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A Triplet Repeat on 17q Accounts for Most Expansions Detected by the Repeat-Expansion–Detection Technique

To the Editor:

Eight hereditary neurogenerative disorders have been identified that result from expansions of CAG trinucleotide repeats (Gilles et al. 1997). Thus, there has been great incentive to develop techniques to efficiently screen for repeat sequences in specific patient populations. The repeat-expansion detection (RED) is a widely used technique that screens for trinucleotide expansions without a requirement of prior knowledge of the disease locus (Schalling et al. 1993; Zander et al. 1997). The method uses genomic human DNA as a template, specific-repeat oligonucleotide primers, and a thermostable ligase to generate oligomers of the primer. However, there are limitations to the technique. Non–disease-related expansions occur frequently in the population, which complicate the interpretation of disease-association studies. For a repeat to be detected by the RED, it must stand out in size, and smaller expansions causing disease can be missed. Also, once an expanded repeat is found, there is no information about chromosomal localization.

Other techniques for identifying trinucleotide expansions have recently been developed (Sanpei et al. 1996; Koob et al. 1998). The direct identification of repeat expansion and cloning technique (DIRECT) was designed to enable the localization and cloning of expanded-repeat regions (Sanpei et al. 1996). By means of the DIRECT technique, a novel, long, and unstable CAG/CTG trinucleotide repeat (Dir I) was identified and localized to chromosome 17q (Ikeuchi et al. 1998). This repeat is highly polymorphic, ranging in size from 10 to 92 repeat copies (30–276 bp) in normal individuals (Ikeuchi et al. 1998). Independently, a second group identified this expanded repeat by cloning the gene fragment from RED positive DNAs (Nakamoto et al. 1997).

DNA samples, obtained with informed consent from psychiatric patients with childhood onset of disease, were studied specifically to detect increased numbers of triplet repeats by use of the RED technique (Burgess et al. 1998). Since children and adolescents with psychiatric disorders appear to have functional brain abnormalities (McKenna et al. 1994), these patients are a valuable resource for such studies. Although expansions of trinucleotide repeats have been associated with several disorders affecting the brain and nervous system, their involvement in the etiology of psychiatric disorders has not been clearly demonstrated (Lindblad et al. 1995; Morris et al. 1995; O'Donovan et al. 1995). Since expanded repeats can be associated with genetic anticipation, patients with an early onset of disease are especially good candidates to evaluate.

The RED technique was used to identify trinucleotide expansions in 227 individuals, including 36 patients diagnosed with childhood-onset schizophrenia (COS); 21 diagnosed with atypical psychosis, termed by us as "multidimensionally impaired" (MDI) (Kumra et al. 1998); 46 patients with attention-deficit hyperactivity disorder (ADHD); 51 screened controls; and 73 relatives of probands. Patients were diagnosed according to standard *Diagnostic and Statistical Manual of Mental Disorders* definitions with standardized interviews as described elsewhere (Gordon et al. 1994; Castellanos et al. 1996). Diagnostic criteria for the MDI group has been discussed elsewhere (Kumra et al. 1998). The RED analysis was performed with a CTG_{10} oligonucleotide in the RED reaction, producing a repeat-size representation at 30-nucleotide intervals (Lindblad et al. 1996; Zander et al. 1997). The same samples were then analyzed for expansions of the polymorphic CAG/CTG Dir I trinucleotide repeat on chromosome 17q, by use of PCR conditions as described elsewhere (Ikeuchi et al. 1998; see fig. 1).

RED expansions of $\geqslant$180 nucleotides were detected in a total of 99 (44%) of the 227 individuals screened, with the distribution of RED scores shown in table 1. When diagnoses were evaluated separately, RED scores of $\geqslant$180 nucleotides were seen in 41% of COS patients ($n = 36$), 43% of MDI patients ($n = 21$), 43% of ADHD patients ($n = 24$), and 29% of the controls ($n = 51$) (fig. 2*A*).

