

Olfactory Receptor–Gene Clusters, Genomic-Inversion Polymorphisms, and Common Chromosome Rearrangements

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The olfactory receptor (OR)-gene superfamily is the largest in the mammalian genome. Several of the human OR genes appear in clusters with ≥ 10 members located on almost all human chromosomes, and some chromosomes contain more than one cluster. We demonstrate, by experimental and *in silico* data, that unequal crossovers between two OR gene clusters in 8p are responsible for the formation of three recurrent chromosome macrorearrangements and a submicroscopic inversion polymorphism. The first two macrorearrangements are the inverted duplication of 8p, inv dup(8p), which is associated with a distinct phenotype, and a supernumerary marker chromosome, +der(8)(8p23.1pter), which is also a recurrent rearrangement and is associated with minor anomalies. We demonstrate that it is the reciprocal of the inv dup(8p). The third macrorearrangement is a recurrent 8p23 interstitial deletion associated with heart defect. Since inv dup(8p)s originate consistently in maternal meiosis, we investigated the maternal chromosomes 8 in eight mothers of subjects with inv dup(8p) and in the mother of one subject with +der(8), by means of probes included between the two 8p-OR gene clusters. All the mothers were heterozygous for an 8p submicroscopic inversion that was delimited by the 8p-OR gene clusters and was present, in heterozygous state, in 26% of a population of European descent. Thus, inversion heterozygosity may cause susceptibility to unequal recombination, leading to the formation of the inv dup(8p) or to its reciprocal product, the +der(8p). After the Yp inversion polymorphism, which is the preferential background for the PRKX/PRKY translocation in XX males and XY females, the OR-8p inversion is the second genomic polymorphism that confers susceptibility to the formation of common chromosome rearrangements. Accordingly, it may be possible to develop a profile of the individual risk of having progeny with chromosome rearrangements.

Introduction

In recent years, several studies (reviewed by Lupski [1998] and Ji et al. [2000]) have shown that repeated sequences, located on the same chromosome at a distance of a few megabases (Mb), predispose to homologous unequal recombination, leading both to chromosome microrearrangements (deletions, duplications, and inversions) and to supernumerary inverted dupli-

cation chromosomes, such as inv dup(15) and inv dup(22). It seems likely that several chromosome rearrangements are mediated by the same mechanisms. Repeated sequences located on the same chromosome could be responsible for pericentric or paracentric inversions (according to their location on different chromosome arms or on the same arm) or for more-complex rearrangements, depending on the number of crossovers occurring between the two duplices (Small et al. 1997). Similarly, repeats located on different chromosomes could be responsible for translocations, as has been demonstrated for the constitutional 11q23;22q11 translocation (Kurahashi et al. 2000a, 2000b). We demonstrate that the olfactory receptor (OR) gene clusters, the largest superfamily in the mammalian genome (Mombaerts 1999), are the substrate for the formation of intrachromosomal rearrangements involving chromosome 8p. Different rearrangements, most of them recurring, are associated with the distal 8p region. Among them, inv dup(8p) (Floridia et al. 1996), del(8p22) (Devriendt et

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al. 1999; Pehlivan et al. 1999; Giglio et al. 2000), and small marker chromosomes der(8)(p23-ppter) (Ohashi et al. 1994; Neumann et al. 1999).

Four years ago, we postulated (Florida et al. 1996) that repeated inverted sequences at the constant breakpoints of inv dup(8p)s were responsible for the rearrangement's formation. Thus, we decided to search for these sequences by cloning the breakpoints and investigating whether any correlation exists between them and the breakpoints of the other common 8p rearrangements.

Material and Methods

Rearrangements

A brief description of the three chromosome rearrangements we studied is given below, using data already reported in the literature.

inv dup(8p).—The abnormal chromosome 8 has an elongated short arm that appears duplicated. Florida et al. (1996) demonstrated, by FISH and microsatellite analysis, that a 10-cM distal deletion is always associated with the duplications and that two regions consistently are the same size: the deleted region and the ~5 cM single-copy region between the two duplicated regions. The duplication is of variable size in different abnormal chromosomes. The finding that some chromosomes are dicentric, with a second inactive centromere at the tip of the inv dup(8p), indicated that the formation of the new chromosome required two events: (1) the formation of a dicentric chromosome 8qter-cen-8p::8p-cen-8qter, and (2) its breakage at anaphase, with the breakpoint either at the level of the second centromere or more proximally along the short arm. The presence of a single-copy region between the two regions of duplication indicates that the breakpoints in 8p are not symmetric. The rearrangement consistently originates in maternal meiosis and has an estimated frequency of 1/10–1/15,000. The phenotype of patients with inv dup(8p) has been extensively delineated (Feldman et al. 1993; Die Smulders et al. 1995; Guo et al. 1995) and is characterized by facial dysmorphism, agenesis/hypoplasia of the corpus callosum, and severe mental retardation.

