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Report

Tightly Clustered 11q23 and 22q11 Breakpoints Permit PCR-Based Detection of the Recurrent Constitutional t(11;22)

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Palindromic AT-rich repeats (PATRRs) on chromosomes 11q23 and 22q11 at the constitutional t(11;22) breakpoint are predicted to induce genomic instability, which mediates the translocation. A PCR-based translocation-detection system for the t(11;22) has been developed with PCR primers flanking the PATRRs of both chromosomes, to examine the involvement of the PATRRs in the recurrent rearrangement. Forty unrelated carriers of the t(11;22) balanced translocation, plus two additional, independent cases with the supernumerary-der(22) syndrome, were analyzed to compare their translocation breakpoints. Similar translocation-specific junction fragments were obtained from both derivative chromosomes in all 40 carriers of the t(11;22) balanced translocation and from the der(22) in both of the offspring with unbalanced supernumerary-der(22) syndrome, suggesting that the breakpoints in all cases localize within these PATRRs and that the translocation is generated by a similar mechanism. This PCR strategy provides a convenient technique for rapid diagnosis of the translocation, indicating its utility for prenatal and preimplantation diagnosis in families including carriers of the balanced translocation.

The t(11;22)(q23;q11) is the only known recurrent, non-Robertsonian constitutional translocation in humans. Carriers of this balanced translocation usually have no clinical symptoms and are often identified after the birth of offspring with an unbalanced form of the translocation, the supernumerary-der(22)t(11;22) syndrome. Patients with the supernumerary-der(22) syndrome have a distinctive phenotype, which consists of severe mental retardation, preauricular tag or sinus, ear anomaly, cleft or high-arched palate, micrognathia, heart defects, and genital abnormalities in the male (Fraccaro et al. 1980; Zackai and Emanuel 1980). This syndrome most often arises through 3:1 meiotic malsegregation of the balanced translocation in meiosis (Shaikh et al. 1999), and clustered breakpoints have been reported in numerous unrelated families (Edelmann et al. 1999; Shaikh et al. 1999).

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In our previous study, the der(11) and der(22) junction fragments of one carrier of the t(11;22) balanced translocation were cloned, which demonstrated that the breakpoints localize within palindromic AT-rich repeats (PATRRs) on both chromosome 11 and chromosome 22 (Kurahashi et al. 2000). Identification of the sequence of both junction fragments permitted the generation of translocation-specific PCR products from both the der(11) and the der(22) in this individual. Analysis of the junction fragments of two additional, unrelated carriers suggested similar breakpoints. These findings provided the impetus to develop a PCR approach for diagnostic and analytic purposes. Initially, we designed primer pairs flanking the PATRRs both on chromosome 11 and on chromosome 22, to amplify the junction fragments of the der(11) and the der(22). However, because the breakpoint regions are extremely AT rich, they were difficult to amplify without generation of nonspecific products from other chromosomal regions. Thus, internal PCR primers were designed, and a nested-PCR method was applied to produce t(11;22) translocation–specific products (fig. 1). With the primers and conditions shown in table 1, junction fragments from the der(11) and from the der(22) could be amplified from a carrier of the t(11;22) (fig. 2, sample 4 [case 16 in table

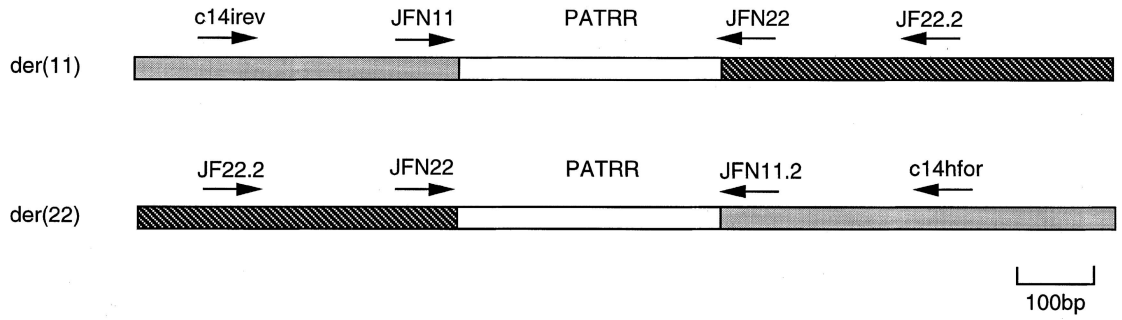

Figure 1 PCR primers used in analysis of the t(11;22) breakpoint. The white portion of the sequences denotes the PATRR, which includes the breakpoint region; gray portions denote chromosome 11; and hatched portions denote chromosome 22. PCR primers are indicated, with their orientation shown by arrows.

