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Failure to Find DUP25 in Patients with Anxiety Disorders, in Control Individuals, or in Previously Reported Positive Control Cell Lines

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Investigation of the co-occurrence of panic and phobic disorders with joint laxity led to the identification of various forms of interstitial duplications involving human chromosome 15q24-q26 (named "DUP25") in a Spanish population. DUP25 was observed in 68 of 70 (97%) patients assigned the diagnosis panic disorder/agoraphobia. DUP25 was also found in 14 of 189 (7%) control individuals. In the present study, we replicated the experimental conditions described by Gratacòs and colleagues in which fluorescence in situ hybridization was used to examine metaphase chromosomes of patients with panic disorder/social phobia and of control individuals from a southern region of the United Kingdom, the primary aim being to determine the prevalence of this chromosomal rearrangement in a geographically and ethnically distinct population. DUP25 was not observed in any of our 16 patients or 40 control samples or in three previously reported DUP25-positive control (Centre d'Etude du Polymorphisme Humain) cell lines, indicating a highly significant difference in the frequency of DUP25 between the study by Gratacòs and colleagues and the present investigation.

Introduction

Gratacòs et al. (2001), published observations on families in which anxiety disorders and joint laxity were segregating suggesting that a duplication of 15q24-q26 (which those authors called "DUP25") was associated with the disorder. The DUP25 occurred in mosaic form in virtually all patients with an anxiety disorder in the seven large families studied, and it occurred in three types: direct telomeric, inverted telomeric, and direct centromeric. In addition, 70 unrelated patients with an anxiety disorder were studied, and DUP25 was seen in no fewer than 68, whereas, among 189 control specimens, DUP25 was seen in only 14. The DUP25-positive cells were almost always in the majority, their average proportion among positive individuals being 59%. Furthermore, the overall size of the duplicated segment was calculated to be in the order of 14–17 Mb, thereby suggesting that it should be visible by conventional microscopy in good-quality metaphase spreads. The inherited mosaicism, together with the absence of segregation of 15q24-q26 markers with DUP25, led the authors to suggest that the mechanism of occurrence of DUP25 must be non-Mendelian.

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These observations are extremely interesting for two reasons: first, if substantiated in other populations of patients with anxiety disorders, the findings provide the first important association between a genetic change and a common psychiatric disorder; and, second, they describe a completely new type of genetic mutation—namely, a large mosaic duplication that occurs in three different forms, that must arise in mitosis, and that is not linked to neighboring loci.

We set out to repeat Gratacòs's study on a geographically distinct population. We studied 40 control individuals and 16 patients with anxiety disorders, (according to the criteria of the Diagnostic and Statistical Manual, 4th edition [DSM-IV] [American Psychiatric Association 1994]) (table 1). We also thought it was important to have a positive control, and Professor X. Estivill kindly provided information on three DUP25-positive control individuals who were studied in the Barcelona laboratory. We obtained the cell lines corresponding to the three members of CEPH families (Corriell Cell Repositories). The positive control cell lines were selected because each represented a different form of the duplication.

Material and Methods

Patient and Control Populations

The patients consisted of a cohort of 16 unrelated individuals that included 9 women and 7 men, with ages ranging from 21 to 65 years of age. All patients were

Table 1

Psychiatric Disorders in the Study Population

Patient	Beighton Hypermobility Score	DSM-IV Diagnosis
1	3	Panic disorder, agoraphobia, social phobia
2	5	Panic disorder, social phobia
3	1	Social phobia
4	1	Social phobia, agoraphobia
5	0	Panic disorder, major depression, agoraphobia
6	0	Panic disorder, agoraphobia, social phobia, general anxiety disorder
7	0	Panic disorder, agoraphobia, social phobia
8	0	Major depressive episodes, agoraphobia
9	0	Panic disorder, general anxiety disorder
10	0	Major depression, panic disorder
11	6	Panic disorder, general anxiety disorder, major depression, panic attacks, general anxiety disorder
12	0	Major depression, panic disorder, agoraphobia
13	5	Depression, panic disorder, agoraphobia
14	3	Depression, social phobia, panic disorder
15	3	Alcohol abuse, depression, agoraphobia, general anxiety disorder
16	3	Major depression, general anxiety disorder, panic disorder, agoraphobia

