tude should be encouraged (cf. Forster 2003). However, we are still skeptical about the corrected results presented in figure 1, for some idiosyncrasies remain and others seem to have been newly introduced. For example, some sites (e.g., 8584, 14318 [YAN0591; C type] and 14783 [TYR0004; D type]), at which Silva et al. (2003 [in this issue]) have now corrected some of the entries in their original data table, still show back mutations. Homoplasy in the coding region is much less than in the control region and may have only a few hot spots (see, e.g., table 2 of Herrnstadt et al. [2002]); the reference to Eyre-Walker et al. (1999) is not really relevant, since those authors have taken quite problematic data at face value (Kivisild and Villems 2000). The recorded variation at 10400 remains highly suspicious. It is hard to believe that 10400 has actually mutated in two B types (KRC0033 and QUE1880) and one L2a type (NGR0522) and reverted in two C types (QTE1875 and YAN0650) and two D4 types (JAP1045 and GRC0131), because no single homoplasious change at this site has been observed in >900 coding-region sequences or fragments that cover site 10400 from Ingman et al. (2000), Maca-Meyer et al. (2001), Derbeneva et al. (2002), Herrnstadt et al. (2002), and Yao et al. (2002). Moreover, site 11177 is found in only 2 of 10 B4b mtDNAs of Silva et al., which contrasts to the co-occurrence of 11177 and 9950 in all 14 B4b mtDNAs of Herrnstadt et al. (2002). To thoroughly settle these anomalies, it is imperative that the authors take notice of the potential processes that might introduce errors, as listed in our letter (Yao et al. 2003 [in this issue]), especially sample crossover. We would encourage the authors to resequence some short fragments that cover the sites listed above.

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A Multicolor FISH Assay Does Not Detect DUP25 in Control Individuals or in Reported Positive Control Cells

To the Editor:

Gratacòs et al. (2001) reported recently that the co-occurrence of panic and phobic disorders with joint laxity was associated with an interstitial duplication of the chromosomal region 15q24-q26 (named "DUP25"). DUP25, which encompasses a region of the size of 17 Mb, was observed only as mosaicism in three different forms (designated as "direct telomeric," "inverted telomeric," and "centromeric"). In each reported case, cells with DUP25 represented the majority (>50%). In addition, DUP25 mosaicism was also observed in 7% of control individuals, indicating that it could represent a