2]). Family members with the same translocation produce the same PCR products (data not shown), whereas family members with a normal karyotype do not yield PCR products (fig. 2, samples 1–3 and 5). The proband who carries the supernumerary der(22) in addition to normal chromosomes 11 and 22 yields only the der(22) junction fragment (fig. 2, sample 6). These results suggested that this nested-PCR approach was t(11;22)-breakpoint specific.

With this PCR approach, analysis of the breakpoints of 40 unrelated carriers of the t(11;22) balanced translocation, as well as of 2 additional cases, who had the supernumerary-der(22) syndrome, was performed. Some characteristics associated with these cases are summarized in table 2. All carriers had been previously diagnosed, by conventional cytogenetic studies, as having the t(11;22)(q23;q11). All 40 carriers of the balanced translocation yielded translocation-specific PCR products from both the der(11) and the der(22), whereas the 2 cases with supernumerary-der(22) syndrome produced PCR products derived from the der(22) only. The generation of PCR products from all individuals suggested that all of the t(11;22) breakpoints are located within the same PATRRs on both chromosome 11 and chromosome 22. In addition, all cases resulted in amplification of PCR products of similar size, with one exception. Case 23 yielded different-sized PCR products such that the product from the der(11) was larger whereas that from the der(22) was smaller. Sequence analysis of this individual's PCR products demonstrated a breakpoint within the same PATRRs but at a location different from that of the others. For the remaining 41 cases, the sequences of the PCR products were almost identical with regard to the breakpoint regions of the derivative chromosomes, suggesting that the breakpoints on both 11q23 and 22q11 are quite similar in their location. However, it should be noted that only the der(22) junction fragment was analyzed in the two cases of supernumerary-der(22) syndrome.

The similarity between the breakpoints of multiple independent families of varied ethnic and racial backgrounds is striking. The majority of the families are white, whereas two are Hispanic (cases 14 and 31), one is Lebanese (case 17), one is African-American (case 32), and another is Palestinian (case 41). Furthermore, our samples included four de novo carriers (cases 8, 16, 39, and 40) and two families (cases 1 and 23) in which there is no +der(22) proband whose breakpoints are all alike. We also utilized this PCR approach to confirm a diagnosis of supernumerary-der(22) syndrome in an individual with 47,XY,+mar (case 42) whose condition had been suspected on the basis of clinical features. In this case, both parents were karyotypically normal, with confirmed paternity, suggesting the occurrence of both the de novo translocation and 3:1 malsegregation events in the same meiosis. Taken together, the similarity of breakpoints in cases of varied ethnicity and de novo rearrangement imply that breakpoint clustering is the result of a common chromosomal breakage mechanism, rather than being due to either founder effect or identity by descent. In our previous work, both the der(11) and the der(22) junction fragments of a t(11;22) have been analyzed (Kurahashi et al. 2000). We identified PATRRs located at the breakpoint regions on 11q23 and 22q11. The data presented here, which demonstrate that all of the translocation breakpoints are within these PATRRs, support the hypothesis that numerous t(11;22) constitutional translocations arise by the same mechanism. We have previously suggested that the palindromic sequences flanking the breakpoint on both 11q23 and 22q11 might facilitate the formation of hairpin or cruciform structures. Furthermore, AT-rich sequences have a lower melting temperature, which permits hairpin structures to form at physiological temperatures. In the presence of these structures, hairpin-nicking activity is likely to create double-stranded breaks on both chromosomes (Akgun et al. 1997). Illegitimate reciprocal

Table 1

Primers Used for PCR Amplification of Junction Fragments

	der(11) PRIMERS		der(22) PRIMERS	
PCR	Name	Sequence	Name	Sequence
First[a]	c14irev	5′-GGAAGTTAGAGAAAACTGAGAA-3′	c14hfor	5′-AACACTCCCACTGACAGCTA-3′
	JF22.2	5′-CCTCCAACGGATCCATACT-3′[b]	JF22.2	5′-CCTCCAACGGATCCATACT-3′[b]
Second[c]	JFN11	5′-CAGAAAGGGAGAGCATGTAG-3′	JFN11.2	5′-GGTTGAAGAATCTTGGCTGG-3′
	JFN22	5′-CGTTGAAGGATGCAGGATGT-3′	JFN22	5′-CGTTGAAGGATGCAGGATGT-3′

[a] Conditions are as follows: 5 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, followed by 35 cycles of 94°C for 30 s, 56°C for 30 s, and 72°C for 1 min.

