

## Letter to the Editor

# Detection of a Large CTG/CAG Trinucleotide Repeat Expansion in a Danish Schizophrenia Kindred

### To the Editor:

To date, 11 different neurodegenerative, neuromuscular, and/or neurodevelopmental disorders, including Huntington disease, myotonic dystrophy, and fragile X syndrome, have been shown to be associated with expansion of unstable trinucleotide repeats [Warren, 1996; Zoghbi, 1996; Zuchenko et al., 1997]. Most of these disorders are associated with a pathology of the nervous system and with unusual genetic features: increased penetrance or disease severity or earlier age at onset in successive generations (= anticipation) along with a parental (maternal or paternal) sex bias in the transmission of the severe form of the disease, which correlates with the degree of instability and allelic expansion of the trinucleotide repeat. Penrose [1948] had suggested that anticipation was apparent in the inheritance of schizophrenia, although he considered the phenomenon likely to be an artifact due to ascertainment bias. Additional studies of schizophrenia have recently provided "evidence" for the existence of anticipation, which could be therefore caused by the expansion of an unstable trinucleotide repeat [Basset and Honer, 1994; Basset and Husted, 1997]. Using the RED (repeat expansion detection) method described by Schalling et al. [1993], a series of independent studies have been recently published, suggesting a possible pathogenic association of (CTG)<sub>n</sub> repeats (mostly in the 180–255-bp range) with schizophrenia [O'Donovan et al., 1995, 1996; Morris et al., 1995; Cardno et al., 1996]. However, the results from these studies are rather equivocal and involve slight differences between the distribution of the maximum repeat lengths of CTG/CAG repeat arrays found in affected individuals compared to the distribution found in normal controls, with the maximum length in affected individuals almost always within normal range. Individuals carrying CTG/CAG arrays in the 180–255-bp range were observed at moderate or high frequency in a broad population-

based survey which includes a normal Danish population sample [Sirugo et al., 1997]. Even larger sizes were observed in one fifth to one third of normal population samples from east Asia, so that interpreting RED results of arrays in the 180–255-bp range as pathogenic is very problematic, even when found in affected individuals.

We systematically analyzed a series of large Danish kindreds, identified through schizophrenic probands, for CTG/CAG expansion mutations using the RED technique as modified by Sirugo and Kidd [1995]. We detected one individual with an extremely long CTG/CAG repeat array in his/her genomic DNA; this individual had a diagnosis of paranoid schizophrenia (K in Fig. 1, and lane 1 in Fig. 2). This result corresponds to at least one array in the genome of at least 300 tandemly repeated CTG/CAG triplets, and has been detected in DNA extracted both from fresh blood samples and from a lymphoblastoid cell line of this individual. This can be considered an abnormally long array, since maximum repeat lengths corresponding to more than 200 tandemly repeated CTGs have only very exceptionally been seen in RED surveys of normal individuals [Schalling et al., 1993; O'Donovan et al., 1995; Sirugo et al., 1997]. A first cousin, also affected by paranoid schizophrenia, had an unusually long CTG/CAG repeat allele (R in Fig. 1, and lane 2 in Fig. 2), as did an affected sibling (T in Fig. 1, and lane 3 in Fig. 2). Other severely affected individuals from this kindred (n = 3 patients) and from other kindreds (n = 7 patients) do not have extremely long CTG/CAG repeat arrays in their genomes. In this kindred there are other individuals with long repeat arrays who do not meet criteria for a diagnosis of paranoid schizophrenia (S and N, Fig. 1). The locus with the unusually long (CTG)<sub>n</sub> array detected by RED has been successfully localized on chromosome 18q21 by using fluorescence in situ hybridization (FISH) on lymphoblastoid metaphase spreads of the schizophrenic individual who carries in the genome the CTG expansion of >300 bp [Haaf et al., 1996]. Because RED CTG/CAG arrays >260 bp are very rare in normal Danes [Sirugo et al., 1997], it is very likely that all long arrays detected by RED in this kindred originate from the same unstable repeat-containing locus on 18q21.

The possible association of the CTG expansion with

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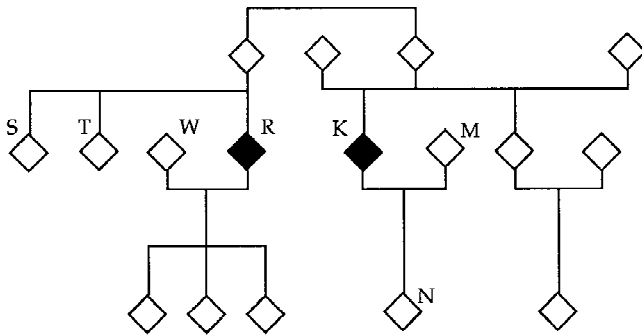


Fig. 1. The Danish kindred analyzed in this study. Solid symbols indicate individuals affected by paranoid schizophrenia. Individuals W, M, N, and S have "schizophrenia spectrum" diagnoses; the phenotypes of individual T and of the offspring of W and R (unlabeled in the figure) fall into the "narrow schizophrenia" category. Large RED (CTG)<sub>n</sub> arrays were detected in individuals K, R, N, S, and T (see Fig. 2).