In analyzing the CAG/CTG repeat on chromosome 17q, we scored the allele in each individual with the largest repeat size. A total of 81 (36%) of the 227 individuals screened had a chromosome 17q Dir I repeat expansion of >150 bp. Interestingly, 80 of the 81 individuals with a repeat size of >150 bp on chromosome 17q had RED scores of $\geqslant$180 nucleotides. Thus, the RED technique appeared to detect this expansion reliably. There was also a strong correlation between the size of the Dir I expansion and the size of the expansion detected by RED (table 1).

Dir I repeats in excess of 50 copies (150 bp) were

observed in 33% of 36 COS patients ($n = 12$), 35% of 46 ADHD patients ($n = 16$), 33% of 21 MDI patients ($n = 7$), and 27% of the 51 controls ($n = 14$). On the basis of these frequencies and P values (COS, $P < .568$; ADHD, $P < .428$; MDI, $P < .607$), it does not appear that expansions at this locus are specifically associated with any of these disease phenotypes.

Thus, expansions of Dir I appear to account for the majority of elevated RED scores observed in our population. However, in each diagnostic group and in the controls there were ~20% of individuals with increased triplet repeats detected by the RED who did not have Dir I expansions (fig. 2*A*). These individuals were screened for expansions of another known heritable expanding CTG repeat in SEF2-1, a gene encoding a transcriptional factor protein found on the human chromosome 18q21.1 (Breschel et al. 1997). A 1.6-kb clone (termed "CTG 18.1"), which consists of portions of SEF2-1, contains an intronic $(CTG)_{24}$ repeat, which is highly polymorphic but not associated with an obvious abnormal phenotype.

In the majority of patients with elevated RED scores without an expansion of Dir I, examination of the CTG18.1 locus revealed an expanded allele of ⩾150 bp. Patients with the expanded CTG18.1 allele generally had very high RED values (many were ⩾240 nucleotides). Removing these samples greatly improved the correlation of RED score with Dir I expansion size (figs. 2*B* and 2*C*), changing the adjusted R^2 value from 0.500 to 0.740.

Table 1

Correlation of Dir I Expansion Size with RED Score in Individuals with RED scores of ⩾180 Nucleotides

Dir I Repeat Size (bp)	RED Score (nucleotides) 180	210	240	270	300	Total
⩽150	2	7	5	4	1	19
151–180	20	1	1	0	0	22
181–210	14	23	3	0	1	41
211–240	0	6	10	0	0	16
>240	0	0	0	1	0	1
Total	36	37	19	5	2	99

