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Searching for Genes for Cleft Lip and/or Palate Based on Breakpoint Analysis of a Balanced Translocation t(9;17)(q32;q12)

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Abstract

Objective—Identification of the breakpoints of disease-associated chromosome rearrangements can provide informative clues to a positional cloning approach for genes responsible for inherited diseases. Recently, we found a three-generation Japanese family segregating balanced chromosome translocation t(9;17)(q32;q12). One of the subjects had cleft lip and palate. We examined whether regions near the breakpoint could be associated with cleft lip and/or palate.

Methods—We determined the breakpoints involved in the translocation by fluorescence *in situ* hybridization analysis and subsequent long-range polymerase chain reaction. In order to study the role of these disrupted regions in nonsyndromic cleft lip and/or palate, we performed mutation analysis and a haplotype-based transmission disequilibrium test using tagging single-nucleotide polymorphisms in the flanking regions of the breakpoints in white and Filipino nonsyndromic cleft lip and/or palate populations.

Results—Sequence analysis demonstrated that two genes, *SLC31A1* (solute carrier family 31 member 1) on chromosome 9 and *CCL2* (chemokine ligand 2) on chromosome 17, were rearranged with the breaks occurring within their introns. It is interesting that *SLC31A1* lies closed to *BSPRY* (B-box and SPRY domain), which is a candidate for involvement with cleft lip and/or palate. Some of the variants in *BSPRY* and *CCL2* showed significant *p* values in the cleft lip and/or palate population compared with the control population. There was also statistically significant evidence of transmission distortion for haplotypes on both chromosomes 9 and 17.

Conclusions—The data support previous reports that genes on chromosomal regions of 9q and 17q play an important role in facial development.

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Keywords

association analysis; balanced chromosomal translocation; *BSPRY*; *CCL2*; cleft lip and palate; haplotype; HapMap; *SLC31A1*

Nonsyndromic cleft lip and/or palate (CL/P) is a common congenital anomaly of complex etiology, with the birth prevalence being approximately 1 per 700 live births (Mossey and Little, 2002; Murray, 2002). Previous family and population studies indicate that both genetic and environmental factors are involved in the occurrence of this malformation. Prior sequencing analysis of CL/P has indicated roles for mutations in *MSX1, FOXE1, GLI2, MSX2, SKI, SPRY2, RYK, FGF1, FGF2*, and *FGF8* in the etiology of the birth defect (Ichikawa et al., 2006; Jezewski et al., 2003; Riley et al., 2007; Vieira et al., 2005). In addition, polymorphisms in *IRF6* have been found to be associated with CL/P (Blanton et al., 2005; Ghassibe et al., 2005; Park et al., 2007; Scapoli et al., 2005; Zucchero et al., 2004).

Because clefts are complex and caused by multiple interacting genes, we do not expect that single gene disruptions in all individuals with a chromosomal break will manifest as CL/P. However, in some cases, identification of the breakpoints of disease-associated chromosome translocations can be an effective way to identify genes and/or chromosomal regions contributing to the occurrence of CL/P. The positional cloning approach for disease-associated genes using chromosome rearrangements has successfully identified four relevant genes (*CLPTM1* [Yoshiura et al., 1998], *SATB2* [FitzPatrick et al., 2003], *SUMO1* [Alkuraya et al., 2006], and *FGFR1* [Kim et al., 2005]) as candidates for CL/P. These findings suggest that identification of the genes disrupted in a cleft/translocation case can give us insight into some of the pathways involved in this birth defect and provide candidates for analysis in cases independent of the index family.

Recently, we identified a Japanese family segregating a balanced chromosome translocation t (9;17)(q32;q12). Of three generations we examined, one individual is affected with cleft lip and palate. Genome-wide linkage studies have suggested that the region 9q21 has significant linkage with CL/P (Marazita et al., 2004). Therefore, genes in this region of chromosome 9 may have an important role in facial development. In this study, we identified the breakpoints of t(9;17)(q32;q12) in the patient by fluorescence *in situ* hybridization (FISH) analysis and identified candidate genes by polymerase chain reaction (PCR) and DNA sequencing. We then carried out a mutation search and a case-control association study of candidate genes. In addition, extended single-nucleotide polymorphism (SNP) analysis of the chromosomal regions adjacent to the translocation breakpoint was performed.

MATERIALS AND METHODS

Clinical Report

The proband was a male infant born prematurely by cesarean section at 24 weeks gestation after an uneventful pregnancy. The maternal age was 25 years and paternal age 26 years at the time of his birth. The parents were nonconsanguineous, and there was no family history of malformations. His birth weight and height were 706 g and 31.8 cm, respectively (10th centile = 560 g, 90th centile = 774 g). The patient was in normal development at 24 weeks gestation. The circumferences of his head and chest were 23 cm and 21 cm at birth, respectively. He had apparent ocular hypertelorism, a bilateral cleft lip and palate, and a mild nasal flattening. Echocardiography showed a small atrial septal defect, which had closed by 2 years of age. At 6 years after birth, his weight and height were normal for age at 20.1 kg and 114.6 cm, respectively. He shows no evidence of developmental delay. Routine chromosome-banding analysis of the patient revealed an apparently balanced translocation between the long arm of

chromosome 9 and chromosome 17: 46,XY, t(9;17)(q32;q12). His father and paternal grandmother had the same translocation seen in the patient; whereas, his mother had a normal karyotype (Fig. 1). Neither of the other translocation carriers had a cleft or malformations, and there was no family history of CL/P.

Identification of Translocation Breakpoint

Fluorescence *In Situ* Hybridization Analysis—Breakpoint mapping of the translocation in the patient was initiated by FISH analysis, according to published procedures (Ono et al., 1997). Bacterial artificial chromosome (BAC) clones were identified using the genome maps provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.org) and University of California, Santa Cruz (UCSC) (http://genome.cse.ucsc.edu) genome browsers. Figure 2 shows the BAC clones on chromosomes 9 and 17 used in the present study.

Cloning of the Translocation Breakpoint—We carried out a series of long-range PCR amplifications of DNA fragments to detect the recombinant sequence caused by the translocation. Primer sequences were designed based on the FISH results, with one forward primer on chromosome 9 and one reverse primer on chromosome 17, and were evaluated by RepeatMasker (http://www.repeatmasker.org/) to avoid nonspecific amplification of repetitive sequence. The PCR products then were subcloned into the pCR2.1-TOPO vector (Invitrogen, Carlsbad, CA). Additional nested primers were designed to obtain a DNA fragment including the breakpoint on der(9) using the subcloned plasmid DNA from the patient and genomic DNA of members of his family.

