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Cytogenetic Analysis of Obsessive-Compulsive Disorder (OCD): Identification of a FRAXE Fragile Site

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Abstract

Obsessive-compulsive disorder (OCD) is a chronic psychiatric disease characterized by recurrent obsessions, compulsions, or both. The prevalence rate of OCD is 2.1% in the general population. Here we report cytogenetic analysis of 26 patients affected with OCD. In one male patient (OCD-K33), we identified a fragile X chromosome by cytogenetic analysis with 21% of cells demonstrating a fragile site at Xq27–q28. Polymerase chain reaction (PCR) and Southern blot analysis demonstrated that the molecular basis of the OCD-K33 fragile X chromosome was expansion of the CCG repeat at FRAXE. The number of the expanded repeats was estimated to be more than 300 copies, qualifying it as a full FRAXE mutation. Further analysis of the family members of OCD-K33 revealed another member with a full FRAXE mutation (630–1,200 copies of the CCG repeat), who had the clinical phenotype of speech impairment, and two other members with normal phenotypes and no FRAXE expansion. The two FRAXE expansions lead to complete methylation at the CCG repeat. The co-segregation of the full FRAXE mutation with apparent neurologic disorders in the same family provides further support to the notion that FRAXE is a genetic neurologic condition. Our findings expand the spectrum of clinical phenotypes associated with FRAXE mutations.

Keywords

obsessive-compulsive disorder (OCD); fragile X chromosome; FRAXA; FRAXE; FRAXF; speech impairment; genetics; *FMR2*; neurologic disorder; mental retardation

INTRODUCTION

Obsessive-compulsive disorder (OCD) is a chronic psychiatric disease characterized by recurrent and persistent ideas, thoughts, impulses, and images (obsessions), or ritualistic, repetitive, purposeful behaviors (compulsions) [American Psychiatric Association, 1994]. OCD can cause marked distress, significant impairment, and severe suffering to patients. The prevalence rate for OCD is estimated to be 2.1% of the general population, affecting 4.3 million people in the United States [Narrow et al., 2002]. The etiology of OCD is unknown, although

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recent findings with neuroimaging techniques have implicated neurologic dysfunction in brain circuits in the production of symptoms [Rosenberg and Hanna, 2000; Neel et al., 2002].

FRAAXE is a fragile site on chromosome band Xq28 that is associated with mild or borderline mental retardation ($50 < IQ < 85$) [Gecz, 2000]. An estimated incidence of FRAAXE is 1/23,423–50,000 males [Brown, 1996; Youings et al., 2000]. About 50 families with FRAAXE have been reported worldwide. The molecular basis of the cytological FRAAXE site is hyperexpansion of the CCG repeat in the 5' untranslated region of the *FMR2* gene, which represses transcription of *FMR2* through methylation [Gecz et al., 1996; Gu et al., 1996]. Normal alleles of FRAAXE range from 10 to 35 repeats, whereas the full mutation has more than 200 repeats. The full FRAAXE mutation completely abolishes transcription of *FMR2* [Gecz et al., 1997b]. *FMR2* consists of 22 exons and encodes a putative transcription factor of 1,311 amino acids [Gecz et al., 1997a; Hillman and Gecz, 2001]. Detailed expression studies using adult mouse brains revealed that *Fmr2* was expressed in the cerebral cortex, hippocampus, amygdala, the purkinje cell layers of the cerebellum, olfactory bulb, striatum, caudate nucleus, epithalamus, thalamus, and entorhinal cortex [Gu et al., 2002].

In this study, we used cytogenetic analysis to characterize 26 patients with OCD. We report here identification of a FRAAXE mutation in an OCD patient.

MATERIALS AND METHODS

Identification of Patients With OCD

OCD patients were ascertained through the Pharmacology Research Corporation at Salt Lake City, UT. All probands satisfied criteria for full OCD [American Psychiatric Association, 1994]. All participants were evaluated for a lifetime history of OCD symptoms by a combination of direct observations and examinations, medical record review, and analysis of data obtained by a direct standardized interview (Yale-Brown Obsessive Compulsive Scale scores, Y-BOCS). The Y-BOCS was administered by an experienced clinician to the patient in order to assess the severity and to confirm the diagnosis of OCD. In general, a higher score indicated the worse symptoms, and a more severe disorder. Informed consent was obtained from participants in accordance with standards established by local institutional review boards.

Karyotype Analysis

Chromosomes were prepared from peripheral blood lymphocyte cultures according to standard procedures with the addition of either thymidine (0.001 M final concentration, Sigma-Aldrich Co., St. Louis, MO) or fluorodeoxyuridine (5×10^{-8} M final concentration, Sigma) to the lymphocyte cultures between 17 and 22 h before the start of the harvest protocol. Chromosomes were GTG banded according to standard protocols.