[b] Corresponds to primer "c" in the study by Kehrer-Sawatzki et al. (1997).

[c] Conditions are as follows: 35 cycles of 94°C for 15 s, 58°C for 15 s, and 72°C for 30 s.

exchange between such disrupted chromosomes 11 and 22 would lead to the formation of a translocation between them.

Despite the striking similarity of these 42 cases, comparisons of the sequence of their junction fragments revealed some slight differences between them. It is not yet clear whether this reflects a subtle difference in the individual breakpoints or some level of polymorphism within the AT-rich repeats. As has been reported elsewhere (Kurahashi et al. 2000), the exact location of the breakpoints at the nucleotide level cannot, as yet, be determined. One reason is that, on both chromosome 11 and chromosome 22, there are numerous ATs within the PATRRs, which makes them resemble one another. Furthermore, despite numerous attempts, there is still a gap in the sequence of this region from the normal chromosome 22 (Dunham et al. 1999; Kurahashi et al. 2000; Shaikh et al. 2000).

Five carriers of the balanced t(11;22) who had an abnormal phenotype (cases 36–40) were identified among the study subjects. One of them (case 38) has mental retardation, lactic acidosis, and autism; another has holoprosencephaly (case 36). The remaining three cases presented with multiple congenital anomalies, of which two are carriers of a de novo balanced translocation (cases 39 and 40). PCR analysis showed that the breakpoints for all five cases localize within the same PATRR, on chromosomes 11 and 22. The breakpoints on chromosome 22q11 are located within LCR-B (low copy repeat B) (Shaikh et al. 2000), one of several large duplications or chromosome 22–specific LCRs (Edelmann et al. 1999; Shaikh et al. 2000). Although the *NF1L* components of LCR-A, LCR-B, and LCR-D are very AT rich, only LCR-B contains a PATRR. Furthermore, the chromosome 22–specific LCRs are composed primarily of pseudogenes and interspersed repeat components and appear to contain few, if any, functional genes (Shaikh et al. 2000). Analysis of the sequence of the breakpoint-spanning bacterial artificial chromosome on 11q23 (i.e., b442e11) identified no genes disrupted by the translocation (Kurahashi et al. 2000), lending further support to the hypothesis that the abnormal phenotype in these five carriers of the translocation is not directly attributable to a variant location of the translocation. In these five individuals, an unidentified gene defect may coexist with the translocation, or some epigenetic factor may be responsible for the abnormal phenotype. Alternatively, low-level somatic mosaicism for +der(22) or −der(22) might be possible in these cases.

In a previous study of families with the t(11;22), the incidence of breast cancer was noted to be significantly higher among carriers of the balanced translocation, suggesting a predisposition to breast cancer (Lindblom et al. 1994). Furthermore, a putative breast cancer gene has been mapped to 11q23, by loss-of-heterozygosity analysis (Carter et al. 1994; Hampton et al. 1994). These findings have led to the suggestion that a putative breast cancer gene could be disrupted by or located in the vicinity of the t(11;22) breakpoint on 11q23. In the present study, one of the carriers of the t(11;22) (case 2) had bilateral breast cancer, diagnosed at 39 and 59 years of age. PCR analysis demonstrated that her translocation breakpoint is located within the same PATRRs, suggesting that a gene at the t(11;22) breakpoint is not responsible for the breast cancer in this individual. This does not eliminate the possibility of either a "position effect" generated by the translocation or linkage to a gene in the vicinity of the translocation, but it should be noted that numerous carriers of the same translocation, in the families that we have previously studied (Zackai and Emanuel 1980; E.H.Z. and B.S.E., unpublished results), are not similarly affected. An alternative explanation for the breast cancer association noted in some carriers of the t(11;22) might be related to the potential for the small der(22) chromosome to be lost by nondisjunction during mitosis. A breast cancer–susceptibility gene (tumor-suppressor locus) either on proximal 22q or on distal 11q could be functionally inactivated by somatic loss of the der(22) in breast tissue, accompanied by a mutation of the gene on the normal homologue. Thus, loss of function of the gene product