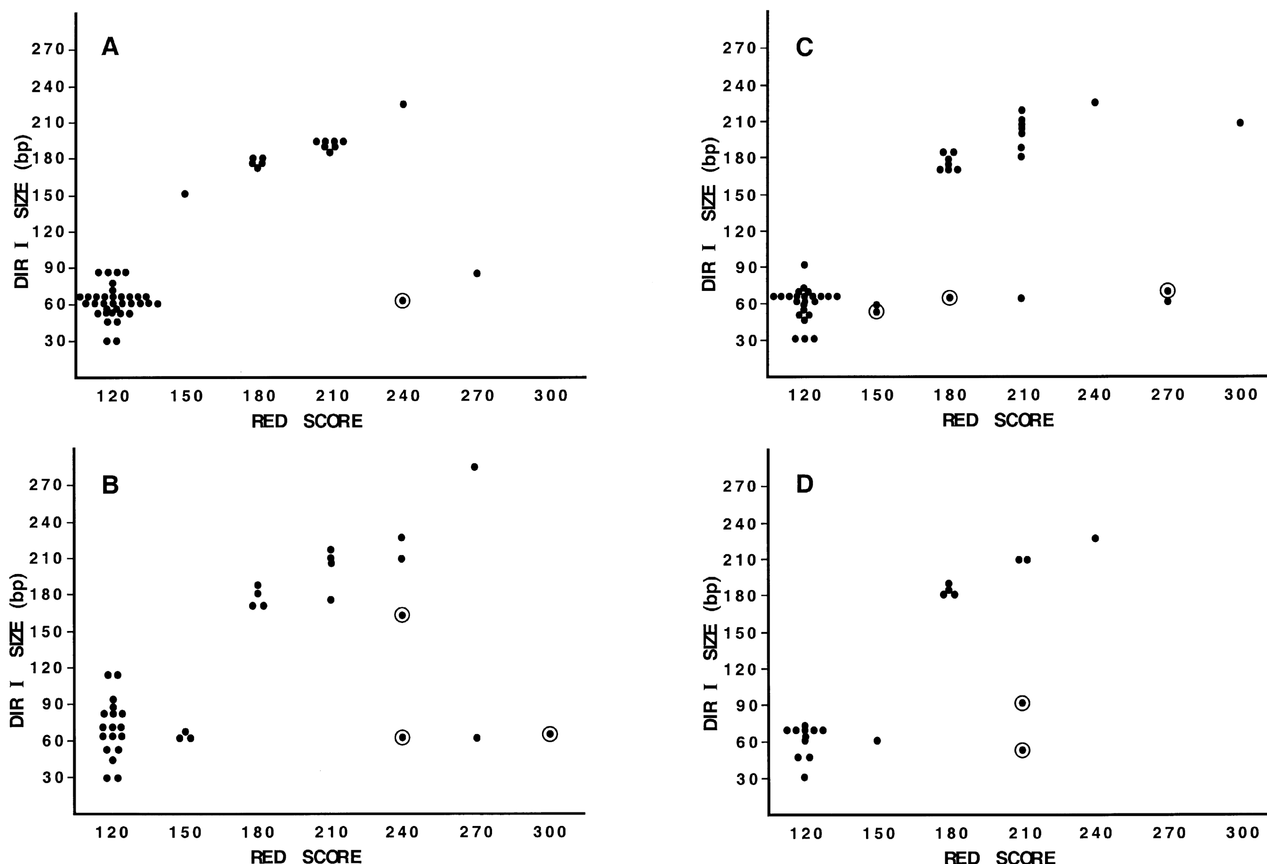

Figure 1 RED score (nucleotides) vs. Dir I size (bp): *A*, normal controls, *B*, patients with COS, *C*, patients with ADHD, and *D*, patients with MDI. Circled individuals were found to have expansions of CTG 18.1.

