The Variant inv(2)(p11.2q13) Is a Genuinely Recurrent Rearrangement but Displays Some Breakpoint Heterogeneity

Ina Fickelscher, Thomas Liehr, Kathryn Watts, Victoria Bryant, John C. K. Barber, Simone Heidemann, Reiner Siebert, Jens Michael Hertz, Zeynep Tümer, and N. Simon Thomas

Human chromosome 2 contains large blocks of segmental duplications (SDs), both within and between proximal 2p and proximal 2q, and these may contribute to the frequency of the common variant inversion inv(2)(p11.2q13). Despite their being cytogenetically homogeneous, we have identified four different breakpoint combinations by fluorescence in situ hybridization mapping of 40 cases of inv(2)(p11.2q13) of European origin. For the vast majority of inversions (35/40), the breakpoints fell within the same spanning BACs, which hybridized to both 2p11.2 and 2q13 on the normal and inverted homologues. Sequence analysis revealed that these BACs contain a significant proportion of intrachromosomal SDs with sequence homology to the reciprocal breakpoint region. In contrast, BACs spanning the rare breakpoint combinations contain fewer SDs and with sequence homology only to the same chromosome arm. Using haplotype analysis, we identified a number of related family subgroups with identical or very closely related haplotypes. However, the majority of cases were not related, demonstrating for the first time that the inv(2)(p11.2q13) is a truly recurrent rearrangement. Therefore, there are three explanations to account for the frequent observation of the inv(2)(p11.2q13): the majority have arisen independently in different ancestors, while a minority either have been transmitted from a common founder or have different breakpoints at the molecular cytogenetic level.

Pericentric inversions are among the most frequent chromosome rearrangements in humans, with a combined frequency of 1%–2%.[1] Although the majority have unique cytogenetic breakpoints, a small number of pericentric inversions are potentially recurrent, with apparently the same breakpoints.[2] When those where both breakpoints lie within centromeric heterochromatin are excluded, the most common pericentric inversion is the inv(2)(p11.2q13),[3] with an estimated frequency between 1 in 500 and 1 in 2,500.[4–7] The inv(2)(p11.2q13) has no direct phenotypic consequences and is considered a polymorphic variant.[5,6,8]

The pericentromeric region of human chromosome 2 contains a remarkable number of highly identical inter- and intrachromosomal segmental duplications (SDs).[9] Human chromosome 2 was formed by the end-to-end fusion of two ancestral chromosomes, and, consequently, the region encompassing the fusion site in 2q13-2q14 contains residual telomeric repeat sequences.[10,11] In addition, 2p11.2 and 2q13 are both sites of human copy-number variation.[12,13] Therefore, the sequence composition of this region makes it prone to genomic instability and the formation of large-scale chromosome rearrangements.[14]

Several hundred cases of inv(2)(p11.2q13) have been reported in the literature, with a wide geographic distribution. However, recurrent detection of chromosome rearrangements does not necessarily mean recurrent formation. For example, we have recently demonstrated that another common pericentric inversion, inv(10)(p11.2q21), appears to be a unique rearrangement with a single ancestral founder.[15] Three explanations may account for the high frequency of inv(2)(p11.2q13): (1) although cytogenetically homogeneous, the inversion represents a collection of similar but unrelated rearrangements; (2) the inversion is genuinely recurrent and has arisen multiple times in different populations; or (3) the inversion has been transmitted identical by descent (IBD) from a single common ancestor or a small number of them. To distinguish among these possibilities, we have applied FISH mapping to characterize the breakpoints in a large series of pericentric inversions involving chromosome 2 and used haplotype analysis to determine what proportion arose as independent mutations and what proportion were transmitted IBD.