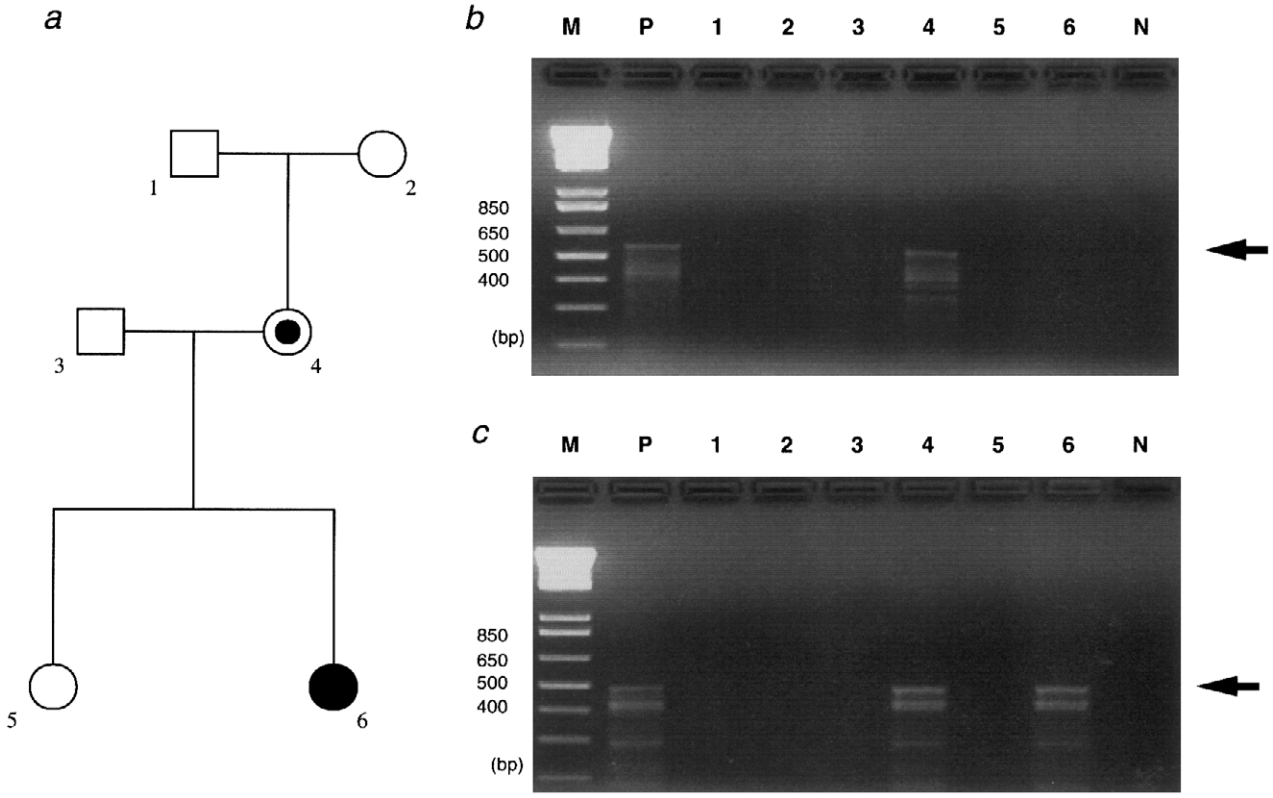

Figure 2 PCR results from one t(11;22) family. *a,* Pedigree of t(11;22) family. The blackened circle denotes the proband, who has the supernumerary-der(22) syndrome, and the circle with a black dot denotes the mother who is a carrier of the balanced translocation. *b,* PCR analysis for der(11). *c,* PCR analysis for der(22). Lanes M show 1-kb plus DNA ladder (GIBCO BRL); lanes P show results for the carrier of the t(11;22) balanced translocation (case 1), who serves as a positive control; the numbered lanes show results for DNA from individuals with the corresponding ID number in the pedigree Size markers (in bp) are indicated. The largest band is the authentic PCR product (*arrows*). The ladder is an artifact that originates from "stutter bands," on amplification of the AT-rich repeat.

could lead to tumor formation. Additional studies of breast cancer specimens from carriers of the t(11;22) that utilize either this PCR assay or a FISH approach to detect loss of the der(22) chromosome in tumor cells might be warranted.

These data highlight the utility of PCR for detection of the 11;22 translocation. The advantages of this PCR assay are that (*a*) it is rapid, (*b*) only a small quantity of DNA is required for screening, and (*c*) the DNA can be acquired by minimally invasive methods. Thus, DNA isolated from buccal swabs can be used to assess carrier or proband status. Since all of the t(11;22) cases studied yielded translocation-specific PCR products that were absent from normal individuals, the specificity of this assay is high. For example, despite the fact that his parents were cytogenetically normal, case 42 was confirmed, by the PCR assay, as being a case of de novo supernumerary der(22). Furthermore, the sensitivity for breakpoint detection should also be quite high, allowing for detection of mosaicism. In this study, one individual is a mosaic for the t(11;22) (case 7). Although the percentage of t(11;22) cells in peripheral blood lymphocytes in this case is 80%, which should be easily detected by standard cytogenetic evaluation, very-low-level mosaicism in other instances might be missed by routine cytogenetic diagnosis. Thus, it is possible that either parent of case 42 might be a low-level t(11;22) mosaic that routine cytogenetic analysis failed to detect. The method that this study provides for detection of low-level mosaicism will allow more-accurate assignment of recurrence risk in such cases.