der(8)(pter-p23.1::p23.2-ppter).—Four patients with such a supernumerary marker chromosome have been reported (Ohashi et al. 1994; Neumann et al. 1999; L. Voullaire, unpublished data). In one patient, it was present in all PHA-stimulated PBL cells; in the other patients, it was present in mosaicism. All of them lacked alphoid sequences and had a neocentromere (Tyler-Smith and Florida 2000). Molecular analysis demonstrated that they contained the distal 8p region in duplication. The paucity of such patients, the young age of one of them, and the mosaicism in the others prevent

any phenotype/karyotype correlation. That only four patients with der(8)(pter-p23.1::p23.2-ppter) have been reported does not exclude the possibility that this supernumerary marker is more frequent. In fact, molecular definition of supernumerary marker chromosomes has been attempted only in recent years. Moreover some markers have been defined by whole-chromosome painting and not by specific probes (Rothenmund et al. 1997), and thus the portion of chromosome 8 that is present has not been defined.

del(8)(p23.1p23.2).—At least eight patients with distal 8p interstitial deletion have been reported (Devriendt et al. 1999; Pehlivan et al. 1999; Giglio et al. 2000). Although different cytogenetic breakpoints were reported in the different studies, molecular investigation has indicated that they have identical breakpoints, thus suggesting that the rearrangement is recurrent. Devriendt et al. (1999) described five identical chromosomes (2–6) as delineated by FISH and microsatellite analysis. The same molecular breakpoints have been reported in patient 8 in Giglio et al. (2000). In patients 1 and 3 in Pehlivan et al. (1999), no molecular definition of the breakpoints was presented, but both patients had haploinsufficiency for GATA4, which is included in the deletion region of Devriendt et al. (1999) and Giglio et al. (2000). The finding that all cases of 8p interstitial deletions have been published only in recent years and that their definition relies on fluorescence in situ hybridization (FISH) analysis, suggests that some “terminal” 8p deletions had been previously misinterpreted. This, in fact, was true in one of our patients (patient 8 in Giglio et al. 2000). The patients' phenotype is characterized by heart defects—including atrioventricular canal, valvar pulmonary stenosis, and tetralogy of Fallot—and by mild mental retardation and behavioral problems.

General Procedure

The rearrangement breakpoints were refined at the YAC level by FISH analysis using CEPH mega-YACs obtained from the YAC Screening Center at DIBIT, Milan. Primers for critical STSs (sequence-tagged sites) were used in PCR experiments to identify corresponding PACs/BACs from libraries FBAC-4434 from GenomeSystemInc and RPCI-11 from Children's Hospital Oakland Research Institute. These were tested by FISH experiments on control slides to verify probe location and status (deleted, duplicated, or single copy) on the inv dup(8p)s, the der(8)s, and the del(8p), using the normal homolog as an internal control. BAC end sequencing was performed to build the breakpoint contigs.

FISH Analysis

FISH analysis was performed on metaphase chromosomes from peripheral blood and/or lymphoblastoid cell lines from control patients, ten inv dup(8p) patients (patients 1–6, 12–14, and 16 in Floridia et al. 1996), two der(8)(pter-p23.1::p23.2-pter) patients (Ohashi et al. 1994; Neumann et al. 1999) and a single del(8p) patient (patient 8 in Giglio et al. 2000). Probe and slide preparation, DNA hybridization, and analysis were performed using conventional methods. At least 20 cells were analyzed by direct microscopic visualization and digital-imaging analysis. Dual-color FISH was performed for interphase FISH experiments to verify if some closely located clones were duplicated both in normal chromosomes 8 and in the inv dup(8p)s. Since all the inv dup(8p)s are deleted for the distal 10 cM, the distal breakpoint was identified by the first probe that was not deleted. The proximal breakpoint was identified by the first probe that showed duplication in the inv dup(8p). Since the duplication regions are separated by an ~5-cM single-copy region, probes around the proximal breakpoint were tested not only in metaphase FISH but also in interphase FISH, using a second probe as a control. With this strategy, we were able to identify as duplicated those probes that could have given a single joined signal in metaphase because of their closeness. The same strategy was used to identify the structure of the der(8), which we suspected to be the reciprocal of the inv dup(8p). Its proximal breakpoint was defined as being between the last clone present and the first clone absent. In the del(8p), the distal breakpoint was identified as the first clone deleted by FISH and the proximal breakpoint as the first not deleted.

FISH analysis was also used for the study of the inversion in mothers of inv dup(8p) patients, in the mother of the der(8p) patient reported by Neumann et al. (1999) and in 72 control subjects of European descent. To this end, FISH was done with GS173o4 (D8S351) and GS257o3 (D8S1130), both of which lie inside the inverted region, and RP11563o19 (D8S1733), ~24 cM proximal to the inverted region.

Clone Isolation by PCR Screening

PCR screening was performed using STSs developed from critical YACs and primers obtained from T7 and SP6 BAC end sequencing. The software package Primer 3 was used to create primers from the sequence information. Standard PCR conditions (35 cycles of 94°C for 30 s, annealing temperature 55°C or 57°C for 30 s, and 72°C for 30 s) were used. Primer pairs were tested and optimized on a monochromosomal hybrid containing chromosome 8, on genomic DNAs from human male and female control subjects, and on genomic DNA from mice and hamsters. The content of clones showing OR

gene cluster FISH signals was verified through PCR with degenerate OR primers, as described by Trask et al. (1998). Confirmation of FISH assignment of clones to the distal repeat (REPD) or the proximal repeat (REPP) was made by colony PCR using STS and BAC end sequence primers.