Inversion carriers were initially identified by G banding of metaphase chromosomes after referral to a genetics laboratory for karyotype analysis (table 1). In all cases, the presence of the inversion was considered coincidental to the clinical reason for referral. In total, the study population comprised 54 independently ascertained families. Of those cases where parental samples were available, 30 were familial and one was de novo. There were 49 families with the "variant" inversion—that is, breakpoints designated as inv(2)(p11.2q13). Of these, four had an addi-

From the Institut für Humangenetik und Anthropologie, Friedrich-Schiller University, Jena, Germany (I.F.; T.L.); Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, United Kingdom (K.W.; V.B.; J.C.K.B.; N.S.T.); Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, Kiel, Germany (S.H.; R.S.); Department of Clinical Genetics, Aarhus University Hospital, Aarhus, Denmark (J.M.H.); and Wilhelm Johannsen Center for Functional Genome Research, University of Copenhagen, Copenhagen (Z.T.)

Received April 23, 2007; accepted for publication June 28, 2007; electronically published August 28, 2007.

Address for correspondence and reprints: Dr. N. Simon Thomas, Wessex Regional Genetics Laboratory, Salisbury NHS Foundation Trust, Salisbury District Hospital, Salisbury SP2 8TE, United Kingdom. E-mail simon.thomas@salisbury.nhs.uk

Am. J. Hum. Genet. 2007;81:847–856. 0002-9297/2007/8104-0029$15.00
DOI: 10.1086/521226

Table 1. Study Population

Case	DNA ID	Country	Origin	Karyotype	Analysis	FISH Class	Haplotype
01	Ger1	Germany	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Unique
02	Ger3	Germany	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Unique
03	Ger5	Germany	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Unique
04	Ger6	Germany	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Unique
05	Ger7	Germany	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Unique
06	Ger11	Germany	Familial	inv(2)(p11.2q13), inv(9)(p11q13)	FISH + PCR	i	Group A
07	Ger20	Austria	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Group A
08	Ger14	Poland	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Unique
09	Ger15	Poland	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Unique
10	Ger17	Austria	Unknown	inv(2)(p11.2q13)	FISH + PCR	i	Unique
11	Ger18	Austria	Unknown	inv(2)(p11.2q13)	FISH + PCR	i	Unique
12	UK11	UK	Unknown	inv(2)(p11.2q13)	FISH + PCR	i	Unique
13	DK2	Denmark	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Unique
14	DK5	Denmark	Familial	inv(2)(p11.2q13)	FISH + PCR	i	Unique
15	…	Austria	Familial	inv(2)(p11.2q13)	FISH only	i	…
16	…	Germany	Unknown	inv(2)(p11.2q13)	FISH only	i	…
17	…	Belgium	Unknown	inv(2)(p11.2q13)	FISH only	i	…
18	…	Belgium	Familial	inv(2)(p11.2q13)	FISH only	i	…
19	…	Belgium	Unknown	inv(2)(p11.2q13)	FISH only	i	…
20	…	Belgium	Familial	inv(2)(p11.2q13)	FISH only	i	…
21	…	Germany	Familial	inv(2)(p11.2q13)	FISH only	i	…
22	…	Germany	Familial	inv(2)(p11.2q13)	FISH only	i	…
23	…	Germany	Unknown	inv(2)(p11.2q13)	FISH only	i	…
24	…	France	Unknown	inv(2)(p11.2q13)	FISH only	i	…
25	…	Israel	Unknown	inv(2)(p11.2q13)	FISH only	i	…
26	…	Germany	Unknown	inv(2)(p11.2q13)	FISH only	i	…
27	…	Germany	Unknown	inv(2)(p11.2q13)	FISH only	i	…
28	…	Germany	Familial	inv(2)(p11.2q13)	FISH only	i	…
29	…	Germany	Familial	inv(2)(p11.2q13)	FISH only	i	…
30	…	Germany	Familial	inv(2)(p11.2q13)	FISH only	i	…
31	…	UK	Unknown	inv(2)(p11.2q13)	FISH only	i	…
32	…	UK	Unknown	inv(2)(p11.2q13)	FISH only	i	…
33	…	UK	Unknown	inv(2)(p11.2q13)	FISH only	i	…
34	…	Germany	Unknown	inv(2)(p11.2q13), t(5;8)(q22;p11.2)	FISH only	i	…
35	Ger16	Belgium	Unknown	inv(2)(p11.2q13), inv(2)(p11.2q13)	FISH + PCR	i, i	Group E
36	…	Germany	Familial	inv(2)(p11.2q13)	FISH only	ii	…
37	…	Germany	Unknown	inv(2)(p11.2q13), +18	FISH only	ii	…
38	DK1	Denmark	Familial	inv(2)(p11.2q13)	FISH + PCR	iii	Group B
39	Ger21	Germany	Unknown	inv(2)(p11.2q13)	FISH + PCR	iii	Group B
40	UK1	UK	Familial	inv(2)(p11.2q13)	FISH + PCR	iv	Group C
41	UK4	UK	Unknown	inv(2)(p11.2q13)	PCR only	…	Group C
42	UK8	UK	Familial	inv(2)(p11.2q13)	PCR only	…	Group C
43	Ger2	Germany	Familial	inv(2)(p11.2q13)	PCR only	…	Unique
44	Ger4	Albania	Familial	inv(2)(p11.2q13)	PCR only	…	Unique
45	UK6	UK	Unknown	inv(2)(p11.2q13)	PCR only	…	Group D
46	UK10	UK	Familial	inv(2)(p11.2q13)	PCR only	…	Group D
47	UK3	UK	Familial	inv(2)(p11.2q13)	PCR only	…	Unique
48	UK5	UK	Unknown	inv(2)(p11.2q13)	PCR only	…	Unique
49	UK7	UK	Unknown	inv(2)(p11.2q13)	PCR only	…	Unique
50	UK2	UK	Familial	**inv(2)(p11.2q14.1)**	FISH + PCR	v	Unique
51	DK3	France	De novo	**inv(2)(p11.2q14)**	PCR only	…	Unique
52	DK4	Norway	Unknown	**inv(2)(p11.2q14)**	PCR only	…	Unique
53	…	Germany	Familial	**inv(2)(p11q21.1)**	FISH only	vi	…
54	UK9	UK	Familial	**inv(2)(p12.2q14.3)**	PCR only	…	Unique