schizophrenia was tested by nonparametric analysis but the test had little power, given the small size of the family analyzed. The association of the CTG expansion with schizophrenia was also tested by analyzing the kindred with a set of 11 polymorphic short tandem repeat markers scattered across 18q21, covering about 30 cM according to the CHLC sex-averaged map [CHLC, 1994]. Pairwise linkage analysis with schizophrenia was performed under a dominant model of inheritance, by taking into account three different phenotype definitions: 1) a very stringent "schizophrenia" definition (SZ1) (as by DSM-III R) [American Psychiatric Association, 1987], and 2) two broader "schizophrenia spectrum" definitions (SZ2, SZ3), corresponding to the application of progressively less stringent diagnostic criteria. Although in some instances a significant exclusionary lod score was obtained (e.g., D18S51, D18S41, and D18S487 with SZ1), the linkage analysis was inconclusive overall. Linkage analysis with phenotype definitions SZ2 and SZ3 also showed a similar trend. Since the STRPs used for analyzing the kindred were very informative (with a mean heterozygosity >70%), the inconclusive or very partially conclusive nature of the linkage analysis results can again be explained by the structure and the small size of the family analyzed and by the very complex nature of the schizophrenia phenotype.

The size of the (CTG)<sub>n</sub> array detected in patient K is extremely unusual. In our RED screening of 244 normal individuals, belonging to six different ethnic populations, we have not detected another (CTG)<sub>n</sub> repeat this large [Haaf et al., 1996; Sirugo et al., 1997]. We have recently been able to identify and isolate the genomic region from which this very long (CTG)<sub>n</sub> repeat originates and to determine that the (unique) unstable locus detected in the Danish kindred is distinct from the RED-1 CTG/CAG expansion mutation locus detected in CEPH family 1334 and mapped to 18q21-23 by Schalling et al. [1993] [Breschel et al., in press; Sirugo et al., in preparation]. The characterization of the genomic region containing this unstable CTG/CAG repeat array is in progress. While the unusual coincidence of two cousins with paranoid schizophrenia having an abnormally long (CTG)<sub>n</sub> array can

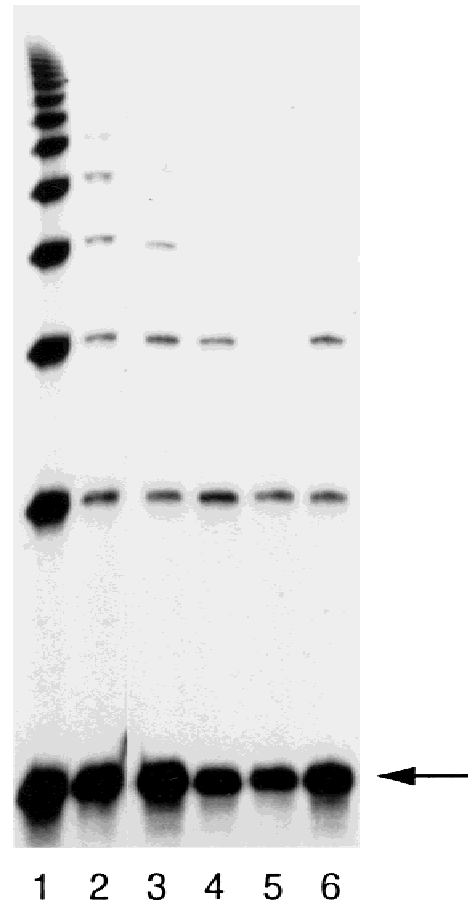


Fig. 2. RED of CTG repeat arrays detected in the Danish kindred. DNAs were all extracted from lymphoblastoid cell lines. Arrow indicates a 102-bp ligation product that corresponds to the ligation of two CTG<sub>17</sub> oligonucleotides. RED reactions were performed as described by Sirugo and Kidd [1995]. Long ladders of ligated CTG<sub>17</sub> products are evident in lanes 1 (individual K) and 2 (individual R). A ligation product from the genomic DNA of individual T is shown in lane 3. RED reactions on additional affected individuals from the same kindred are shown in lanes 4-6. These individuals (not represented in the pedigree in Fig. 1) were all proven to be negative for instability of the chromosome 18q21 CTG/CAG tandem repeat detected in individuals K, R, and T.

only be considered anecdotal, because of the small sample size of the kindred, it is not less significant than the partial trisomy of 5q identified by Bassett et al. [1988]. This (CTG)<sub>n</sub> expansion may have important phenotypic consequences and may partly determine and/or modulate the schizophrenia phenotype in the Danish kindred. For example, in individuals carrying the abnormally large arrays, there could be local alteration of the chromatin structure which could cause transcriptional suppression or gene(s) expression perturbation [Otten and Tapscott, 1995; Wang et al., 1994] at and around the expanded triplet repeat locus.

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