Genomic DNA, Polymerase Chain Reaction (PCR), and Southern Blot Analysis

Isolation of human genomic DNA was prepared from peripheral blood leukocytes using DNA Isolation Kits for Mammalian Blood according to the manufacturer's instructions (Roche Diagnostics Co., Indianapolis, IN), or from lymphoblastoid cell lines as described [Sambrook et al., 1989].

PCR for amplification of FRAAXA, FRAAXE, and FRAAXF trinucleotide repeats was performed with Exo-Pfu DNA polymerase (Stratagene, La Jolla, CA) according to standard protocols. Southern blot analysis was carried out with *EcoR* I-digested genomic DNA and a FRAAXE probe (570-bp *EcoR* I-*Sac* II DNA fragment containing exon 1 of *FMR2*).

RESULTS

Identification of a Fragile X Chromosome in OCD Patient OCD-K33

Although twin and family-based studies suggest that genetic factors contribute to pathogenesis of OCD, genetics of OCD is expected to be complex and current association and linkage techniques may not be sufficient for mapping most OCD genes [Rosenberg and Hanna, 2000]. An alternative approach for mapping an OCD gene is to identify cytogenetic abnormalities in OCD patients. Cytogenetic abnormalities, including translocations, deletions, and other anomalies, have repeatedly led to the isolation of genes responsible for the phenotype in a wide variety of human genetic disorders. To identify a cytogenetic abnormality associated with OCD, we carried out conventional cytogenetic analysis of G-banded metaphase chromosomes from 26 OCD patients. These patients include 15 males and 11 females. In each analysis, 20–30 G-banded cells were examined. The karyotypes for 25 patients were normal. Interestingly, a fragile X chromosome was identified in male patient OCD-K33 at the distal end of Xq (Xq27–q28). To confirm this finding, we re-examined 70 G-banded cells from OCD-K33, and found that 15 cells (21%) were positive for the fragile X chromosome.

Molecular Basis of the Fragile X Chromosome in OCD-K33

There are three fragile sites identified at Xq27–q28: FRAXA and FRAXE are associated with expanded, hypermethylated, and unstable CCG repeats, and FRAXF has an expansion at (GCCGTC)_n(GCC)_n compound array [Parrish et al., 1994]. In order to identify which of the three fragile sites was responsible for the fragile X chromosome observed in patient OCD-K33, we performed PCR analysis of trinucleotide repeats from FRAXA, FRAXE, and FRAXF with genomic DNA from OCD-K33 and three normal controls. As shown in Figure 1A,C, the numbers of trinucleotide repeats in FRAXA and FRAXF are comparable between OCD-K33 and three normal controls, suggesting that FRAXA and FRAXF are not involved in the OCD-K33 fragile X chromosome. In contrast, the CCG repeat of FRAXE was easily PCR-amplified in three controls, but could not be amplified in patient OCD-K33 (Fig. 1B) presumably due to expansion of the repeat. These data suggested that the molecular basis of the OCD-K33 fragile X chromosome is the expansion of the CCG repeat at FRAXE.

To confirm that the OCD-K33 fragile X chromosome involved FRAXE, we carried out Southern blot analysis with the 570-bp *EcoR I*-*Sac II* *FMR2* probe detecting the FRAXE CCG repeat *EcoR I* fragment. As shown in Figure 2, patient OCD-K33 has a full FRAXE mutation with more than 300 copies of the CCG repeat (individual II-1, lane 2). These data confirm that the molecular basis of the fragile X chromosome in patient OCD-K33 is expansion of the CCG repeat at FRAXE.

To further characterize the FRAXE fragile site associated with OCD, we studied the family members of OCD-K33. The mother of OCD-K33 is phenotypically normal and has one normal X chromosome, and one X chromosome with moderately expanded repeats (66 copies) (Fig. 2, individual I-1, lane 3). Individual I-2, the maternal uncle with normal phenotype, has the normal X chromosome (Fig. 2, lane 4). However, individual I-3 has hyper-expansion of FRAXE CCG repeat, a full mutation with 630–1,200 copies of the repeat (Fig. 2, lane 5). Phenotypic evaluation of individual I-3 revealed that he has speech impairment. In this family, expansion of FRAXE CCG repeats co-segregates with neurological abnormalities: OCD was found in one individual and speech impairment was found in another affected individual.

The methylation pattern of the FRAXE CCG repeat was analyzed on genomic DNA from patient OCD-K33 and his family members by Southern blot analysis. The DNA was digested by either *EcoR I* or *EcoR I*/*Not I*. Two individuals with full FRAXE mutations (Fig. 2, I-3 with speech impairment; II-1 with OCD) showed complete methylation (lack of digestion) of the

Not I site, whereas individual I-2 with normal phenotype and a normal control are not methylated at the FRAXE CCG repeats (data not shown). The mother of the OCD patient showed random methylation of both X chromosomes (data not shown). Our results suggest that the two expansions reported here are full FRAXE mutations that inactivate expression of *FMR2*.