NOTE.—Cases 50–54 (karyotype in bold) have nonvariant pericentric inversions.

tional structural or numerical chromosome abnormality, including one case (Ger16) with a pericentric inversion of both chromosome 2 homologues. All other cases were heterozygous for the inversion. The study also included five families with a "nonvariant" pericentric inversion of chromosome 2, having a breakpoint in or very close to 2p11.2 but with a long-arm breakpoint distal to 2q13.

BAC clones for FISH mapping were selected from the 37k clone set by use of Ensembl Cytoview, and standard techniques were used for probe labeling, detection, and

Table 2. FISH Characterization of Inversion Breakpoints

Class, Karyotype, No. of Cases, Chromosome Arm, and BAC	BAC Result	Location (kb)
i, inv(2)(p11.2q13) (n = 35):		
2p:		
RP11-81F3	Normal	86,721–86,889
RP11-153P14	Split	87,386–87,542
RP11-50B16	Inverted	88,147–88,311
2q:		
RP11-438K19	Inverted	111,492–111,673
RP11-1429F20	Split	112,145–112,356
RP11-80K12	Split	112,116–112,283
RP11-399B17	Normal	112,563–112,728
ii, inv(2)(p11.2q13) (n = 2):		
2p:		
RP11-316G9	Normal	89,562–89,773
RP11-433C18	Split	89,902–89,958
2q:		
RP11-1429F20	Split	112,145–112,356
iii, inv(2)(p11.2q13) (n = 2):		
2p:		
RP11-269K22	Normal	86,591–86,755
RP11-81F3	Split	86,721–86,889
RP11-223J6	Inverted	87,235–87,386
2q:		
RP11-528G9	Inverted	110,255–110,349
RP11-404O21	Split	111,009–111,101
RP11-480O8	Normal	111,110–111,259
iv, inv(2)(p11.2q13) (n = 1):		
2p:		
RP11-316G9	Split	89,562–89,773
RP11-433C18	Split	89,902–89,958
2q:		
RP11-67L14	Inverted	113,259–113,410
RP11-395L14	Normal	113,968–114,143
v, inv(2)(p11.2q14.1) (n = 1):[a]		
2p:		
RP11-548D17	Normal	83,448–83,632
RP11-312D1	Inverted	85,154–85,341
vi, inv(2)(p11.2q21) (n = 1):[a]		
2p:		
RP11-156D20	Normal	Unknown
RP11-368I13	Split	~91,400
RP11-721A22	Split	91,460–91,590