DNA Sequencing—The PCR products were labeled with the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit according to the manufacturer's instruction (Applied Biosystems, Carlsbad, CA). The products were purified, and then analyzed on an ABI 3700 automated sequencer (Applied Biosystems). The Applied Biosystems sequence software (version 2.1.2) was used for lane tracking. Chromatograms were transferred to a UNIX workstation, base-called with PHRED 4.0 (http://droog.mbt.washington.edu/poly_doc40.html) and results viewed with the CONSED program (version 4.0) (Nickerson et al., 1997). The UCSC and the Ensembl databases (http://www.ensembl.org) were used to detect potential genes around the breakpoint.

Mutation Search of Candidate Genes in Case/Control CL/P Population

To examine the association of the disrupted genes with the occurrence of CL/P in individuals unrelated to this family, we screened for mutations of the untranslated regions (UTR), exons and exon-intron boundaries of the two rearranged genes on chromosome 9 and chromosome 17. In addition, any plausible candidate gene adjacent to the breakpoint was screened. We sequenced 90 individuals affected with isolated CL/P from the Philippines and 90 from Iowa. The control group comprised 90 samples from unrelated Filipinos and whites provided by Centre d'Etudes du Polymorphisme Humaine (Dausset et al., 1990). Standard chi-square tests of association were used to compare the frequencies of each variant found by sequencing between groups of patients and controls.

Haplotype Analysis Adjacent to the Translocation Breakpoint

To determine whether the chromosomal regions adjacent to the breakpoint could play a role in the development of CL/P, we carried out the haplotype-based transmission disequilibrium test. Four tagging SNP markers (rs974230, rs1330691, rs4596714, rs3750534) adjacent to the breakpoint on chromosome 9 and three markers (rs16561, rs725276, rs1029719) on chromosome 17 were selected, based on the HapMap (http://www.hapmap.org/index.html.en) database. The TaqMan genotyping for these SNPs

was performed with the ABI PRISM 7900 Sequence Detection System (Applied Biosystems) using 371 case/parent families from the Philippines and 206 from Iowa. Haplotype-based transmission disequilibrium statistics were calculated using the software FBAT (Horvath et al., 2001; Rabinowitz and Laird, 2000).

Bioethics Approval

Committee approval was obtained from both the ethical committee at Toyohashi Municipal Hospital and Aichi-Gakuin University to obtain whole blood from the patient, his parents, and his paternal grandparents after signed informed consent. For the association analysis, patients were identified at the University of Iowa Hospital and Clinics and in the Philippines through Operation Smile (Murray et al., 1997). Blood samples were obtained with informed consent following institutional review board (IRB) approval in the United States (University of Iowa, IRB Committee) and in the Philippines (Hope Foundation, Bacolod City, Negros, Philippines).

RESULTS

Identification of the Translocation Breakpoint

We found that two clones, RP11-73G18 (164,488 base pairs [bp]) and RP11-999I23 (160,162 bp), included the breakpoint region, judging from a hybridization pattern in which FISH signals were detected simultaneously on the normal chromosome 9 as well as both der(9) and der(17) chromosomes (Fig. 3a). The breakpoint on chromosome 17 was covered by two clones, RP11-770A19 (194,408 bp) and RP11-799L9 (178,732 bp), according to the hybridization pattern in which FISH signals were detected simultaneously on the normal chromosome 17 as well as both der(9) and der(17) chromosomes (Fig. 3b).

To examine sequences of the translocation breakpoints, we tried PCR amplification involving the breakpoint region (Fig. 4a). A primer set of F2 (5′-TCTTAACT-TACCTGTTGCTGCCGTTCCCTG-3′) derived from chromosome 9 and R1 (5′-TGGGCTGAGATTGTGG-TAACCGTAGGAAAG-3′) derived from chromosome 17 successfully amplified a DNA fragment of approximately 3 kbp in length. The PCR product amplified by 9f1 (5′-GTGTATGATACCATAACAATTAC-3′) and 17r1 (5′-GCACTGAGCAAACAGGAAGTG-3′) was sequenced after subcloning into the pCR2.1-TOPO vector. The nucleotide sequence of this PCR product demonstrated that the breakpoint of chromosome 9 lies in intron 1 of *SLC31A1* (solute carrier family 31 member 1; GenBank accession number NM001859, between nucleotides 113,072,186 and 113,072,187) according to the UCSC database (hg17). The breakpoint on chromosome 17 is assigned to the 5′UTR of chemokine ligand 2 (*CCL2*; GenBank accession number MN002982, between nucleotides 29,568,964 and 29,568,965). These three exons also form exons 4, 5, and 6 of a predicted six-exon gene of which *CCL2* may be one isoform, according to the Ensembl database (Transcript ID; GENSCAN00000016010). The function of this predicted gene is unknown. The translocation examined in this study shows no gain or loss of nucleotides at the breakpoint, confirming a perfectly balanced reciprocal translocation in translocation in the proband, his father, and his paternal grandmother (Fig. 4b and 4c).

Mutation Analysis of Candidate Genes in Case/Control CL/P Population

In order to determine whether genes at or near the breakpoint play a role in CL/P, we screened for mutations in three candidate genes: *SCL31A1* and *BSPRY* on chromosome 9 and the predicted gene that includes exons of *CCL2* on chromosome 17. Although *BSPRY* is located approximately 120 kbp downstream from the breakpoint, we considered it a plausible candidate because it is induced by the fibroblast growth factor receptor (*FGFR*) pathway (Welsh et al., 2007) and impaired FGF signaling has been found to be associated with 3% to 5% of CL/P (Riley et al., 2007). In addition, variants in a related gene, *SPRY2*, that also has a sprouty

domain, already have been identified in CL/P (Vieira et al., 2005). We hypothesized that disruption of regulatory elements of *BSPRY* might lead to a cleft phenotype.

Table 1 shows nucleotide variants found in *BSPRY* and the predicted gene that include *CCL2*. In *BSPRY*, two common polymorphisms (rs752757 and rs2296074) and two new variants (positions 113202320 and 113202593) were detected. One variant (position 113202320) shows a significant *p* value (.012) in the Filipino CL/P population compared with matched controls. In the predicted gene containing *CCL2*, six new variants were detected. Four variants (positions 29527634, 29540781, 29540817, and 29540841) possibly result in a change of amino acid, R15C, M33T, N45S, and T53M, respectively, according to the Ensembl database (Transcript ID; GENSCAN-00000016010). R15C, N45S, and T53M were not found in Filipino controls, and T53M was not found in either Filipino or white controls. Five known variants were also identified (rs4795884, rs4795885, rs1860188, rs28730833, and rs4586). The variant at position 29540817 was present with a significant *p* value (.007) in an affected Filipino population compared with control population. No variants were identified in *SLC31A1* (data not shown).