BAC End Sequencing

BAC DNA was isolated using an automated nucleic acid system (AutoGen 740, Integrated Separation System) or with QIAGEN plasmid Midi kit and purified with Microcon 100 columns (Amicon). One microgram of BAC DNA and 40 pmol of T7 and SP6 primers were used for sequencing with the ABI PRISM big-dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). The sequences of the T7 and SP6 primers and the sequencing reaction time have been reported elsewhere (Matsumoto et al. 1997). Sequence analysis was completed on an ABI 377 automated DNA sequencer.

Pulse Field Gel Electrophoresis (PFGE) analysis

Clone DNA was digested with *Not*I. High-molecular weight DNA was isolated by incubation of agarose plugs from inv dup(8p)s, del(8p), and control lymphoblastoid cell lines (Sambrook et al. 1989). Plugs were then incubated with *Not*I. Digestion products were size fractionated on a 1% agarose gel. For a resolution program of 10 kb–1.5 Mb, the following conditions were used (two blocks): 90 s pulse time, 6 V/cm, 8 h.

Contig Construction

Clone sizing.—Each clone DNA was completely digested with *Not*I and was put in low-melting 1% agarose and electrophoresed by PFGE with use of a CHEF gel apparatus (Biorad). For a resolution program of 5–300 kb, the following conditions were used: 0.2–26.3 s pulse time, 6 V/cm, 12 h.

Restriction mapping.—To define the size of overlap between clones, restriction mapping was performed. BACs and PACs were partially digested with *Eco*RI, *Pst*I, and *Hind*III and were electrophoresed on 0.8% agarose gels at 9V/cm for 5 h.

Bioinformatics.—We conducted database searches through BLAST, using sequenced regions (STSs and cloned BAC ends), and we extended and completed the contigs through NIX. The distal (8p23.1) and the proximal (8p23.2) contig share several sequences; the presence of the specific STS we used in the first screening allowed us to anchor unambiguously the clones to either contig, whereas some BAC end sequences containing OR gene clusters matched both contigs.

Microsatellite Analysis

The consistent maternal origin of inv dup(8p) was previously demonstrated by Floridia et al. (1996). Among the other rearrangements, we could only study the parental origin of the rearrangement in the patient with der(8p) reported by Neumann et al. (1999). DNA was extracted from lymphoblastoid cell lines of the proband and his parents. For polymorphic loci D8S277 and D8S1819, belonging to the distal 10-cM 8p region that is duplicated in the proband, locus information and primer sequences are available from the Genome Database. For all amplifiers, unlabeled reverse primers were purchased from Life Technologies, and forward primers, labeled with 5-FAM, Hex, or Tet dyes, were obtained from MWG Biotech. PCRs were performed using standard protocols (PE Applied Biosystems). Samples were analyzed on an ABI PRISM310 Genetic Analyzer (PE Applied Biosystems). The sizes of the alleles and the areas of the peaks were calculated with GeneScan 3.1 (PE Applied Biosystems). Statistical analysis was performed with SPSS 4.0. To assess whether duplication had occurred at any given locus, a quantitative analysis was performed. DNAs were amplified, and samples were collected at several points, depending on the kinetics of amplification of each amplifier. The area of each allelic peak (a measure of the amount of amplified material) and the ratio ($R_{A1/A2}$) between the areas of the shortest (A1) and the longest (A2) allele were calculated. Analysis of samples at multiple points during amplification helped to minimize PCR artifacts and gave additional information about the kinetics of amplification at each locus.

Results

Comparison between experimental data in 10 inv dup(8p) patients and *in silico* data, allowed us to build the two breakpoint contigs, as shown in figure 1. All probes at and around the breakpoints (REPD and REPP in fig. 1) gave a consistent pattern of FISH signals not only on chromosome 8 but also on several other chromosomes and mimicked that of the OR gene clusters (Trask et al. 1998). Sequencing and *in silico* studies demonstrated that REP probes have high sequence homology. Although all STSs recognizing REPP clones did not recognize the REPD ones, and vice versa (in colony PCR experiments), the two REPs share a 95%–97% degree of homology, as demonstrated by *in silico* data and by the finding that end primers from some REPD clones recognized clones belonging to REPP.

On normal chromosomes 8, these probes identified two repeats (REPD: distal repeat; REPP: proximal repeat) of ~400 kb each. Each of the REP probes gave two sets of signals, at 8p23.1 and 8p23.2, that were

identifiable in prometaphase and interphase, whereas, in metaphase, a single, very large signal was usually seen (figs. 2a, 2b). The inv dup(8p)s showed a single set of signals, clearly smaller with probes GS42i21 and GS214h7, the first clones not deleted, which should thus encompass the distal breakpoint (figs. 2a, 2b). The proximal breakpoint should be within GS38o7, the first clone that shows a duplicated signal (data not shown).