[a] Long arm breakpoint distal to 2q13.

analysis.[16] DNA was available from at least one inversion carrier for 31 of the 54 families. Table 1 details which analyses were performed on each inversion family. Haplotype analysis was performed using 24 microsatellites located within the inverted region. Additional loci outside of this interval were also tested on a subset of related cases. Microsatellites were selected from the human Genome Database and Ensembl, and fluorescently labeled PCR products were analyzed on an ABI 3100 sequencer.

Cell suspensions were available on at least one carrier of variant inv(2)(p11.2q13) from each of 40 families. Although these were apparently homogeneous cytogenetically, FISH mapping identified four different breakpoint combinations. The majority of the inv(2)(p11.2q13) variants, 35 of 40, had the same or closely related breakpoints in both 2p11.2 and 2q13, with a split signal in the BAC clones RP11-153P14 and RP11-1429F20, respectively. These are referred to as "class i breakpoints." See table 2 for full classification details. Although these BACs showed split signals on both the normal and the inverted chromosomes, it was possible to differentiate the two homologues, because of the difference in intensity of the 2p and 2q signals (fig. 1*a*). The breakpoints of a further four inv(2)(p11.2q13) have also been analyzed by Goidts et al., and, in all four cases, the breakpoint-spanning BACs overlapped with RP11-153P14 and RP11-1429F20,[13] corresponding to our class i.

There were three other rare breakpoint combinations (defined as classes ii, iii, and iv) among the remaining five variant inv(2)(p11.2q13) (fig. 1*b* and 1*c*). All four variant inversion classes were approximately the same size and spanned 22–25 Mb of DNA. The short-arm breakpoints of two nonvariant inversions in 2p11.2 were also characterized and shown to be unique. These are designated as classes v and vi.

The class i and ii inversions appear to share a common breakpoint on 2q13 within BAC RP11-1429F20, and the short-arm breakpoints of the class ii and iv inversions are close together, around the BAC RP11-433C18, but not identical. Thus, in total, we have identified six different breakpoints in 2p11.2 and three different breakpoints in 2q13.

Haplotype analysis was used to measure the degree of allele sharing between families to determine whether the inversions arose independently or were IBD. Theoretically, inversion haplotypes that are completely conserved or differ at only a small number of loci are likely to be IBD. In contrast, inversion haplotypes with only limited allele sharing are likely be independent. This approach could potentially misclassify inversions with a very ancient origin (such that large numbers of microsatellite mutations could accumulate) and inversions in which two crossovers had occurred (resulting in balanced progeny but with loss of the ancestral haplotype). However, such double recombinants are very unlikely, because the class i inversion covers a genetic distance of only 12 cM (sex averaged).

To interpret the haplotype data, the background level of allele sharing due to chance was first estimated by comparison of the variant inversions with four of the nonvariants. Since these rearrangements have different breakpoints, they must be independent. Allele sharing was observed at 6–18 of the 24 loci tested (table 3). Statistically, the mean of all comparisons was 12.07, and the standard deviation was 2.80. The expected range of allele sharing, calculated as the mean ± 2.5 standard deviations, is 5.06–19.67.