Haplotype Analysis in Markers Adjacent to the Translocation Breakpoint

Although no statistically significant evidence of transmission distortion was seen for either SNP individually, there was statistically significant evidence of transmission distortion for haplotype h2-F9 (C-G-T-T; bi-allelic *p* value = .017) and haplotype h2-C9 (G-A-C-T; biallelic *p* value = .028) on chromosome 9 region (Table 2). We also confirmed statistically significant evidence for haplotype h5-F17 (G-G-T; biallelic *p* value = .042) and haplotype h8-F17 (G-A-A; biallelic *p* value = .017) on chromosome 17 region (Table 3). These *p* values, however, did not reach the level of significance, if we account for the number of tests done and use the conservative Bonferroni correction (0.0071).

DISCUSSION

It has been demonstrated that CL/P is associated with trisomy 9q (Metzke-Heidemann et al., 2004), tetrasomy 9q (Wyandt et al., 2000), and chromosomal translocations involving the 9q region: t(6;9)(p23;q22.3) (Donnai et al., 1992); 46,XX,−222,+der(9)t(9;22)(q22;q11.2) (Pivnick et al., 1990); and 46,X,t(X;9)(p22.1;q32) (Zori et al., 1993). Naritomi et al. (1989) divided trisomy 9q syndrome into four groups (group 1: trisomy for 9q11>q32, group 2: 9q32>qter, group 3: 9q34, and group 4: 9q13/q21>qter) according to the length of the trisomic segment. Of these, the patients in groups 2 and 4 showed cleft palate, suggesting that the 9q32 segment may have important roles for facial development. Genome-wide linkage studies have revealed that the region 9q21 has significant linkage with facial clefting (Marazita et al., 2004). A number of candidate genes for CL/P in this region, 9q21–23, have been reported previously (*ROR2, PTCH, FOXE1*, and *TGFBR1*) (Ichikawa et al., 2006; Loeys et al., 2006; Mansilla et al., 2006; Mizuguchi et al., 2004; van Bokhoven et al., 2000; Vieira et al., 2005). Using a positional cloning approach, we demonstrated that *SLC31A1* on chromosome 9 was disrupted within its intron. The *SLC31A1* encodes a copper transporter and is itself not a strong candidate for involvement in facial development; although, unbalanced copper regulation can disturb cell metabolism (Lee et al., 2001). However, it is increasingly recognized that point mutations in regulatory elements located far from gene structural elements can be damaging. For example, mutations located up to ~1 Mbp from the gene *SHH* are capable of causing congenital abnormalities (Gurnett et al., 2007; Lettice et al., 2003) and campomelic dysplasia has resulted from a balanced translocation breakpoint 1.3 Mbp downstream of the *SOX9* gene (Velagaleti et al., 2005). In the present report, although we did not detect any mutations in the *SLC31A1* gene itself, we did detect a significant *p* value for CL/P in the case-control study of a strong candidate for craniofacial development, the *BSPRY* gene, which is located 120 kbp

downstream from the breakpoint (Table 1). Thus the translocation break may disrupt not the gene itself but regulatory regions. Moreover, there was significant evidence of transmission distortion for haplotypes on chromosome 9 (Table 2). In summary, it is possible that this region of chromosome 9q contains one or more genes playing a role in facial development.

It is also possible that facial development may be influenced by genes on chromosome 17q. Case reports of chromosome translocations involving 17q24 (Czako et al., 2004; Luke et al., 1992), 17q25 (Bridge et al., 1985), 17q21.1 (Martinet et al., 2006), and 17q23.3 (Stalker et al., 2001) are associated with CL/P. Additively, genome-wide linkage analysis also shows significant association of the 17q21 region to the occurrence of CL/P (Marazita et al., 2004). Three candidate genes for CL/P in this region, 17q23–25, have been described (*SEPT9*, *MKS1*, and *GAA*) (Huie et al., 1999; Jeannet et al., 2001; Paavola et al., 1995). In addition, *RARA*, which is located approximately 6 Mbp downstream from the breakpoint studied here, is associated with CL/P (Chenevix-Trench et al., 1992). In this study we identified a predicted gene containing *CCL2* in the 17q breakpoint region. *CCL2* (monocyte chemotactic protein-1) is a member of the small inducible gene family and plays a role in the recruitment of monocytes to sites of injury and infection and so is not an obvious candidate for CL/P. We did, however, observe multiple amino acid variants in the predicted gene. One of them, a heterozygous variant in position 29540817 (possibly N45S), showed a significant *p* value in the Filipino CL/P population compared with the control population (Table 1). Moreover, there was statistically significant evidence of transmission distortion for two haplotypes on chromosome 17 in Filipinos but not in the white population (Table 3). These data suggest that the participation of this region in facial development may vary among different populations, and these results warrant an extension of these studies to larger numbers for confirmation.

Although three generations were examined here segregating the same chromosome translocation, only one individual was clearly affected with cleft lip and palate. CL/P is commonly nonpenetrant even in disorders caused by single gene mutations. Mutations in *IRF6* cause the autosomal dominant van der Woude syndrome, but there is only 70% penetrance for the CL/P phenotype (Burdick, 1986; Zucchero et al., 2004). Mutations in *MSX1* and several of the FGF and FGFR genes may also cause CL/P, but again only a subset of individuals with the mutation have a cleft phenotype (van den Boogaard et al., 2000). Isolated clefts are complex traits, not single-gene Mendelian disorders. They are likely caused by several interacting genes, each with small effects. In our case, the presumed loss of 50% of gene product may be the tipping point in an individual already having several other of the predisposing alleles/genes that may be absent in other family members with the translocation, hence their unaffected status. Clearly, future work is required to develop a better understanding of penetrance in CL/P.

In summary, we report an extensive study of candidate genes for CL/P based on a family with CL/P and segregating a balanced chromosome translocation t(9;17)(q32;q12). Analysis of the translocation shows disruption of *SLC31A1* on chromosome 9 and a predicted gene that includes *CCL2* on chromosome 17. It is intriguing that *SLC31A1* lies close to *BSPRY*, an excellent candidate gene for CL/P, and the results of a case-control study support a role for *BSPRY* in CL/P. In the aggregate, these data may provide additional support for an intensive search for genes/mutations and also for micro-deletions in the 9q/17q region that would play a role in CL/P.