currently attending the outpatient clinic at the Psychiatric Department of the Royal South Hants Hospital and had a DSM-IV diagnosis of either panic disorder or social anxiety. The diagnoses were confirmed using the Mini International Neuropsychiatric Interview (Sheehan et al. 1998) and either the Liebowitz Social Anxiety Scale (Liebowitz 1987) or the Hamilton Rating Scale for Anxiety (Hamilton 1959). At interview, each patient underwent the Beighton hypermobility test (results ranged from 0 to 6) (Beighton 1993) (table 1) and gave a sample of blood for karyotyping and FISH testing. All patients gave written informed consent to participate in the study, and the protocol was approved by the Southampton and South West Hants Local Research Ethics Committee. The control population consisted of 40 anonymized blood samples from unrelated adults referred for cytogenetic analysis because of recurrent miscarriages and were reported to have a normal karyotype. Three cell lines reported to be positive for the DUP25 (Gratacòs et al. 2001) were obtained from the Corriell Cell Repositories. These were derived from unrelated samples of the CEPH families.

Conventional Cytogenetic and FISH Slide Preparation

Conventional cytogenetic analyses were performed using standard procedures, slides for conventional cytogenetic analysis were GTG-banded using standard methods, and high-resolution cytogenetic analysis was reported at a minimum 550-band resolution.

Selection of FISH Probes

The probes selected for FISH were as described by Gratacòs et al. (2001) (i.e., cosmids c251-3 and t216-1, located at the centromeric and telomeric ends of the DUP25 region, respectively; fig. 1). However, because t216-1 was not available until late in the study, only c251-3 was used in combination with the pTRA-25 alphoid repeat probe for the chromosome 15 centromere (D15Z3) (Choo et al 1990); the latter was to facilitate the rapid localization of the 15 homologues in the metaphase spreads. In addition, BACs were chosen (Ensembl Genome Browser) at the centromeric end of DUP25, RP11-81A1 at 15q25.1 (GenBank accession number AC015871.4; NT_010356), which maps to D15S989/AFM333zh1 and contains the *BCL2A1* gene, and, at the telomeric end of DUP25, RP11-285I14 at 15q25.3 (GenBank accession number AC011966.7; NT_024670), which contains WI-4034 and part of the *NTRK3* gene. Furthermore, BAC RP11-285I14 is in the same location as cosmid t216-1 (fig. 1).

FISH

Slides for the FISH experiments were prepared as described elsewhere (Crolla et al. 1997). DNA samples extracted and purified from cosmid, BAC probes were labeled using a standard nick-translation reaction, and the labeled probe was coprecipitated with Cot-1 DNA prior to resuspension in a hybridization buffer containing five parts deionized formamide plus 20% dextran sulfate, two parts 10 × SSCP, and three parts dH_2O. Targets and probe DNAs were denatured simultaneously on a hot plate for 5 min at 65°C and were left overnight at 37°C in a humid atmosphere. After two 5-min stringent washes at 0.4 × SSC/0.1% Tween 20 at 75°C, followed by 2 min in 2 × SSC at room temperature, the indirectly labeled probes were detected with fluorophores (antidigoxigenin-TRITC [tetramethylrhodamine isothiocyanate]) (Roche) or avidin–fluorescein isothiocyanate (Vector Laboratories). The posthybridization