For the majority of the inv(2)(p11.2q13) variants, allele sharing was in the same range as the estimated background level (table 4). Thus, they are unlikely to be related to each other, demonstrating for the first time that the inv(2)(p11.2q13) is a genuinely recurrent rearrangement.

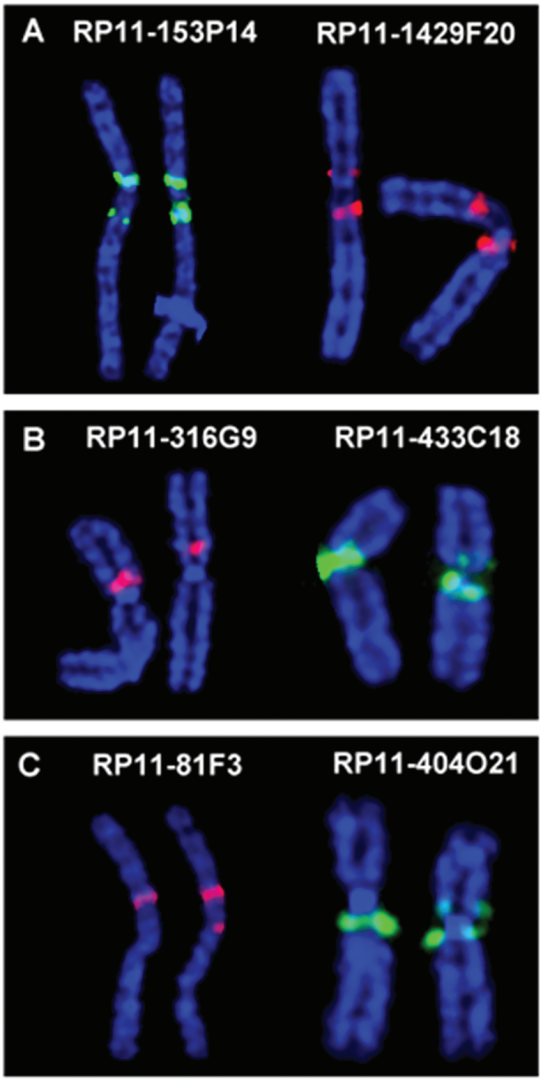

Figure 1. *A,* Spanning BACs for class i inversion breakpoints in 2p11.2 and 2q13. Probe RP11-153P14 (*green*) shows an intense signal in 2p11.2 and a much smaller hybridization signal in 2q13 on the normal chromosome 2 (*left*). In contrast, the inverted homologue shows a much more intense signal in 2q13 (*right*). Probe RP11-1429F20 is labeled in red. The normal chromosome 2 (*left*) shows a large signal on 2q13 and a smaller signal on 2p11.2. In contrast, the inverted homologue (*right*) shows two signals of approximately equal intensity, one on each chromosome arm. *B,* Spanning BACs for class ii inversion breakpoint in 2p11.2. Probe RP11-316G9 (*red*) shows only a single intense signal on both the normal chromosome 2 (*left*) and on the inverted homologue (*right*) in 2p11.2, outside the inversion. Probe RP11-433C18 (*green*) shows only one intense signal on the normal chromosome 2 (*left*). In contrast, the inverted homologue (*right*) shows an asymmetric split signal with a predominant signal in 2q13. *C,* Spanning BACs for class iii inversion breakpoints in 2p11.2 and 2q13. RP11-81F3 (*red*) shows no cross hybridization on the normal chromosome 2 (*left*).The inverted homologue (*right*) shows an asymmetric split signal with the smaller signal in 2q13. RP11-404O21 (*green*) shows a intense signal in 2q13 on the normal chromosome 2 (*left*). The inverted homologue (*right*) shows two signals of approximately equal intensity, one on each chromosome arm.