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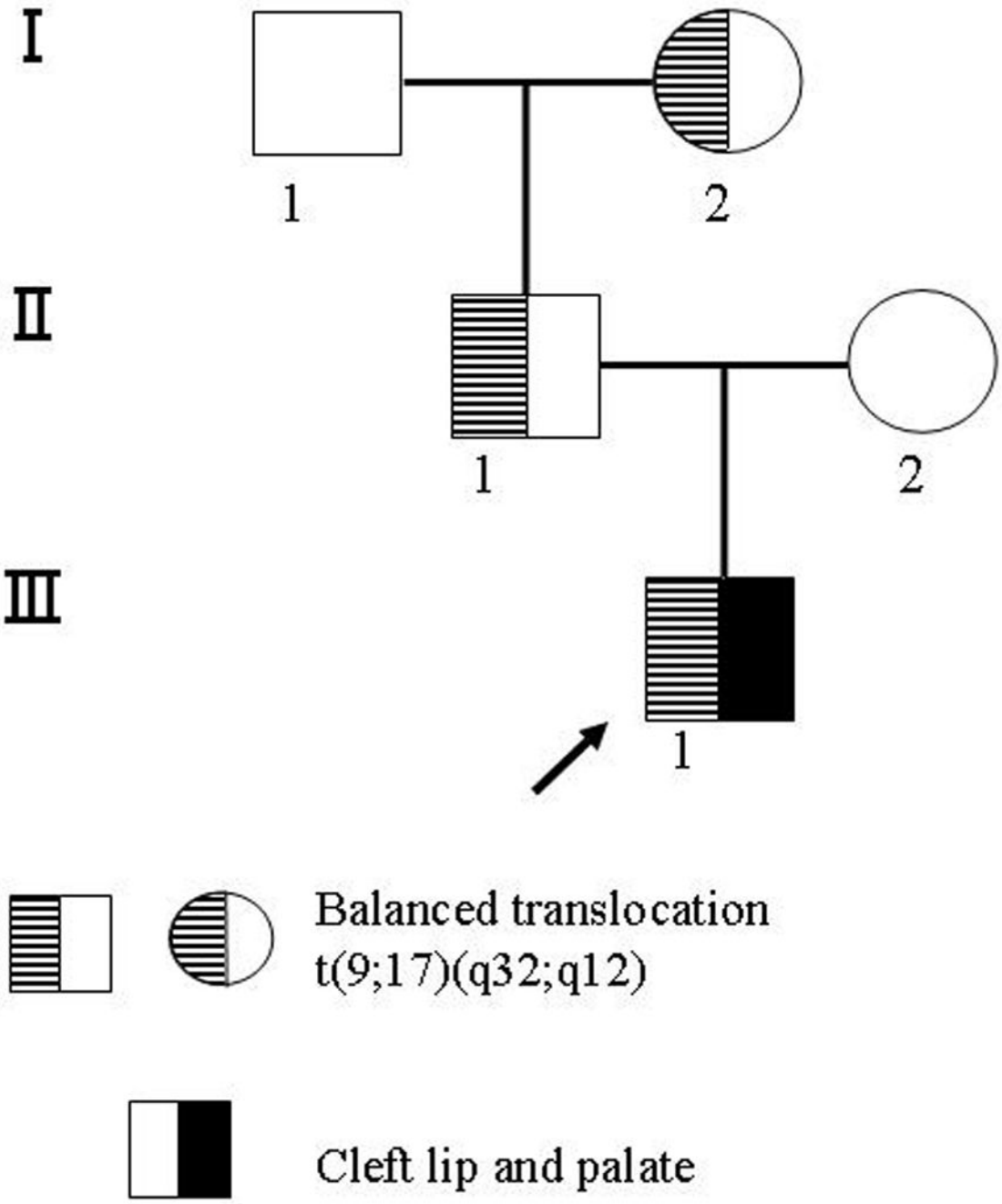


