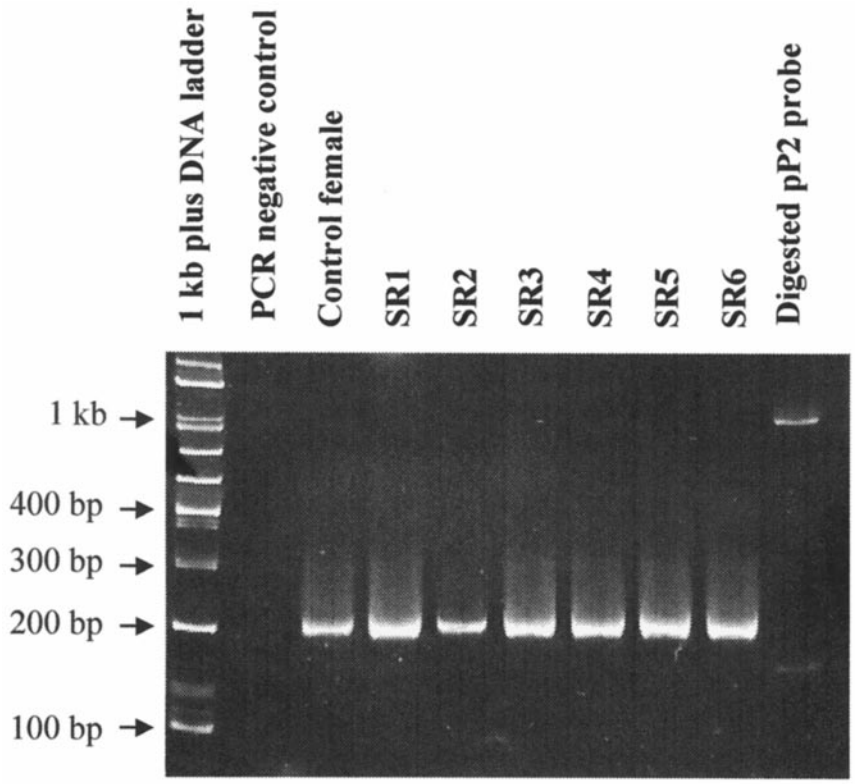

Figure 1. Direct detection of 2835 C→T mutation at 16S rRNA. Ethydium bromide stained polyacrylamide gel exhibiting results of SacI-digested mismatch polymerase chain reaction product in one control female (lane 3) and six patients with RTT (lanes 4-9). All subjects exhibit the band corresponding to the normal undigested product of 196 bp. The presence of the 2,835 C→T mutation should cut the product into 165-bp and 30-bp fragments. Lane 10: probe pP2 with a known internal SacI restriction site was used to monitor the digestion.

Subjects and Methods

Twenty-seven Spanish patients with classic RTT who were 2-18 years of age were analyzed. All patients fulfilled the diagnostic criteria. Forty unrelated female subjects were also included as a control population.

DNA was prepared from blood lymphocytes by classic phenol extraction and ethanol precipitation. 16S rRNA mutation analysis was performed as described by Tang et al. [4]. The direct method of mismatch polymerase chain reaction and SacI digestion was used to detect the 2,835 C→T transition. To ensure that lack of digestion was really caused by the absence of a mutation at 2,835, 1 μg of a cloned probe with a known internal SacI site was used for the control of digestion. To search for other putative changes the 800-bp fragment corresponding to 16S rRNA was amplified; polymerase chain reaction products were digested with PvuII and analyzed by SSCP.

Results

The C→T transition at nucleotide 2,835 was not detected in any of the 27 patients with RTT nor in the 40 healthy control subjects. Figure 1 presents the 196-bp wild type of product of the mismatch polymerase chain reaction not digested by SacI. Moreover, SSCP analysis of the 350-bp PvuII-digested 16S rRNA fragment failed to detect any difference between the patients and control subjects. Our results are in agreement with those reported by Cardaioli et al. [7] in a series of 26 Italian patients with RTT, in whom no 2,835 C→T mutations were evident either.

Discussion

RTT is known to be caused by mutations at the Xq28-linked MECP2 gene, encoding methyl-CpG binding protein 2 [2]. De novo mutations within the coding region of the gene have been described in 60% of the affected females [8]. Supporting these results, we detected MECP2 heterozygous mutations in 17 of the present group of 27 Spanish patients (data not presented).

The existence of a second locus causing RTT cannot be excluded until the complete gene sequence has been screened. Nevertheless, it is difficult to explain the finding of consistent 16S rRNA mutations in a specific Chinese population with RTT but not in other series of patients of a different ethnic origin. Although it is not likely that mtDNA plays a primary role in the etiopathogenesis of RTT, the relationship between the changes observed in the mtDNA by Tang et al. and the pathologic findings of their patients deserves further investigation.

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