In only five cases has a pericentric inversion of chromosome 2 been shown to arise de novo[7,8,17,present study]; therefore, the high proportion of independent inversions is likely due to lack of selection against inversion carriers rather than to a high rate of new mutations. In contrast with class i, the other breakpoint combinations were rare; these inversions are likely to have arisen from single mutation events and to be IBD in all carriers.

Only 9 of the 27 inv(2)(p11.2q13) families appear to be related, with shared alleles at ⩾22 of the 24 loci. The related families formed four distinct groups, each with a unique haplotype (A, B, C, and D) (table 5). The group A inversion has a class i breakpoint, the group B inversion a class iii breakpoint, and the group C inversion a class iv breakpoint. The breakpoints of the group D inversion were not determined, and DNA was unavailable from the two families with class ii inversions.

The families in each haplotype group were generally from similar geographical locations. Thus, inv(2)(p11.2q13) displays a limited founder effect within specific population groups. The relative contribution of independent cases and founder effects is likely to depend upon the population being tested.

One interesting observation from the genotyping data is that the two inversions identified in the homozygous carrier (Ger16) had almost identical haplotypes: Ger16 was homozygous at 22 of 24 microsatellite loci tested within the class i inverted region (table 5). The region of homozygosity extended beyond the 2q13 inversion boundary into 2q14 but not distal to the 2p11.2 breakpoint. Elsewhere on chromosome 2, no excess of homozygosity was observed. The heterozygous loci within the inversion itself make segmental uniparental isodisomy and mitotic duplication/gene conversion unlikely. A de novo event cannot be excluded, but the parents would still have to carry nearly identical haplotypes in proximal chromosome 2. The most likely explanation is that one inv(2)(p11.2q13) was transmitted from each parent and the inversion derives from the same ancestral founder. The Ger16 inversion haplotype is distinct from haplotypes A–D and so represents a possible fifth haplotype group (group E).

Recombination, which would normally be low in pericentromeric regions, appears to be completely suppressed within the inverted segment. Unbalanced recombinant products involving an inv(2)(p11.2q13) are very rare and have involved crossing-over adjacent to, rather than within, the inverted segment.[18,19] Typing of microsatellites outside the inverted segment showed that recombination suppression appeared to extend for variable distances beyond the breakpoints (table 5). Two of the group C families shared the same haplotype well beyond the inversion breakpoints, particularly on proximal 2p. In the third group C family, recombination must have occurred just distal to the breakpoints on both 2p11.2 and 2q13 to break up the ancestral haplotype. In group D, the conserved haplotype extends beyond the inverted region on both

Table 3. Level of Allele Sharing between Variant and Nonvariant Inversion Carriers

	No. of Alleles Shared																															Standard
	UK2	UK9	DK3	DK4	UK1	UK3	UK4	UK5	UK6	UK7	UK8	UK10	UK11	Ger1	Ger2	Ger3	Ger4	Ger5	Ger6	Ger7	Ger11	Ger14	Ger15	Ger16	Ger17	Ger18	Ger20	Ger21	DK1	DK2	DK5	Mean	Deviation
UK2		11	14	15	10	17	10	12	7	15	10	8	16	9	11	11	12	15	11	13	14	12	15	7	14	13	15	11	11	13	16	12.267	2.677
UK9	11		17	11	7	13	7	11	9	10	7	11	16	11	16	12	12	15	13	12	13	12	16	11	11	14	13	8	8	13	11	11.700	2.731
DK3	14	17		13	11	12	11	11	8	14	11	10	10	14	13	12	11	14	13	15	14	10	14	6	10	11	12	11	10	13	13	11.933	2.212
DK4	15	11	13		9	12	9	8	7	16	9	8	15	11	12	14	12	17	14	14	17	18	16	11	13	12	18	6	7	10	17	12.367	3.518
All comparisons																																12.067	2.801

NOTE.—Comparison of allele sharing between variants and nonvariants. Each figure indicates the number of shared alleles between inversion haplotypes in any two families.