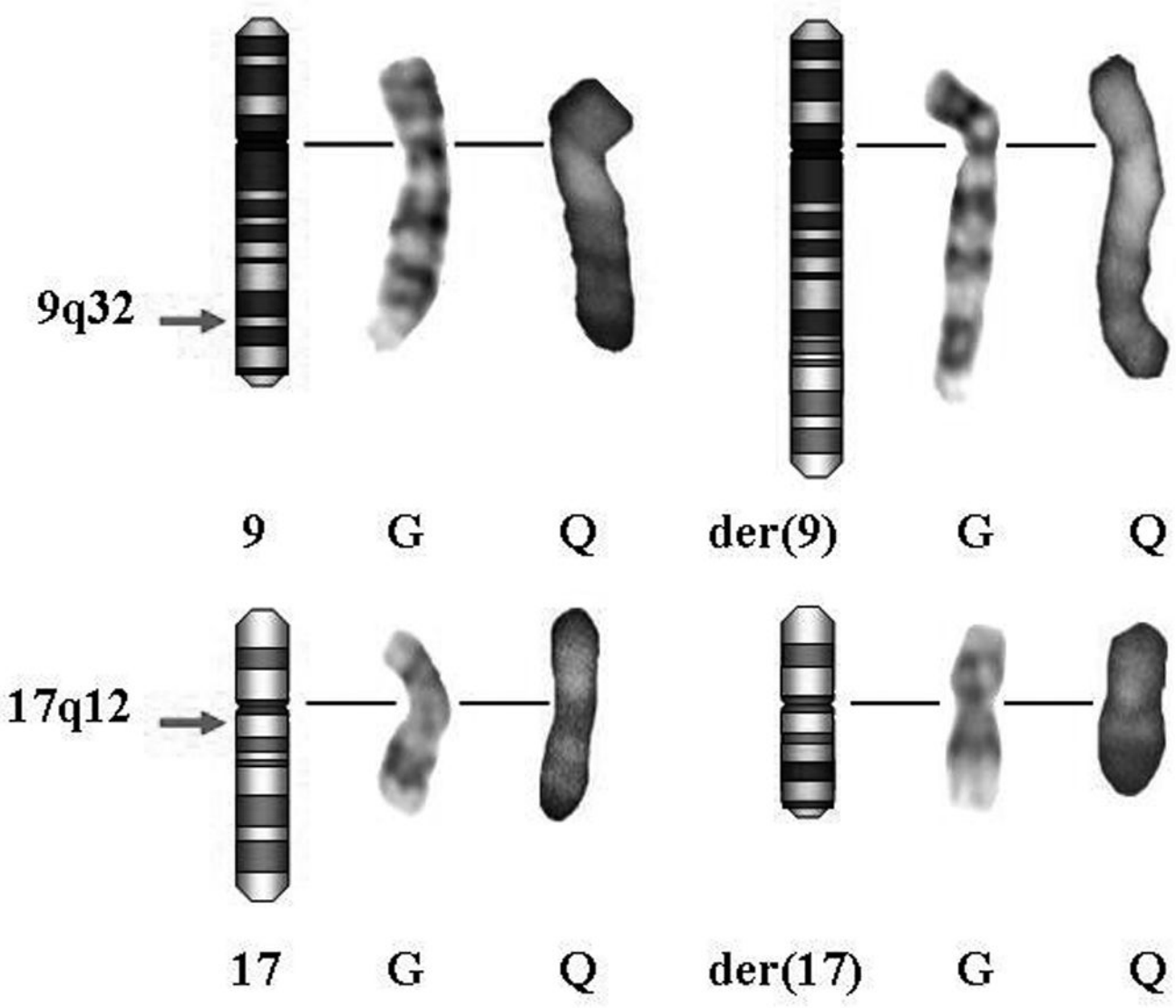
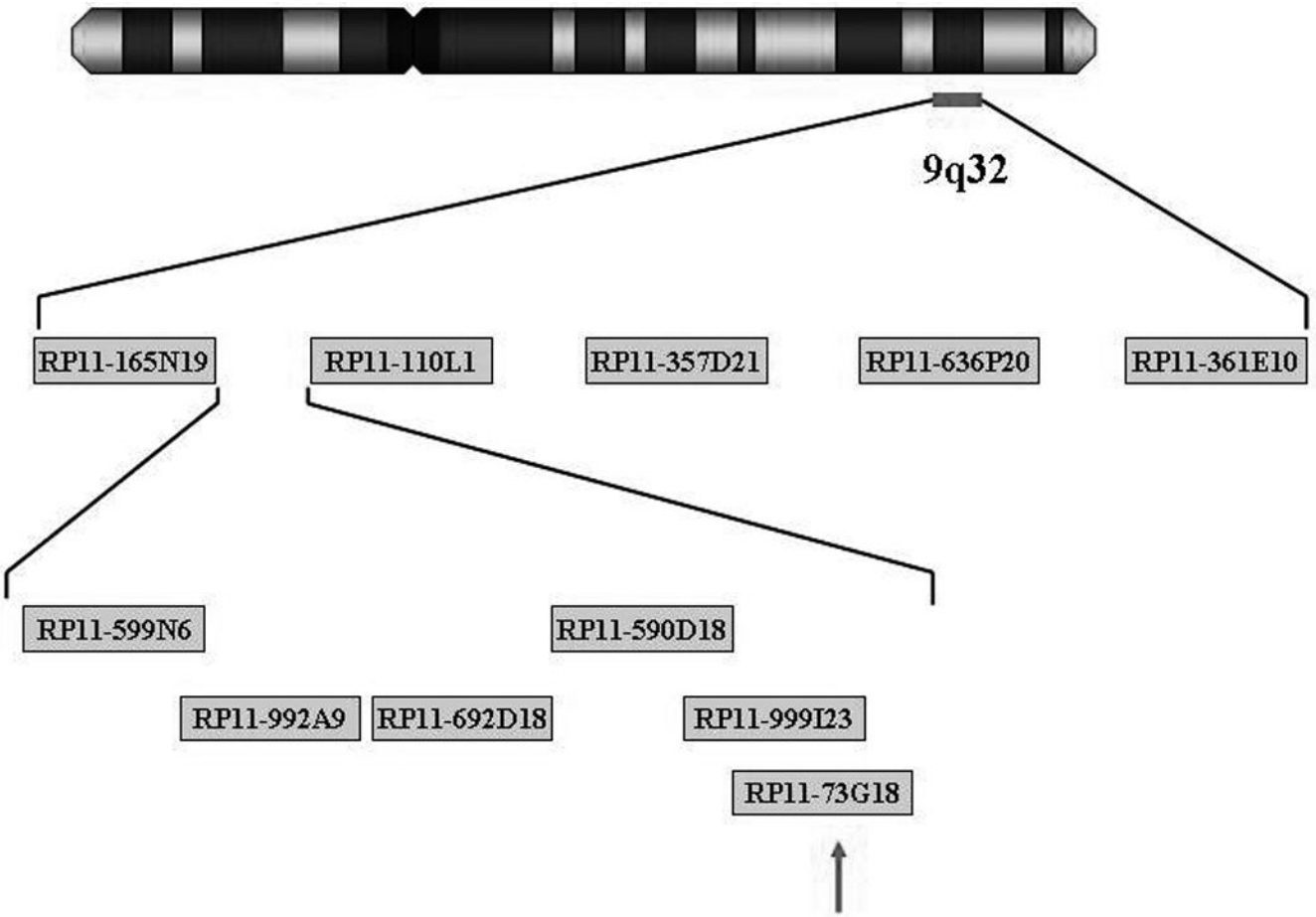


FIGURE 1.
Case presentation. a: Pedigree of the family examined. b: Chromosomal breakpoints in the patient (III-1). Ideogram and a partial karyotype are shown. Chromosomal breakpoints are indicated by arrows.