Molecular definition of the +der(8p) patients, demonstrated that the marker is the reciprocal of the inv dup(8p). In fact, clones in red (fig. 1), which are deleted in the inv dup(8p), appeared duplicated in the der(8p), OR probes GS42i21 and GS214h7 gave clearly bigger signals (fig. 2c) and GS38o7 was the last probe present. All probes included between the two REPs, from GS173o4 to GS257o3 (fig. 1), showed single-copy signals. The structure of the marker was clearly demonstrated by two-color FISH with GS77p24 (D8S1695, D8S265) in single copy and GS143G5 (D8S1819) duplicated (square, fig. 2c). Microsatellite analyses of one of the der(8p) patients and of his parents demonstrated the maternal origin of the marker (data not shown).

Since inv dup(8p)s consistently originate in maternal meiosis (Floridia et al. 1996), and since the single +der(8p) we could investigate also was of maternal origin, we studied the critical 8p region in eight inv dup(8p) mothers and in the +der(8p) mother. We found that in their metaphase chromosomes the FISH signals for GS173o4 and GS257o3, that delimit the region between REPD and REPP, were inverted in one homolog in all mothers (fig. 3). The same submicroscopic inversion was found in a heterozygous state in 19 (26%) of 72 normal control subjects of European descent. Initial evidence for a submicroscopic inversion polymorphism in 8p came from examination of meiotic product in the CEPH reference families (K.W.B. and J.L.W., unpublished observation).

We also mapped the breakpoint of the interstitial del(8p) patient, predicting that the same 8p-REPs mediating the inv dup(8p) and the der(8p) might well mediate this deletion. In fact, all probes included between the two REPs, from GS173o4 to GS257o3 (fig. 1), were deleted by FISH in the del(8p) whereas GS110p23 was present. Thus GS257o3 and GS110p23 flank the proximal breakpoint. The distal breakpoint should be within GS42i21 and GS214h7 which shows smaller and single FISH signals in the del(8p) (fig. 2d). Probes distal to REPD (GS143g5 and GS29g18) and proximal to REPP (RP11-813l8 and CIT-HSP2244f17) were not deleted.

Discussion

These results demonstrate that homologous recombination between the two 8p-REPs is responsible for four chromosome rearrangements. Although exact break-