Table 4. Level of Allele Sharing between Variant Inversion Carriers

	No. of Alleles Shared																									
	UK1	UK3	UK4	UK5	UK6	UK7	UK8	UK10	UK11	Ger1	Ger2	Ger3	Ger4	Ger5	Ger6	Ger7	Ger11	Ger14	Ger15	Ger16	Ger17	Ger18	Ger20	Ger21	DK1	DK2	DK5
UK1		7	**24**	9	7	11	**24**	8	10	8	10	12	10	9	7	5	5	8	10	6	8	13	7	6	5	11	9
UK3			7	9	9	15	7	9	13	11	12	12	12	17	15	15	14	13	13	11	13	15	13	8	8	17	15
UK4				9	7	11	**24**	8	10	8	10	12	10	9	7	5	5	8	10	6	8	13	7	6	5	11	9
UK5					4	12	7	5	9	9	13	10	11	8	6	7	9	9	9	7	7	6	8	7	6	8	9
UK6						11	7	**22**	6	5	9	8	8	15	8	6	9	10	9	7	5	5	5	3	2	9	11
UK7							11	12	15	12	17	10	13	15	14	11	15	16	18	11	15	13	15	7	6	12	15
UK8								8	10	8	10	12	10	9	7	5	5	8	10	6	8	13	7	6	5	11	9
UK10									8	6	10	9	9	15	9	8	7	11	10	8	6	6	6	5	4	10	10
UK11										11	12	13	13	15	12	12	15	13	14	12	14	14	15	5	6	10	15
Ger1											15	8	10	9	9	12	9	15	10	7	14	14	9	7	7	12	13
Ger2												14	11	16	13	12	13	14	12	12	16	11	12	7	8	10	15
Ger3													12	18	13	15	12	10	13	10	13	18	12	10	11	16	16
Ger4														15	15	12	12	13	14	9	10	14	12	13	14	13	11
Ger5															18	15	18	14	14	13	12	16	18	6	6	15	16
Ger6																13	12	15	15	8	12	16	12	9	10	13	15
Ger7																	15	13	12	9	10	15	15	10	11	14	16
Ger11																		13	12	6	12	13	**23**	6	6	12	17
Ger14																			12	8	15	13	12	7	8	14	15
Ger15																				13	10	10	12	8	8	14	16
Ger16																					6	11	5	3	4	7	10
Ger17																						13	11	4	5	12	15
Ger18																							14	7	8	18	15
Ger20																								5	6	12	16
Ger21																									**23**	9	8
DK1																										8	8
DK2																											15
DK5																											

NOTE.—Each figure indicates the number of shared alleles between inversion haplotypes in any two families. For families with identical or nearly identical haplotypes, the number of shared alleles is shown in bold type.