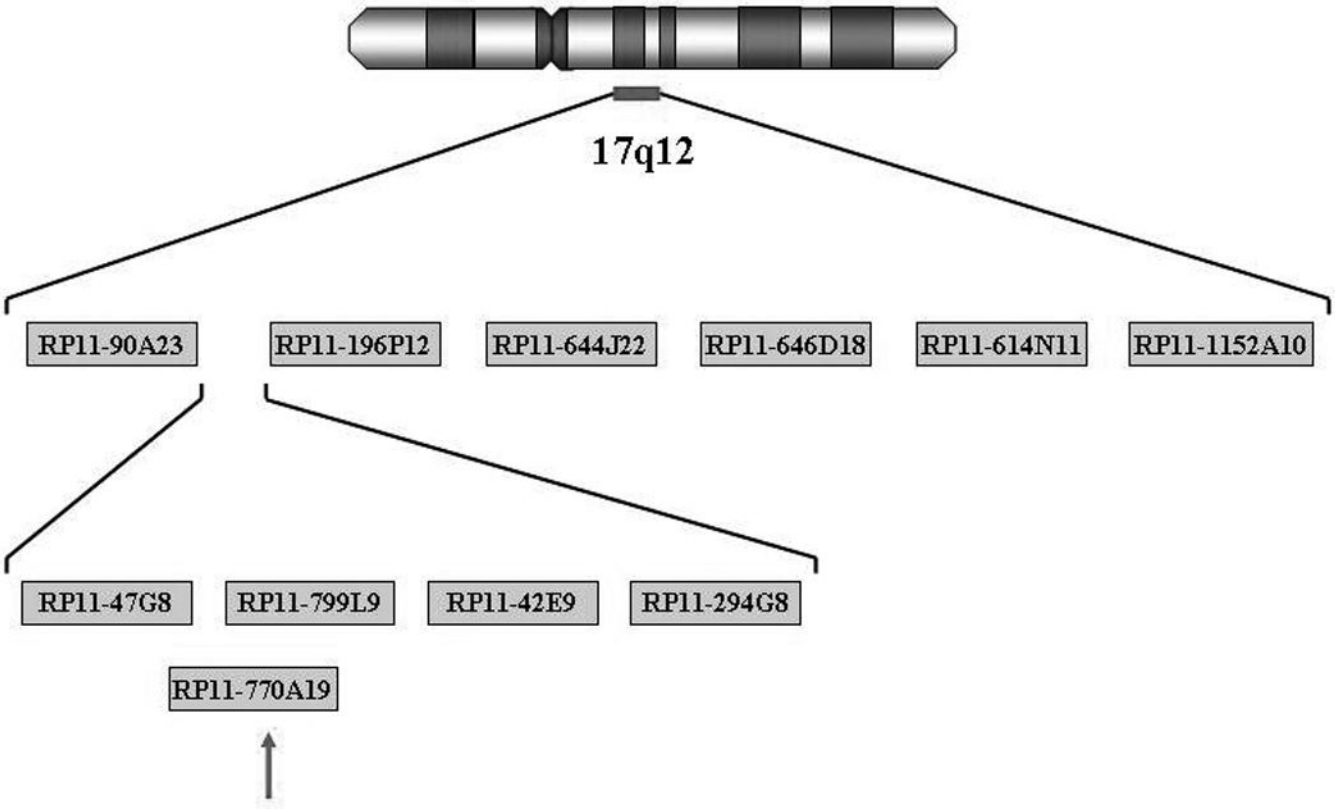
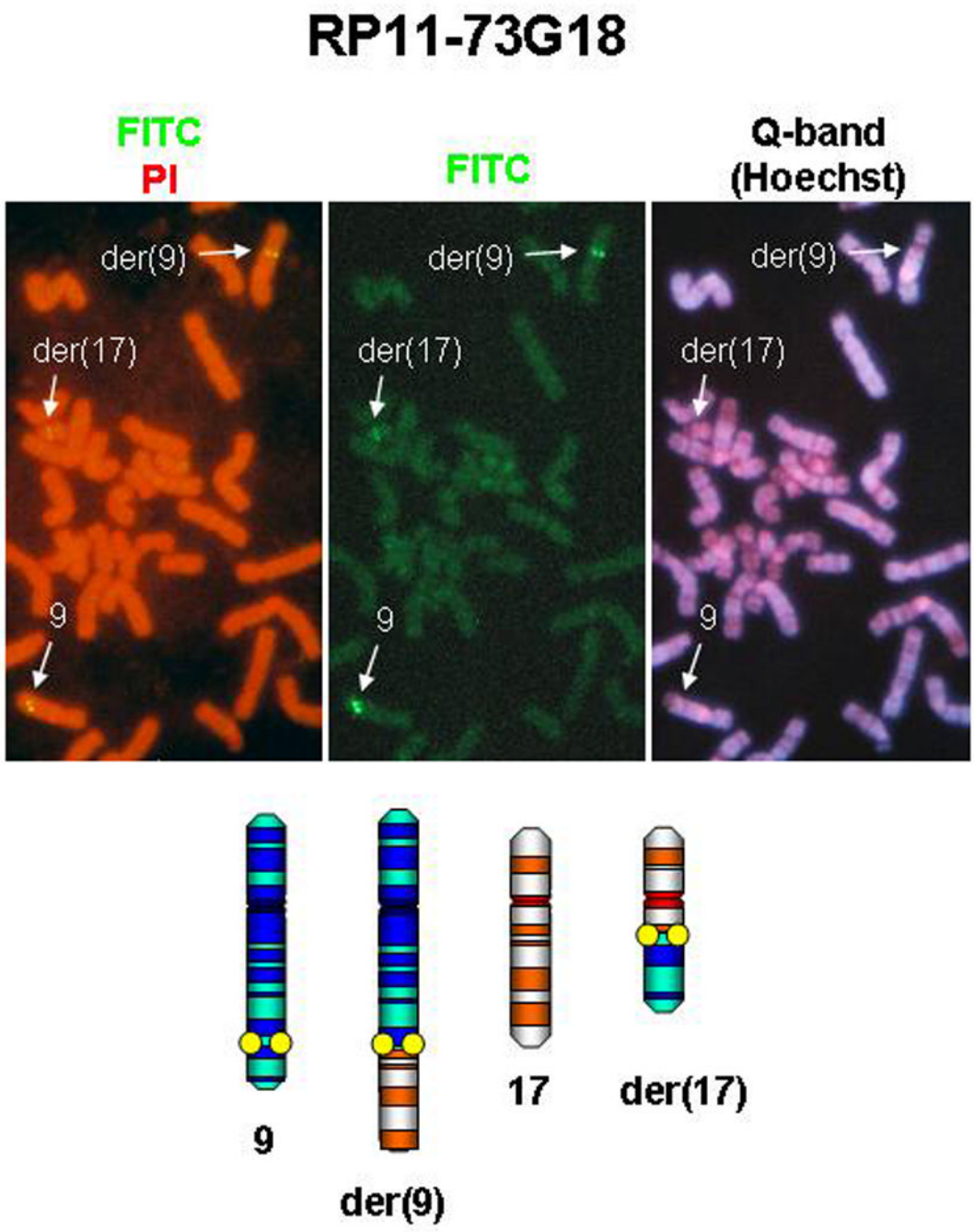


FIGURE 2.

BAC clones used for FISH analysis. a: A physical map of BAC clones covering the 9q32 subchromosomal band. b: A physical map of BAC clones covering the 17q12 subchromosomal band.