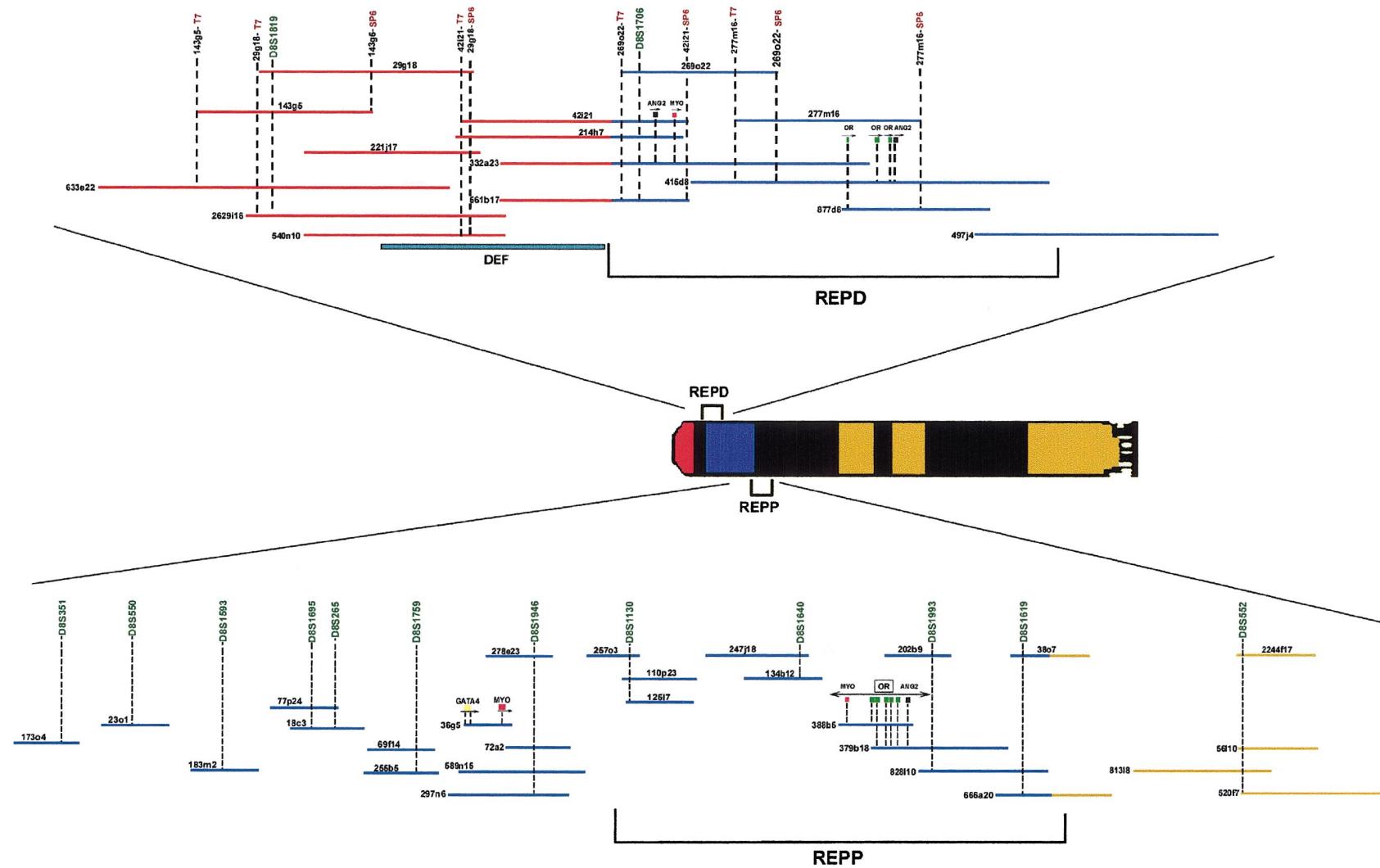


Figure 1 Inv dup(8p) breakpoint contigs. *Middle*, 8p ideogram. Shown in red is the region deleted in inv dup(8p); in blue, the region in single copy; and in brown, the region that can be totally or partially duplicated (Floridia et al. 1996). REPD = distal repeat; REPP = proximal repeat. All clones inside REPs give an OR FISH pattern. *Top* and *bottom*, STSs (in green) and BAC ends (SP6 and T7) anchored to the respective clones. Red/blue and blue/brown clones indicate the inv dup(8p) and the der(8p) breakpoints (see text). In the interstitial del(8p), red clones lie in the nondeleted region, red/blue clones indicate the distal breakpoint, and blue clones in REPD and between REPD and REPP up to 257o3 are deleted; all the proximal blue and brown clones are present. Colored squares indicate genes and pseudogenes found in the region. GATA4 = GATA-binding protein 4 gene; MYO (large square) = myosin gene; MYO (small squares) = myosin pseudogenes; OR = olfactory receptor genes, pseudogenes, and gene fragments; ANG2 = angiotensin II genes; and DEF = defensin genes (other defensins are located 100–150 kb proximal to REPD).