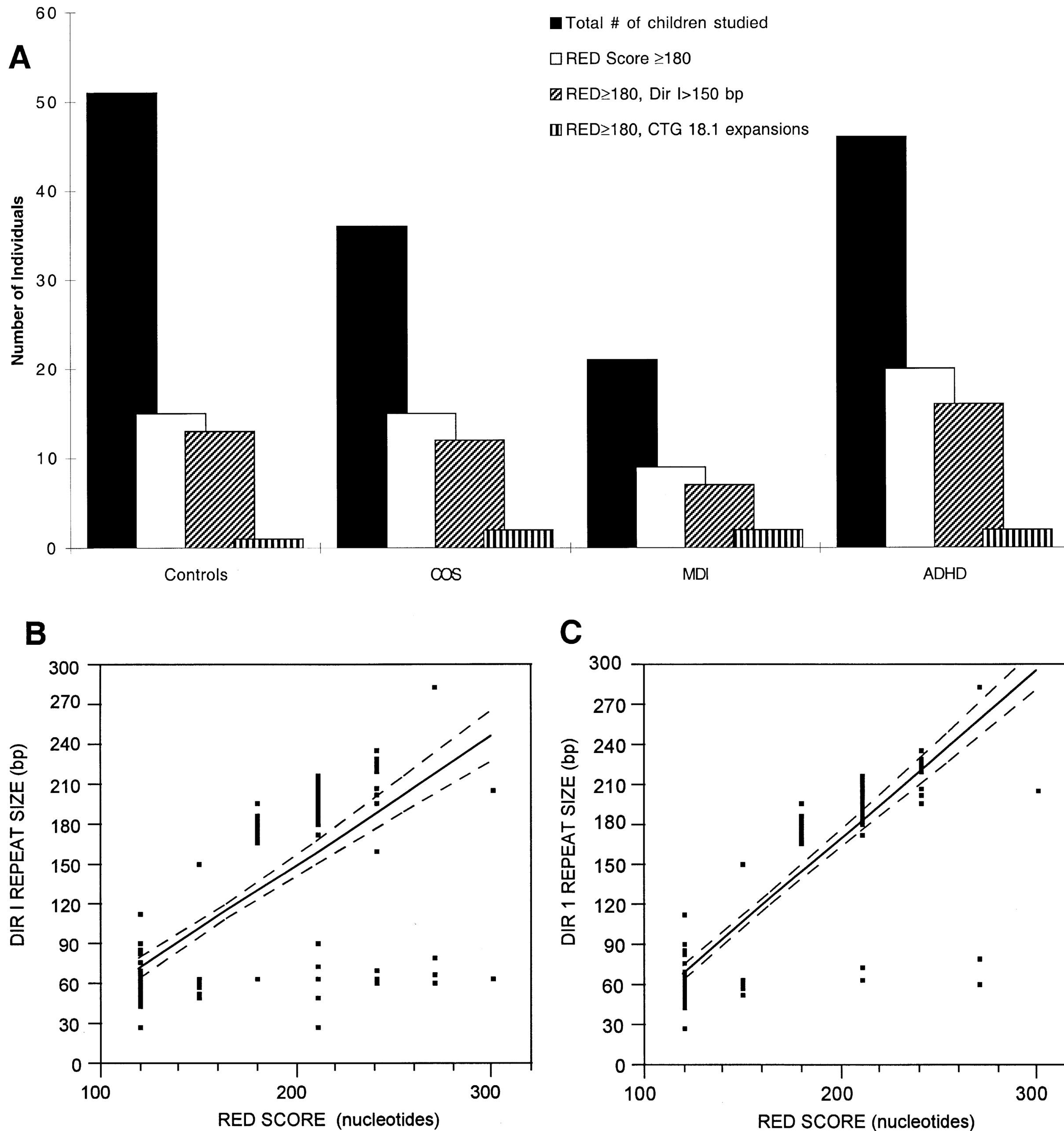

Figure 2 *A*, Distribution of elevated RED scores by diagnosis. *B*, Linear fit of RED scores plotted against Dir I repeat size: observations = 227, adjusted $R^2 = 0.500$, root mean square error = 15.62, analysis of variance $P \leqslant .001$. *C*, Linear fit when 15 individuals with expansions at CTG18.1 are removed: observations = 212, adjusted $R^2 = 0.740$, root mean square error = 11.42, analysis of variance $P \leqslant .001$.

This study demonstrates that up to 94% of trinucleotide-repeat expansions detected by RED can be accounted for by PCR analysis of two specific loci, Dir I and CTG18.1. The etiology of the remaining expansions requires further study. The combination of RED analysis with PCR of the Dir I and CTG18.1 loci may improve the ability to identify other disorders associated with repeat expansions. Clearly, although the RED technique reliably detects large trinucleotide repeats in the genome, many of the large repeats identified are not those related to illness.

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Paternal Isodisomy of Chromosome 7 Associated with Complete Situs Inversus and Immotile Cilia

To the Editor:

Uniparental disomy (UPD) refers to the inheritance of two homologous chromosomes from one parent, in a diploid individual. Heterodisomy is the inheritance of both parental homologues, whereas isodisomy implies the inheritance of two copies of a single parental homologue (Engel 1980). Uniparental inheritance of a human autosome in a cytogenetically normal individual was first recognized by Spence et al. (1988), on the basis