In families segregating the translocation, pregnancies can now be monitored earlier and more expeditiously, to detect the supernumerary der(22) or the t(11;22) by PCR analysis of uncultured amniotic fluid or villi. However, since most families are ascertained after 3:1 meiotic segregation of the +der(22) from female carriers of the t(11;22) (table 2), caution must be exercised, to avoid maternal-cell contamination in such cases. Furthermore, we recommend confirmation on cultured amniocytes or villi. This PCR approach could also be applied as a more sensitive method for preimplantation genetic diagnosis

Table 2

Summary of 42 Cases of t(11;22)

Case	Patient ID	Race	Associated Finding(s)
Carriers of balanced translocation:			
Unaffected:			
1[a]	CH99-200	White	No +der(22) in pedigree
2	CH97-82	White	+der(22) in twins; bilateral breast cancer
3[a]	CH97-62	White	
4	CH92-224L	White	
5	CH95-257	White	
6	CH94-188L	White	
7	P91-007F	White	Mosaic for t(11;22), 46,XX(20%)/46,XX,t(11;22)(80%)
8[b]	CH97-78	White	Carrier of de novo t(11;22)
9[a]	CH97-120	White	
10	CH96-180	White	
11	CH97-03	White	
12[c]	CH97-181	White	
13[d]	CH98-40	White	
14	CH98-120	Hispanic	
15	CH98-169	White	
16	CH97-48	White	Carrier of de novo t(11;22)
17	CH91-221L	Lebanese	
18	P89-43	White	Aunt of +der(22) meiosis II nondisjunction
19	CH96-123	White	
20	CH00-09	White	
21[d]	CH00-32	White	
22	CH91-258L	White	
23	CH93-075L	White	No +der(22) in pedigree
24	P90-34L	White	
25	CH2227	White	
26	D00-28	White	
27	CH98-229	White	
28	CH94-037L	White	
29	CH2217	White	
30	CH91-182L	White	
31	CH92-360L	Hispanic	
32[e]	GM03372	African American	
33[e]	GM04403	White	
34[e]	GM06229	White	
35	IARC322	White	
Abnormal phenotype:			
36	CH91-150F	White	Carrier of balanced translocation who had holoprosencephaly
37	CH97-33	White	Carrier of balanced translocation who had an abnormal phenotype
38	P91-015L	White	Carrier of balanced translocation who had mental retardation, lactic acidosis, and autism
39	D97-309	White	Carrier of de novo translocation who had an abnormal phenotype
40	D98-78	White	Carrier of de novo translocation who had an abnormal phenotype
Supernumerary-der(22) syndrome:			
41[d]	CH93-166L	Palestinian	
42	D9-61	White	De novo +der(22)

[a] Reported in our previous work (Kurahashi et al. 2000).
[b] Previously reported by Zackai and Emanuel (1980), as "family C."
[c] Previously reported by Zackai and Emanuel (1980), as "family B."
[d] +der(22) transmitted by a father who was a carrier.
[e] Obtained from Coriell Cell Repositories (Camden, NJ).

(PGD) during in vitro fertilization (Fung et al. 1999). We recommend blastomere biopsy on an eight-cell embryo derived from a parent who is a carrier of the t(11;22). Since we have identified one individual with supernumerary-der(22) syndrome who had a karyotype of 47,XY,t(11;22)(q23;q11),+der(22)mat (the index case for the unbalanced translocation in family 18) caused by nondisjunction during meiosis II, we recommend that biopsy of the first polar body be avoided for PGD in t(11;22) diagnosis. Furthermore, it is important that FISH or quantitative PCR be undertaken, to confirm the presence of only a single der(22) when both derivative chromosomes are detected prenatally by PCR. In conclusion, this PCR assay provides a cost-effective

method to rapidly detect translocation-specific junction fragments derived from either the der(11) or the der(22). PCR analysis can provide an alternative to conventional cytogenetics, as a rapid diagnostic procedure to detect carriers of the t(11;22) as well as to diagnose the supernumerary-der(22) syndrome in affected individuals.

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Electronic-Database Information

The URL for data in this article is as follows:

Unbalanced 11/22 Translocation, http://www.nt.net/~a815/index.html

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