DISCUSSION

Due to its rare occurrence, the FRAXE clinical phenotype has not yet been clearly defined. To date, the phenotype that is most likely to be associated with FRAXE fragile site is mild mental retardation and developmental delay [Gecz, 2000]. However, the causal relationship between FRAXE and mental retardation is inconsistent because the FRAXE full mutation and abolished *FMR2* transcription did not always co-segregate with the mental retardation phenotype in some families [Gecz et al., 1997b]. Some FRAXE patients were found to exhibit speech delay, learning difficulties, and reading and writing problems, mild attention deficit hyperactivity disorder, autism, and premature ovarian failure (reviewed by Gecz, 2000). Our finding of a FRAXE fragile site in family members affected with OCD or speech impairment expands the spectrum of clinical phenotypes associated with this genetic condition.

Increasing evidence suggests that OCD is linked to neurologic dysfunction of the orbitofrontal cortex, caudate nucleus, and thalamus [Rosenberg and Hanna, 2000; Neel et al., 2002]. Expression of *Fmr2* in cerebral cortex (orbitofrontal cortex is part of cerebral cortex), the caudate nucleus, and thalamus is consistent with the association of OCD with FRAXE. Based on the positive therapeutic effects of serotonin reuptake inhibitors in some OCD patients, alternations in serotonergic neurotransmission have been hypothesized as a pathogenic mechanism underlying OCD [Pigott and Seay, 1999]. The findings that *lilliputian* (the *Drosophila* homolog of *FMR2*) regulates expression of *huchebein* [Tang et al., 2001] that is essential for development of serotonin neurons provide an indirect connection between OCD and *FMR2*. Gu et al. [2002] generated knockout mice deficient in *Fmr2*. The knockout animals exhibited a delay-dependent conditional fear impairment. It remains to be investigated whether the fear impairment in *Fmr2* knockout mice is related to OCD phenotype in humans.

In the family reported here, two individuals with full FRAXE mutations have associated neurologic abnormalities, whereas the two individuals who do not have FRAXE mutations are phenotypically normal. These data are consistent with the hypothesis that FRAXE mutations are associated with neurologic disorders. It is interesting that the two affected individuals in this study have different phenotypes, one with OCD and the other with speech impairment. Their phenotypic difference may be explained by the different sizes of the FRAXE CCG repeat expansions, which may be associated with a difference in a time window in development when the expansion is unmethylated. Alternatively, the timing of methylation is variable between individuals (and probably between cells), resulting in variation in phenotype. The same hypothesis may be applicable to the finding that not all individuals in the same families with the FRAXE full mutations display the mental retardation phenotype. To further understand the contribution of FRAXE mutations in patients with OCD, these individuals should be screened for FRAXE expansions. If FRAXE expansions can explain a proportion of OCD, screening for these mutations may provide helpful information for genetic counseling and reproductive options to the families of affected members.

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Fig. 1.

The fragile X chromosome in male patient OCD-K33 with obsessive-compulsive disorder (OCD) is caused by expansion of the CCG repeat at FRAXE. Amplification of the CCG repeat at FRAXA (**A**) and FRAXE (**B**), and the (GCCGTC)_n(GCC)_n compound array at FRAXF (**C**) were carried out with polymerase chain reaction (PCR) using primers that span the corresponding repeat. The controls include no DNA, and DNA samples from three normal individuals (HG DNA1, HG DNA2, and HG DNA3). The size of the FRAXA and FRAXF repeats is comparable between OCD-K33 and the three normal controls, excluding FRAXA and FRAXF as the molecular basis of the OCD-K33 fragile X chromosome. Failure of amplification of the FRAXE repeat in OCD-K33 suggests that expansion of the FRAXE CCG repeat may be the cause of the OCD-K33 fragile site. PCR primers for FRAXA are 5'-gctcagctccggtttcggttcacttcggt-3' (forward) and 5'-agccccgcacttcaccaccagctcctcca-3' (reverse); FRAXE, 5'-gcgaggaagcggcggcagtggtgctggcg-3' (forward) and 5'-ctgtgagtgtgaagtgt-gtgaatgctg-3' (reverse); FRAXF, 5'-cagggggcggttggtcaggtt-3' (forward) and 5'-cctgcgccttacagcggttca-3' (reverse).

Fig. 2.

Southern blot analysis of patient OCD-K33 with OCD and his family members for expansion of FRAAXE CCG repeat. **Lane 1:** λ Hind III molecular weight marker; **lane 2,** proband patient OCD-K33 with OCD (individual II-1); **lane 3,** proband's mother (individual I-1) with normal phenotype; **lane 4,** proband's maternal uncle (individual I-2) with normal phenotype; **lane 5,** proband's maternal uncle (individual I-3) with speech impairment; **lane 6,** a normal control. The human genomic DNA was digested with *EcoR I*, transferred to a nylon membrane, and probed with a 32 P-labeled 570-bp *EcoR I*-*Sac II* DNA fragment that contains exon 1 of *FMR2* just distal to FRAAXE CCG repeats. The size and corresponding CCG repeat number are indicated at the right.