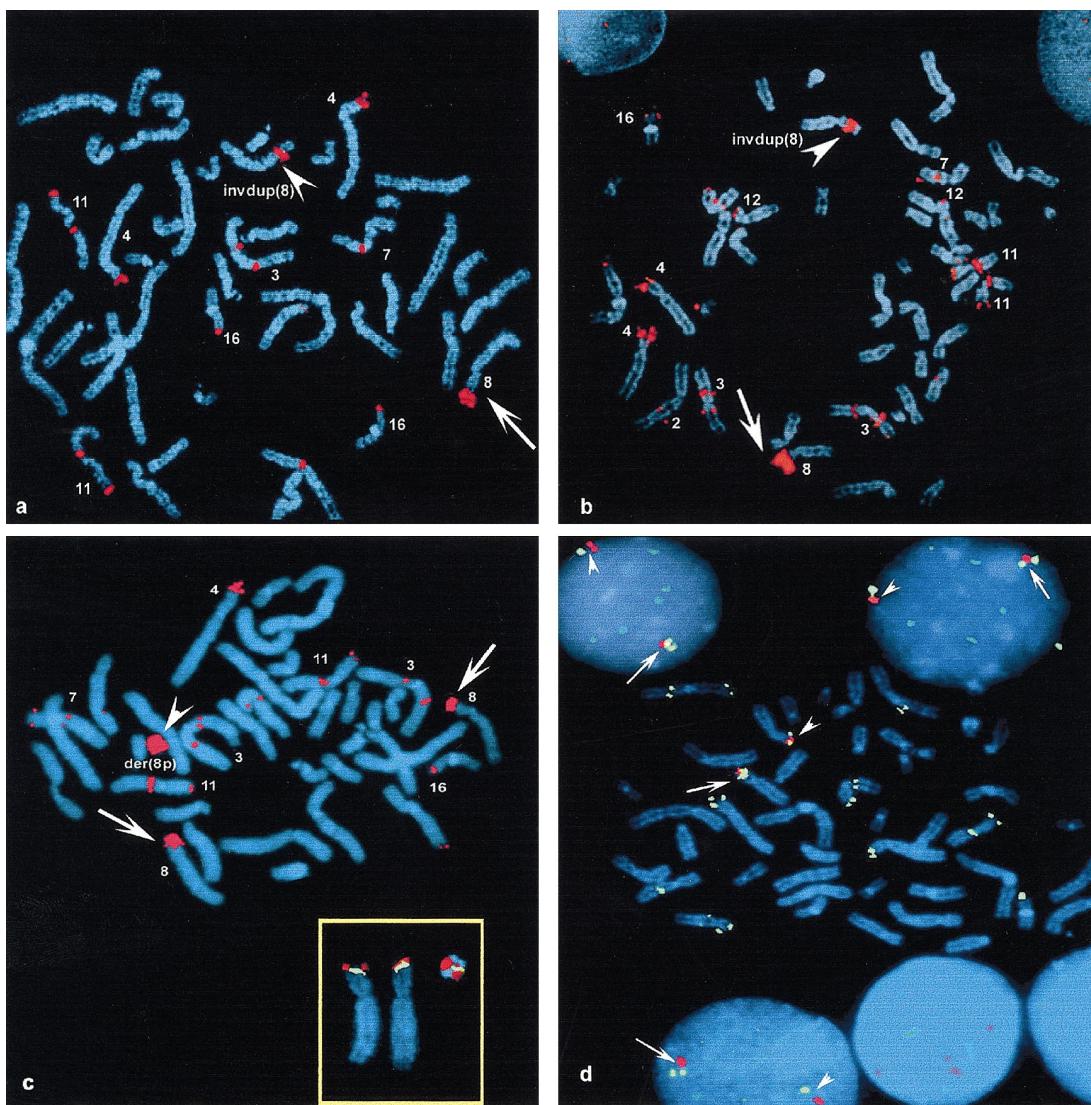


Figure 2 *a* and *b*, FISH with GS214h7 (*a*) and GS42i21 (*b*) in a prometaphase and a metaphase, respectively, from an inv dup(8p) subject. The two clones show a double set of signals (*a*) and a large signal (*b*) on the normal chromosome 8 (arrows) and a single smaller signal on the inv dup(8p)s (arrowheads). These BACs, which partially overlap and lie at the distal edge of REPD, also hybridize to other chromosomes (identified with numbers) containing OR gene clusters. *c*, FISH with GS42i21 in a metaphase from a subject with +der(8p). The signal pattern is the same as in *a* and *b*; the +der(8p) shows very large signals. Box, cut-out of chromosomes 8 and +der(8p), showing duplication of the red region of the contig in figure 1 (GS143g5) and monosomy of the contig's blue region (GS77p24). *d*, FISH in a metaphase from the del(8p) subject with GS42i21 (green) and GS143g5 (red), the latter mapping distal to the two REPs. In the del(8p) (arrowhead), the green signal of the OR gene cluster is single and small, whereas, in the normal 8 (arrow), it is double and big. This FISH pattern is visible both in metaphase and interphase nuclei.

point definition has not been determined, because of high sequence homology of the two REPs, the inv dup(8p), +der(8p), and inv(8p) breakpoints should fall at the red/blue and blue/brown contig clones (fig. 1), so that the two recombinant OR clusters are almost eliminated in the inv dup(8p) (fig. 4*a*), whereas both of them are present in the der(8p) (fig. 4*b*). In the del(8p), the distal breakpoint coincides with that of the inv dup(8p) and +der(8p), whereas the proximal breakpoint falls at

D8S1130. Accordingly, REPD is almost completely eliminated by the deletion. Experimental and *in silico* data demonstrated that the two REPs contain a repeat unit formed by MYO-ORs-ANG2 (fig. 1), flanked and embedded among repeats belonging mainly to the long interspersed nuclear element (LINE) and short interspersed nuclear element (SINE) families and to other DNA sequences. Both the repeat unit and the other sequences are common to several OR gene clusters (Brand-Arpon

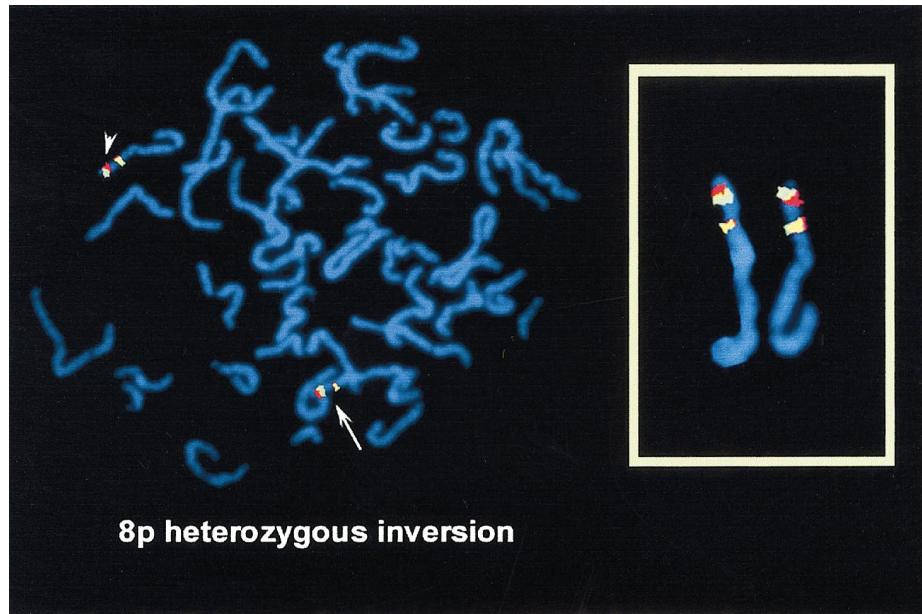


Figure 3 Metaphase from the mother of a subject with inv dup(8p), showing the normal (arrow) and the inverted (arrowhead) chromosomes 8 (magnified in the box). FISH was done with GS173o4 (red, D8S351), GS257o3 (green, D8S1130), both inside the inverted region (see fig. 1), and RP11-563o19 (yellow, D8S1733), ~24 cM proximal to the inverted region.

et al. 1999; Glusman et al. 2000; S.G. and V.C., unpublished observation). Although all the rearrangement breakpoints fall inside the two 8p-REPs, they seem to fall outside the repeat unit.