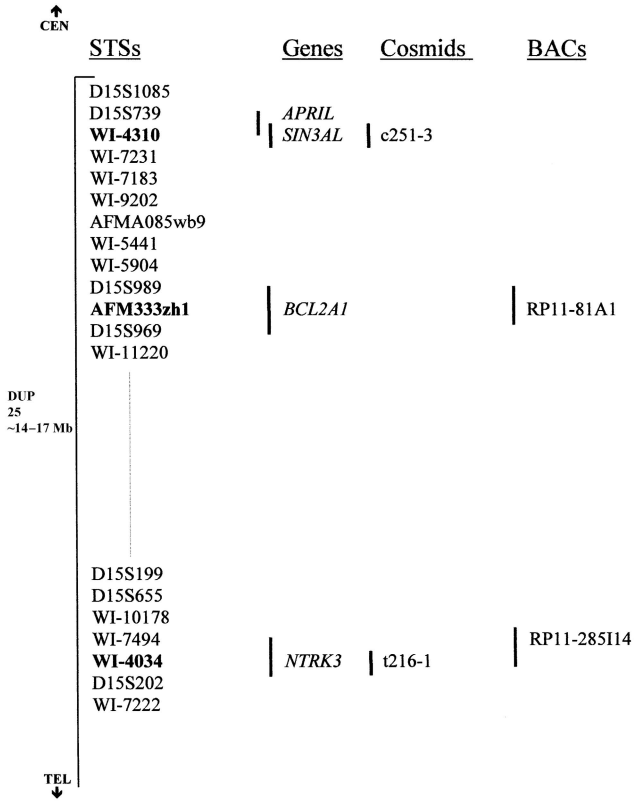

Figure 1 Physical map of the 15q24-q26 (DUP25) region showing STSs and genes surrounding the BACs and cosmids used as FISH probes (Gratacòs et al. 2001). The centromeric (CEN) and telomeric (TEL) ends are indicated, as is the estimated size of the region.

stringency conditions used in the present study are therefore equivalent to those previously reported by Gratacòs et al. (2001 and references therein). Slides were mounted in Vectashield (Vector Laboratories) antifade solution containing 4′,6-diamino-2-phenylindole. Cells were examined using a Zeiss epifluorescence microscope fitted with an 8300 Chroma filter set, and images were captured using MacProbe Applied Imaging software.

Results and Discussion

Each patient sample was tested with two sets of FISH probes: (1) RP11-81A1 (biotin) at the centromeric end of DUP25 and RP11-285I14 (digoxigenin) at the telomeric end; and (2) c251-3 (digoxigenin) at the centromeric end of DUP25 and pTRA25 (biotin). pTRA25 (D15Z3) is an alphoid centromere repeat probe that was used as an internal FISH control, primarily to facilitate rapid identification of the 15 homologues within the metaphase spreads.

A minimum of 40 metaphase spreads were scored on each sample independently by two observers, one of whom (J.A.C.) is extremely experienced in all aspects of FISH. There was no evidence of a duplication of signals in distal 15q that would be indicative of DUP25 in any of the 16 patient samples or in any of the 40 control samples. Because Gratacòs et al. (2001) reported an average of 59% of examined cells to be DUP25 positive, we felt it was unnecessary to extend our analyses to >40 metaphase spreads from each of the patient and control samples. The absence of any DUP25-positive patients is significantly different from the results obtained by Gratacòs et al. (2001) ($P = 1.58 \times 10^{-15}$; Fisher's exact test), whereas the result on the control sample ($P = .062$; Fisher's exact test) is not significantly different from that of Gratacòs et al. (2001) but does suggest that DUP25, if it occurs, does so with a different frequency in the two control populations.

The three CEPH cell lines obtained as positive controls for the present study were tested with both sets of probes. Fifty metaphase spreads were scored for each set of probes (30 by M.T. and 20 by J.A.C.), and no evidence of DUP25 was observed. Because it seemed possible that the duplication might have been selected against during culture, suspensions of the cell lines in fixative were sent to the Barcelona laboratory for confirmation of our findings. Surprisingly, L. Armengal and his colleagues, who scored 22–30 metaphase spreads on each of our cell line suspensions, reported 40%, 15%–20%, and 45% respectively to be positive for DUP25.

It is difficult to think of any logical scientific or technical explanation for the differences between the two laboratories in scoring the positive control cultures. However, we were unable to detect any DUP25-positive cells, either in the positive control samples from CEPH or in our patient or control samples. Furthermore, we have never had a report of such a duplication in any of the thousands of diagnostic samples that have been scored on high-resolution chromosomes in our laboratory. These results make it important that other groups try to confirm or refute the presence of a polymorphic large mosaic duplication involving chromosome band 15q25 and to determine its association with anxiety disorders.

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Electronic-Database Information

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

Corriell Cell Repositories, http://locus.umdnj.edu/ (for positive control CEPH cell lines)

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

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for BAC probes RP11-81A1 [accession number AC015871.4] and RP11-285I14 [accession number AC011966.7])

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