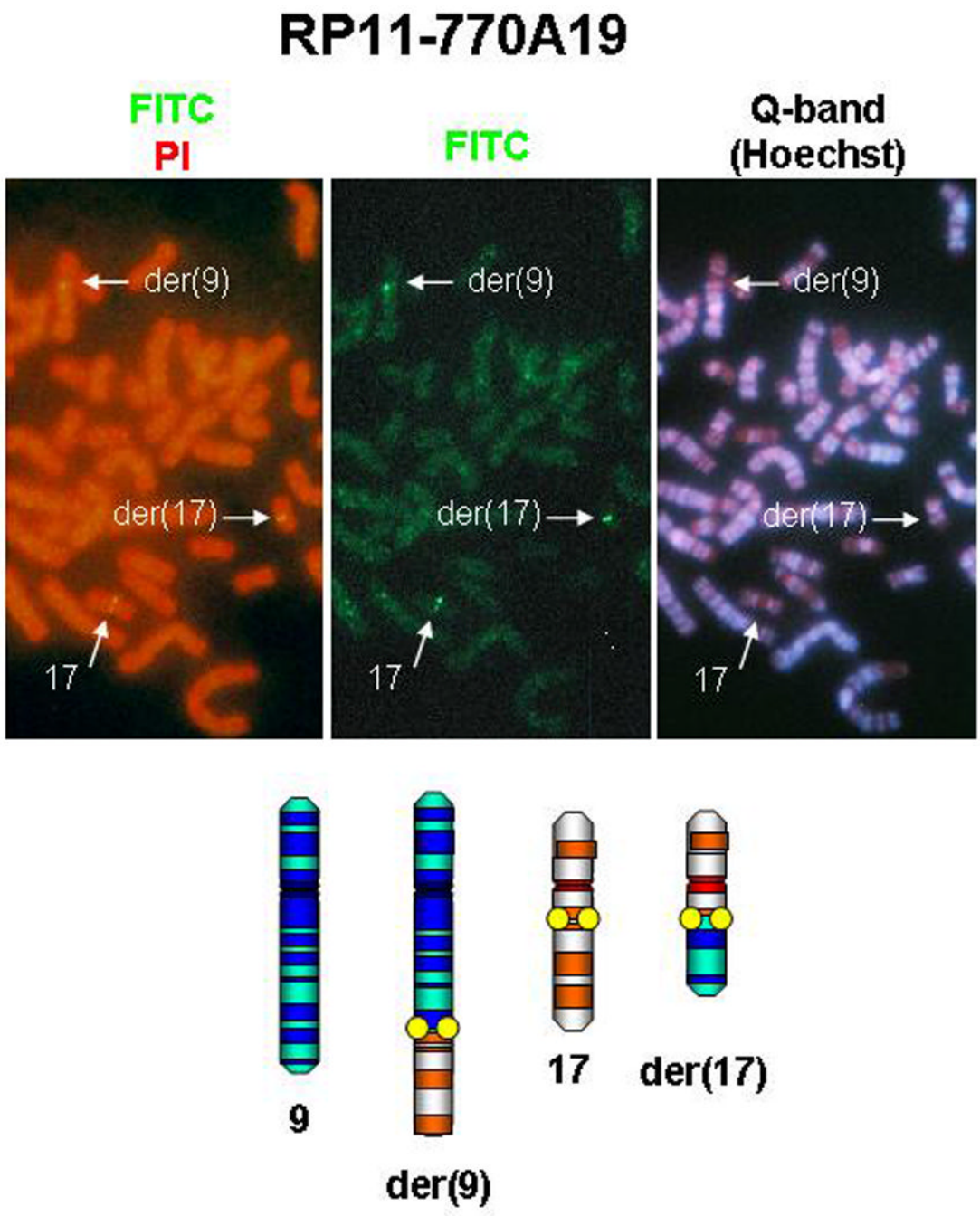


FIGURE 3.

Molecular cytogenetic characterization of the t(9;17)(q32;q12). Each left panel shows FISH signals (FITC) on propidium iodide-stained chromosomes (PI). Each right panel exhibits Q-banded chromosomes (Hoechst). The RP11-73G18 clone simultaneously hybridized to normal 9, der(9), and der(17) chromosomes. The RP11-770A19 clone simultaneously hybridized to normal 17, der(9), and der(17) chromosomes. Bottom panels show schematic diagrams of FISH results.

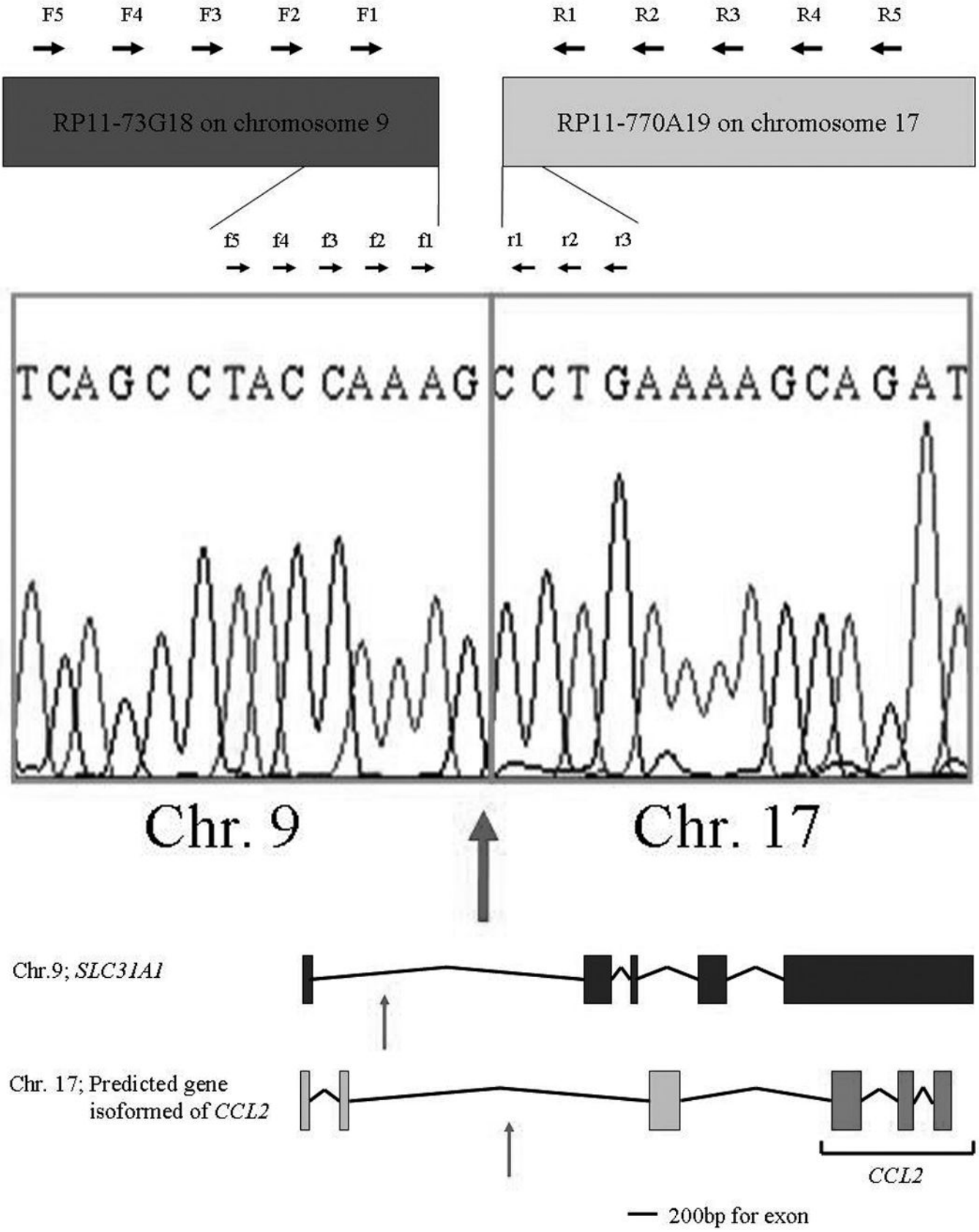


FIGURE 4.

Fine mapping of the breakpoints. a: Primers used for long-range PCR. Forward and reverse primers were designed every 5 kbp of RP11-73G18 on chromosome 9 and RP11-770A19 on chromosome 17, respectively (arrows in upper). Additional primers flanked regions of the breakpoint (arrows in lower). b: Identification of the breakpoint on der(9), using a PCR product with a primer set of 9f1 and 17r1. c: The breakpoint on chromosome 9 is located between exons 1 and 2 of *SLC3A1*. The breakpoint on chromosome 17 is located between exons 2 and 3 of a predicted gene, according to the Ensembl database. Arrows indicate the positions of the breakpoints.

