Screening for Mutations in the MECP2 (Rett Syndrome) Gene in Gilles de la Tourette Syndrome

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Gilles de la Tourette syndrome (TS) is a relatively common neurobehavioral disorder of childhood onset with a prevalence of 0.7% to 4.2%. Tourette syndrome is characterized by multiple motor and vocal tics, and it is frequently associated with attention-deficit/hyperactivity disorder, obsessive-compulsive disorder, or both. Although studies of families with TS suggest that the disease is inherited as an autosomal trait, the inheritance pattern has not been clearly determined. Using different phenotypic definitions of TS, autosomal dominant, autosomal recessive, and “bilineal” transmission models have been postulated. The dominant model assumes reduced penetrance, and in the bilineal transmission, both parents would have TS or a form fruste of the disease. Effects of additional loci or epigenetic factors may also modify the phenotypic expression. Linkage and sib-pair analyses in families with TS, as well as cytogenetic studies, point to involvement of several autosomal susceptibility loci for TS, including 4q, 7q31, 8p, 11q23, and 18q. X-linked inheritance does not appear to be a major cause of the disease as a high frequency of male-male transmission has been observed. However, the marked preponderance of TS for males (male-female ratio of about 4.3:1) has raised the possibility of an X-linked modifier gene affecting the clinical expression of TS.

Rett syndrome (RTT) is an X-linked dominant disorder affecting girls (with a prevalence in the range of 1:10000 to 1:15000 female births) with onset after the first 6 to 18 months of life. Rett syndrome is characterized by severe developmental delay, acquired microcephaly, autism, lose of acquired speech and purposeful hand use, stereotyped motions, seizures, and ataxia and gait apraxia, as well as sleep and breathing abnormalities. The disorder is caused by mutations in the MECP2 gene on Xq28, encoding methylcytosine binding protein 2 (MeCP2). Mutations in MECP2 have also been found in male patients with either Angelman syndrome, Klinefelter syndrome, severe neonatal encephalopathy, autism, mental retardation, resting tremors, and/or progressive spasticity. The wide phenotypical variability in patients carrying MECP2 mutations appears to be dependent on the type and position of each particular mutation as well as on the skewed X-inactivation patterns in carrier females.

Tourette syndrome and RTT are both considered neurodevelopmental disorders in which affected individuals have an apparent normal neonatal development that is followed by the delayed onset of motor and behavioral disturbances. Post-mortem examinations of the brains of patients with RTT and TS have not revealed any specific pathological change. The co-occurrence in the same families of patients affected by TS and RTT has been described. Comorbid early-onset TS with reversible loss of various abilities and the emergence of autistic behavior have recently been described. Thus, although there are significant phenotypical differences between RTT and TS, these observations and the growing number of neurological diseases resulting from defects on epigenetic and/or DNA methylation pathways during brain development led us to hypothesize that RTT shares a genetically or mechanistically common underlying defect with TS. This defect could
affection the TS phenotype in a sex-dependent fashion. In this current study, we tested the possibility that patients with TS have either rare polymorphisms or mutations in the MECP2 gene.

Thirty-one male patients with no family history of TS were diagnosed as having TS according to the criteria of the Tourette Syndrome Classification Study Group. Under signed consent, DNA was purified from peripheral blood samples. The complete nucleotide sequence of the coding regions of the MECP2 gene (ie, exons 2, 3, and 4), and the corresponding intron-exon flanking regions, was determined in the 31 patients with TS. The following 3 pairs of specific oligonucleotide primers were used for polymerase chain reaction (PCR) amplification of the MECP2 coding regions: (1) F1/R1 (5'-taagcgggaatccctagc-3'/5'-cgtgccccaaatgtgcctataa-3') for the intron 1/exon 2/intron 2 region (418 base pairs [bp]); (2) F2/R2 (5'-aggacatcaagatctgagtgtat-3') for the intron 2/exon 3/intron 3 region (626 bp); and (3) F3/R3 (5'-ccaggaagctttgtagctgcggcagg-3'/5'-ttggggatgtttttcttaccg-3') for the intron 3/exon 4/intron 4 region (1447 bp). These PCR conditions were as described elsewhere, except that high-fidelity Platinum (Invitrogen, Carlsbad, NM) or Hot-Start (QIAGEN Inc, Valencia, Calif) Taq polymerases were used. The PCR products were purified with the use of a purification kit (QiAquick PCR; QIAGEN Inc), characterized in agarose gel electrophoresis, and sequenced by using the big dye terminator fluorescent technology. Primers used for sequencing were F1 for exon 2 and its flanking regions; F2 and R2 for exon 3 and its flanking regions; and F3, R3, intF3 (5'-ggaggagaagctttgtagctgcggcagg-3'), intR3 (5'-taccttttgcgccccgggctg-3'), and intR3bis (5'-tccttaccttctctgccgcggcggcgg-3') for the large exon 4 and its flanking regions.

No mutations were found in the MECP2 gene in these 31 male patients. A previously described +1233 C→T polymorphism (S411) was found in 1 patient. A new intronic variant was found in twins with TS (IVS3-19delT). This intronic polymorphism is contiguous to an already-described nonpathogenic variant, IVS3-20delT, suggesting that IVS3-19delT is not pathogenic. These results suggest that mutations or rare polymorphic variants in the coding sequences and intron-exon boundaries of the MECP2 gene are neither a frequent cause nor an important modifier of the clinical phenotype in TS.

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REFERENCES

11. Comings DE, Comings BD. Evidence of an X-linked modifier gene affecting the expression of Tourette syndrome and its relevance to the increased frequency of speech, cognitive, and behavioral disorders in males. Proc Natl Acad Sci USA. 1986;83:2551-2555.