Several recurrent chromosomal changes such as deletions, duplications or inversions, are due to unequal homologous recombination at meiosis between region-specific low-copy repeats (duplicons) flanking the rearranged region (Lupski et al. 1998; Ji et al. 2000). These duplicons are comprised of genes, pseudogenes, and gene fragments as in the OR clusters (Brand-Arpon et al. 1999; Glusman et al. 2000). The type of rearrangement is predominantly defined by the orientation of recombining duplicons and the number of crossovers (Small et al. 1997; Lupski et al. 1998; Ji et al. 2000). Deletions and reciprocal duplications result from misalignment of direct repeats and a single crossover between the distal and the proximal repeat. Inversions result through mispairing of inverted repeats and a double recombination event between them (Small et al. 1997; Saunier et al. 2000). In fact, a single crossover would produce a dicentric and an acentric product (Small et al. 1997).

8p-REPs mediate a deleted chromosome, two inverted duplicated chromosomes—the inv dup(8p) and the der(8p), the first of which originates as a dicentric (Floridia et al. 1996; see also fig. 3a)—and a submicroscopic inversion. Thus, recombining sequences may lie in both orientations, at least in one cluster. Such a sit-

uation has been reported at the NPHP1 locus in 2q13 (Saunier et al. 2000), in which the NPHP1 deletion is mediated by direct repeats and the benign inversion is mediated by inverted repeats, respectively flanking and including the two direct repeats. In the OR gene clusters, a unidirectional organization has been found in the 3p13 cluster (Brand-Arpon et al. 1999) and a complex bidirectional one on 17p (Glusman et al. 2000) with OR genes oriented cen→tel, interspersed with genes oriented tel→cen. We found that both 8p-REPs have a unidirectional organization largely overlapping that of the 3p13 cluster (Brand-Arpon et al. 1999) and of other OR clusters (S.G. and V.C., unpublished observation). Consistent data demonstrate that the REPD order is tel-ANG2-MYO-ORs-ANG2-cen, whereas the inconsistent Genome Database information concerning the proximal region prevented determination of whether the orientation of REPP is tel-MYO-ORs-ANG2-cen or vice versa. The orientation of the other LINE and SINE repeats also is unknown. Thus, at the moment, no obvious explanation can be offered for the formation of rearrangements requiring both repeats with the same orientation and repeats with opposite orientation.

The finding that inv dup(8p) and der(8p) mothers are heterozygous for a benign inversion polymorphism indicates that, at pachytene, asynapsis at the inverted region promotes the refolding of one chromosome onto itself, favoring the formation of the inv dup(8p) and the der(8p) (fig. 4). After the Yp inversion that predisposes