TABLE 1

Polymorphisms Found (Entries in the Table are Numbers of Individuals With Homozygous Common Allele/Heterozygous Rare Allele/Homozygous Rare Allele)

	Filipino Genotyping Distributions			White Genotyping Distributions			
Variant (Position*)	**Case (AF†)**	**Control (AF)**	**p Value**	**Case (AF)**	**Control (AF)**	**p Value**	**Comment**
BSPRY							
new (113202320)	62/0/0 (1.000)	68/5/0 (0.966)	.012	62/0/0 (1.000)	55/0/0 (1.000)	~.0	
rs752757 (113202508)	41/19/2 (0.814)	33/32/6 (0.690)	.058	55/22/2 (0.835)	25/7/1 (0.864)	0.756	
new (113202593)	49/2/0 (0.980)	65/3/0 (0.978)	.895	75/1/0 (0.993)	25/0/0 (1.000)	.449	
rs2296074 (113210279)	45/23/1 (0.819)	33/18/0 (0.825)	.565	42/4/0 (0.957)	33/6/0 (0.923)	.341	
Predicted gene (GENESCAN00000016010) containing *CCL2*							
rs4795884 (29527481)	52/32/6 (0.756)	42/36/1 (0.747)	.103	17/47/17 (0.500)	24/40/19 (0.530)	.396	
new (29527634)	82/0/0 (1.000)	79/0/0 (1.000)	~.0	74/6/0 (0.963)	57/4/0 (0.967)	.828	R15C‡
Rs4795885 (29527655)	83/2/0 (0.988)	79/0/0 (1.000)	.103	53/23/3 (0.816)	38/20/3 (0.787)	.830	
new (29540781)	48/30/4 (0.768)	16/12/4 (0.688)	.364	60/19/3 (0.848)	53/25/2 (0.819)	.489	M33T‡
new (29540817)	79/6/0 (0.965)	69/0/0 (1.000)	.007	57/21/4 (0.823)	53/29/1 (0.813)	.198	N45S‡
new (29540841)	86/0/0 (1.000)	69/0/0 (1.000)	~.0	81/1/0 (0.994)	84/0/0 (1.000)	.234	T53M‡
new (29541048)	86/0/0 (1.000)	69/0/0 (1.000)	~.0	69/11/0 (0.931)	75/7/1 (0.981)	.290	
rs1860188 (29602669)	31/21/3 (0.755)	35/21/2 (0.784)	.833	49/12/2 (0.873)	22/13/0 (0.814)	.073	
new (29602875)	54/4/1 (0.949)	47/5/0 (0.952)	.463	63/0/0 (1.000)	73/0/0 (1.000)	~.0	
rs28730833 (29606607)	64/1/0 (0.992)	80/0/0 (1.000)	.204	56/0/0 (1.000)	80/0/0 (1.000)	~.0	
rs4586 (29607382)	10/19/8 (0.527)	11/27/14 (0.471)	.755	20/30/5 (0.636)	5/9/4 (0.528)	.363	

*Position according to UCSC database (hg17).

†AF = allele frequency.

‡Amino acid change in predicted gene containing *CCL2* according to the Ensembl database (Transcript ID; GENSCAN00000016010).

TABLE 2

Results of Haplotype-based Transmission Disequilibrium Test for Single-Nucleotide Polymorphisms (SNPs) Around the Breakpoint on Chromosome 9

	SNP					
Filipino Haplotype	**a***	**b**	**c**	**d**	**Estimated Frequency**	**p Values (Biallelic)**
h1-F9	C	A	T	T	0.282	.456 (−)
h2-F9	C	G	T	T	0.246	.017 (+)
h3-F9	C	G	C	T	0.206	.095 (−)
h4-F9	C	A	T	C	0.053	.809 (+)
h5-F9	C	G	T	C	0.052	.892 (+)
h6-F9	G	A	T	T	0.047	.638 (−)
h7-F9	C	G	C	C	0.031	.093 (−)
h8-F9	G	G	C	T	0.027	.367 (+)
h9-F9	C	A	C	T	0.015	.594 (+)
h10-F9	G	G	T	T	0.013	.815 (+)
h11-F9	G	A	T	C	0.010	.956 (−)
h12-F9	G	G	C	C	0.010	.476 (+)
	SNP					
White Haplotype	*a*	*b*	*c*	*d*	*Estimated Frequency*	*p Values (Biallelic)*
h1-C9	G	A	T	T	0.137	.111 (−)
h2-C9	G	A	C	T	0.121	.028 (−)
h3-C9	G	A	T	C	0.110	.441 (+)
h4-C9	C	A	C	T	0.102	.342 (−)
h5-C9	G	A	C	C	0.087	.224 (+)
h6-C9	C	A	T	T	0.085	.122 (+)
h7-C9	G	G	T	C	0.065	.103 (+)
h8-C9	G	G	T	T	0.062	.624 (+)
h9-C9	G	G	C	T	0.059	.246 (+)
h10-C9	C	G	T	T	0.056	.198 (−)
h11-C9	C	G	C	T	0.039	.819 (+)

* a = rs1330692 C/G; b = rs3750534 A/G; c = rs4596714 C/T; d = rs974230 C/T.

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TABLE 3

Results of Haplotype-Based Transmission Disequilibrium Test for Single-Nucleotide Polymorphisms (SNPs) Around the Breakpoint on Chromosome 17

	SNP				
Filipino Haplotype	**e***	**f**	**g**	**Estimated Frequency**	**p Values (Biallelic)**
h1-F17	A	G	T	0.322	.451 (+)
h2-F17	A	A	T	0.316	.991 (+)
h3-F17	A	A	A	0.103	.782 (+)
h4-F17	A	G	A	0.102	.973 (+)
h5-F17	G	G	T	0.065	.042 (−)
h6-F17	G	A	T	0.054	.450 (−)
h7-F17	G	G	A	0.025	.765 (−)
h8-F17	G	A	A	0.013	.017 (+)
	SNP				
White Haplotype	*e*	*f*	*g*	*Estimated Frequency*	*p Values (Biallelic)*
h1-C17	A	A	A	0.180	.275 (+)
h2-C17	A	A	T	0.164	.592 (+)
h3-C17	G	A	T	0.136	.597 (+)
h4-C17	G	A	A	0.120	.684 (−)
h5-C17	A	G	A	0.115	.899 (+)
h6-C17	G	G	T	0.112	.547 (−)
h7-C17	A	G	T	0.099	.534 (−)
h8-C17	G	G	A	0.075	.161 (−)

*e = rs16561 A/G; f = rs1029719 A/G; g = rs725276 A/T.