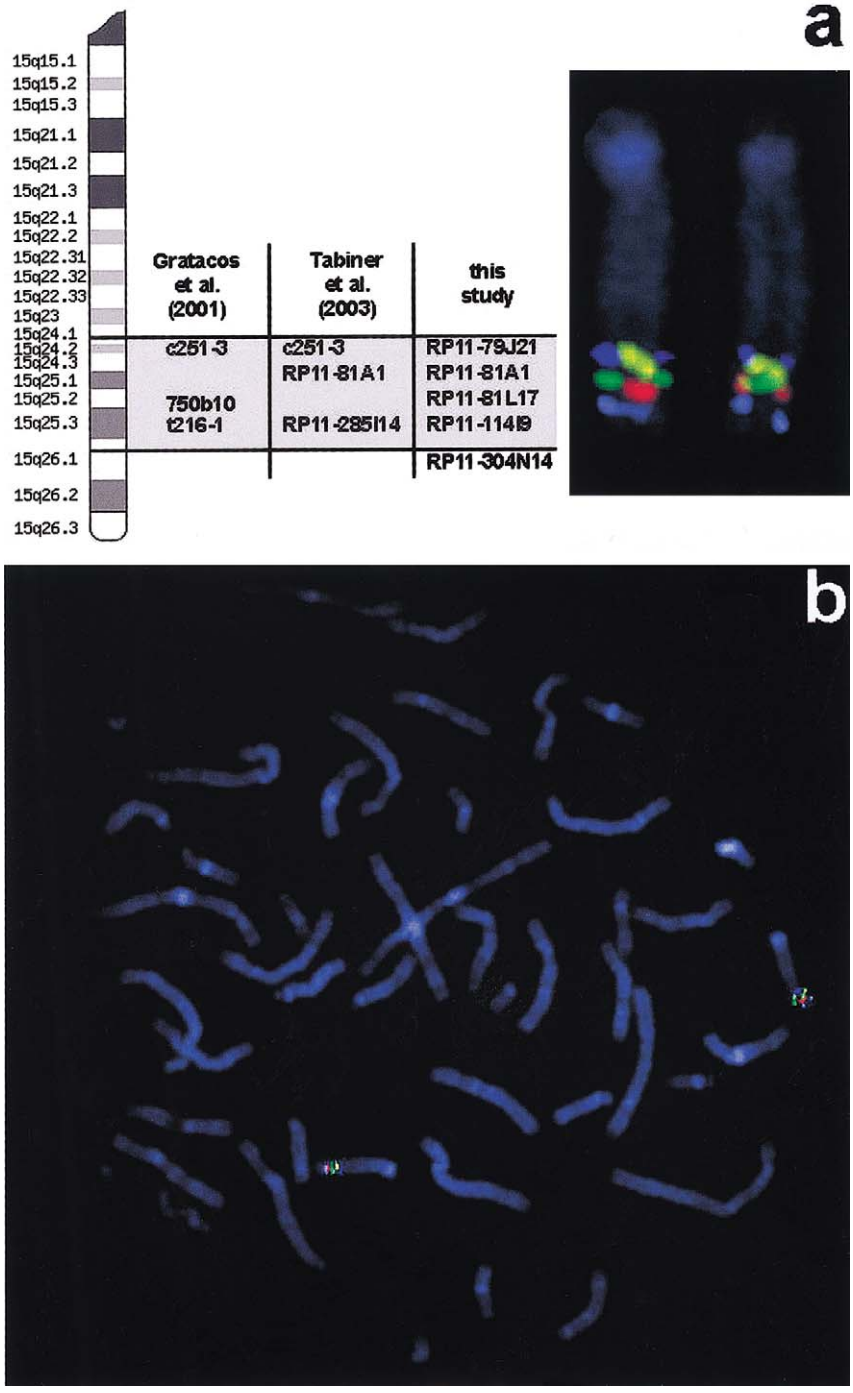

Figure 1 *a,* Ideogram of the distal part of the long arm of chromosome 15. The DUP25 region is gray shaded. For comparison, the probes used by Gratacòs et al. (2001), by Tabiner et al. (2003), and by us are shown. The FISH image shows a representative chromosome 15 pair with the normal sequence of signals (from proximal to distal): RP11-79J21 (*dark blue*), RP11-81A1 (*yellow*), RP11-81L17 (*green*), RP11-114I9 (*red*), RP11-304N14 (*light blue*). *b,* Representative metaphase spread of case B33. Ninety percent of cells were supposed to have a DUP25. In our analysis, all metaphase spreads showed a normal hybridization pattern, as depicted here.

common polymorphism. Another important implication was a proposed new, non-Mendelian mode of inheritance for DUP25.

Tabiner et al. (2003) reported in the *Journal* analyses of 40 control individuals, 16 patients with anxiety disorders, and three reported positive control cell lines. However, the authors could not find DUP25 in any sample, including the previously reported positive control cell lines. Consequently, Tabiner et al. (2003) demanded "that other groups should try to confirm or refute the presence of a polymorphic large mosaic duplication involving chromosome band 15q25 and to determine its association with anxiety disorder."

Here, we used a multicolor FISH assay to screen samples for DUP25. We assembled a FISH probe panel consisting of five different BAC probes, each labeled with a different color. The probe set includes four probes that, according to Gratacòs et al. (2001), map within DUP25 (RP11-79J21, RP11-81A1, RP11-81L17, and RP11-114I9) and one probe that is distal to DUP25 (RP11-304N14) (fig. 1*a*). Probe labeling and hybridization was done essentially according to protocols that we previously published in the *Journal* (Uhrig et al. 1999; Azofeifa et al. 2000). The cells were analyzed by use of single fluorescence filters for each fluorochrome. After image acquisition, the gray-scale images were pseudocolored and overlayed.

We analyzed slides from 70 randomly selected anonymous individuals from the southern part of Germany (Bavaria). If we assume that, indeed, 7% of the general population should have DUP25 mosaicism, the likelihood to find none among 70 randomly selected individuals is 6×10^{-3}. In each case, we evaluated at least 25 metaphase spreads, which should be sufficient, since DUP25 was reported to occur in an average of 59% of cells (Gratacòs et al. 2001). All control individuals showed a normal hybridization pattern for all probes (fig. 1*a*). We never observed duplicated probes or a change in the order of the hybridization signals.

In the next step, we requested positive control slides from the Barcelona laboratory for confirmation. Lluis Armengal kindly provided us with slides from an established cell line (P3) and a case, which was prepared directly from blood of a patient (B33). According to the analysis done in the Barcelona laboratory, DUP25 should have been present in up to 90% of cells in either case. However, our FISH assay could not identify any hint of duplication in all analyzed cells (fig. 1*b*). The slides were also evaluated by GTG-banding analysis. The average resolution was 450–500 bands/metaphase spread. As the draft human genome had been significantly improved since the publication of the Gratacòs et al. (2001) paper, we used the Ensembl Genome Browser (release 10.30.1, last updated on January 30, 2003) of the Sanger Institute for a precise and updated assessment of the DUP25 size. The distance between the genes *LOXL1* and *IQGAP1,* which corresponds to DUP25, is 16.81 Mb. Although a duplication of such a size present in 90% of cells should be detectable, experienced cytogeneticists did not find any evidence of any structural rearrangement in the respective region.

Our results indicate a difference in the frequency of DUP25 as reported by Gratacòs et al. (2001) and confirm the observations made by Tabiner et al. (2003). Most importantly, there are now two groups, which achieved different results on reported positive control cell lines as the Barcelona group.

It is difficult to explain the differences between the laboratories in scoring the positive controls. It is known that this chromosome 15 region is rich in low copy repeats (Pujana et al. 2001). Therefore, this region may be prone for structural rearrangements. However, although our multicolor FISH assay should have a high sensitivity (four clones map in DUP25), we did not find any evidence for an increased rate of structural rearrangements in the distal part of chromosome 15 in all analyzed metaphases. The differences cannot be explained by different hybridization protocols, since these are basically identical in all three laboratories (Nadal et al. 1997; Uhrig et al. 1999; Tabiner et al. 2003). Therefore, additional data by other groups should add to the clarification of the proposed causative role of large genomic duplications involving chromosome bands 15q24-q26 in panic and phobic disorders.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Ensembl Genome Browser, http://www.ensembl.org

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