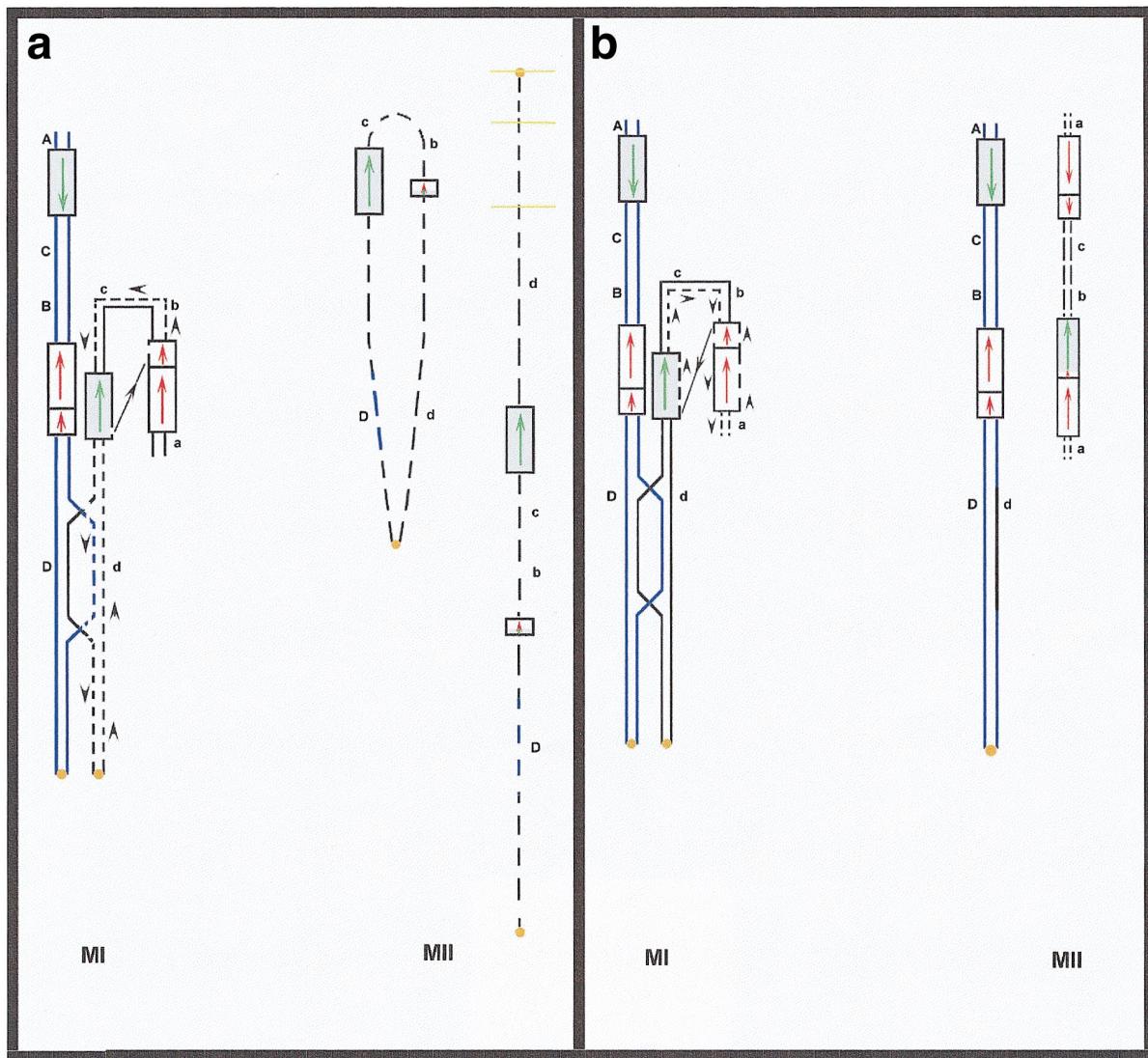


Figure 4 Ideogram showing the mechanism of origin of the inv dup(8p) and of the +der(8p) at pachytene. Only the short arm of chromosome 8 is shown. *a*, At the first meiotic division (MI), the two maternal homologous chromosomes 8 (blue and black) undergo canonic recombination along the synapsed portion (in all informative inv dup(8p)s, part of the duplication region contains both maternal alleles [Florida et al. 1996]). The region delimited by the two REPs (boxes) is inverted in the blue chromosome. Presence of heterozygous inversion leads to homologous synapsis interruption and to the refolding of the black chromosome, allowing intrachromatid synapsis and ectopic recombination between the two REPs (recombination is indicated by arrowheads). Red and green arrows show the orientation of the sequences inside each REP. As can be seen in fig. 1, two angiotensin II genes with the same orientation tel→cen, are present at the proximal and the distal portion of REP. The orientation of REPP is hypothetical. Since a single FISH signal is visible in the inv dup(8p) with probes related to the OR gene clusters (see text), ectopic recombination should take place between the proximal portions of REPP and the distal one of REP, thus leading to a very small OR cluster in the recombinant chromosome (hatched line). At MII, the recombinant chromosome (middle) shows a normal size gray OR cluster and a very small white/gray cluster. At anaphase II (right), when the centromere divides, the two chromatids, linked together, can join the opposite poles, provided that a breakage between the two centromeres occurs. If the breakage occurs at the level of one of the yellow lines, an inv dup(8p) is formed whose duplication size is determined by the position of this breakage. *b*, The same mechanism that produces the inv dup(8p) is responsible for the supernumerary der(8p) formation. The type of recombination is shown by arrowheads. The other chromosome 8 (blue) presumably undergo canonic recombination in the portion proximal to the inversion. This chromosome and the der(8p) segregate together in the oocyte. After anaphase II, the egg will contain a normal chromatid 8 and the der(8p). Formation of a neocentromere in the der(8p) will assure its preservation.

to translocations producing XX males and XY females (Jobling et al. 1998), this is the second polymorphism giving susceptibility to chromosome rearrangements. Two observations suggest that heterozygous females have only a low risk of having children with inv dup(8p): (1) heterozygotes are common in the population, whereas the rearrangement is rare; and (2) none of the >50 subjects reported to date with inv dup(8p) have sibs with the same rearrangement.

Since human OR gene clusters are located on almost all human chromosomes and some chromosomes contain more than one cluster (Trask et al. 1998; Mombaerts 1999), the role of OR gene clusters in mediating chromosomes abnormalities is likely not limited to the four 8p rearrangements. Perhaps the most striking coincidence is that with the t(4p;8p)(p15;p22) breakpoints (Wieczorek et al. 2000). In fact, analysis of four unrelated patients with this chromosome abnormality showed preliminary evidence that the translocation is indeed mediated by OR gene clusters (S.G., unpublished data).

In conclusion, our data demonstrate not only the involvement of OR gene clusters in mediating common chromosome rearrangements, but also the fact that at least some of the recurrent rearrangements are due to specific genomic polymorphisms, thus indicating the possibility of developing a profile of the individual risk of having progeny with chromosome rearrangements.

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Electronic-Database Information

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Center for Genome Research, STS-Based Map of the Human Genome, http://carbon.wi.mit.edu:8000/cgi-bin/contig/phys_map

Center for Medical Genetics, Marshfield Medical Research Foundation, <http://research.marshfieldclinic.org/genetics/>
Genome Database, <http://gdbwww.gdb.org/> (for locus information and primer sequences)

NIX-Identity Unk nown Nucleic Sequence, <http://www.hgmp.mrc.ac.uk/Registered/Webapp/nix/>

Primer 3 Input, <http://www.genome.wi.mit.edu/cgi-bin/primer/primer3-www.cgi> (for Primer 3 software)

YAC Screening Center, DIBIT, Milan, <http://www.spr.it/iger/home.html> (for CEPH mega-YACs)

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