Table 5. Extent of Haplotype Sharing

Locus	Heterozygosity	No. of Alleles	Location (Mb)	Size of PCR Product (in bp) for Haplotype Group (FISH Classification)				
				A (i)	B (iii)	C (iv)	D (NT)	E (i)
D2S1262	88	6[a]	79.1	NT	NT	—	249	229/239
D2S329	74	8	79.3	NT	NT	234	234	NT
D2S139	82	9	79.7	NT	NT	—	—	110/122
D2S2191	62	8	80.3	NT	NT	178[b]	—	NT
D2S253	75	8[a]	80.3	NT	NT	335[b]	335	335
D2S2180	73	9	80.5	NT	NT	220[b]	206	220/224
D2S2343	65	7	81.7	NT	NT	238[b]	240	NT
D2S289	70	7	82.7	NT	NT	188[b]	182	NT
D2S428	62[a]	7	82.9	NT	NT	156[b]	152	158
D2S1396	78[a]	5[a]	82.9	NT	NT	112[b]	116	112
D2S2162	61	4	83.4	NT	NT	140[b]	138	138/140
D2S2951	66[a]	5	83.7	—	NT	232[b]	—	220/224
D2S440	78[a]	6	84.9	—	NT	204[b]	196	200
D2S1387	61[a]	7[a]	85.0	—	NT	160[b]	156/160	160
D2S2371	63	5	85.0	179	—	177[b]	—	177
D2S1790	73[a]	9	85.1	—	—	290[b]	290	290
D2S2161	77	9	85.3	190/192	—	192[b]	192	192/200
D2S2333	82	8	85.5	239	229	225[b]	229	229/237
D2S2232	78	8	86.0	—	214	216[b]	204/208	206
D2S388	78	6	86.1	252	256	252[b]	262	258/262
D2S1331	75[a]	5[a]	86.5	309	—	305[b]	317	305/309
D2S417	76	9	86.9	—	200	196	202	206
D2S2216	77	8	88.3	135/143	135	141	141	141
D2S2181	58	8	88.5	180	172	180	180	180
Centromere	...	...	...	...	...	...	...	...
D2S2159	74	7	95.5	174	172	176	174	176
D2S113	78	10	96.8	213	—	223	223	221
D2S2222	70	7	97.8	222/224	222	216	224	218
D2S2175	76	7	98.4	121	125	125	125	127
D2S2311	65	10	98.5	152	148	156	154	152/156
D2S2209	74	7	100.7	182	182	194	184	192
D2S2264	77	7	101.9	227/245	237	243	247	243
D2S278	83	9[a]	102.0	286/302	286	286	274	298
D2S373	74	8	102.6	230	220	216/230	230	220
D2S1343	65[a]	7[a]	104.9	—	268	264	270	268
D2S2229	83	12	105.8	185	199	195	175	189
D2S274	86	11[a]	105.8	161	129	145	—	141
D2S176	70	6	106.0	238	242	246	238	238
D2S1897	88	17	106.0	217	225	223	224	227
D2S293	83	12	106.7	168	178	182	176	174
D2S2386	72	7	107.0	273	271	271	277	277
D2S1784	70[a]	5	107.8	204/208	204/208	200	—	204
D2S1890	73	7	107.9	214	204	214	210	210
D2S340	70	9	108.4	161	167	167	159	169
D2S1893	75	7	109.5	252	256	252	258	258
D2S1888	77	7	111.5	82/86	84	84	84	82/86
D2S1892	80	12	111.6	221	233	227	227	227
D2S1896	78	10	112.4	174	184	188	180	174
D2S2269	88	12	112.7	—	250	272	272	272
D2S160	79	7	113.1	210	208	210	206	214
IL1A	75	7	113.3	128	132	128	126	126
D2S1895	80	8	113.8	123	—	125	121	115
D2S121	81	10	114.4	—	—	176	178	180
D2S276	80	6 [a]	114.8	208	—	208	208/216	208
D2S2953	54[a]	6[a]	114.9	150	150	150	133	158
D2S308	68	4	114.9	231	NT	225	225	225
D2S1265	75	5[a]	115.3	275	NT	—	279	275/279
D2S410	83[a]	7[a]	116.0	—	NT	—	167	169/173
D2S1771	62[a]	7	117.0	—	NT	—	132	132
D2S363	83[a]	7	117.1	NT	NT	254	260	251/253
D2S1277	80	8[a]	117.3	NT	NT	—	172	175
D2S2254	76	11	119.7	NT	NT	—	213	195/197

NOTE.—For each microsatellite locus tested, the heterozygosity, number of alleles, and physical location (in Mb) are given. The information is taken from the UCSC Genome Browser and the Genome Database, unless otherwise marked. For each haplotype group, the shared alleles are indicated as the size in base pairs of the relevant PCR product(s). Where two values appear, the haplotype contains one of two possible alleles, and it is not possible to determine which. A minus sign (−) indicates no allele sharing. NT = not tested.

[a] Value calculated using data from this study.

[b] Allele shared by only two of the three families.

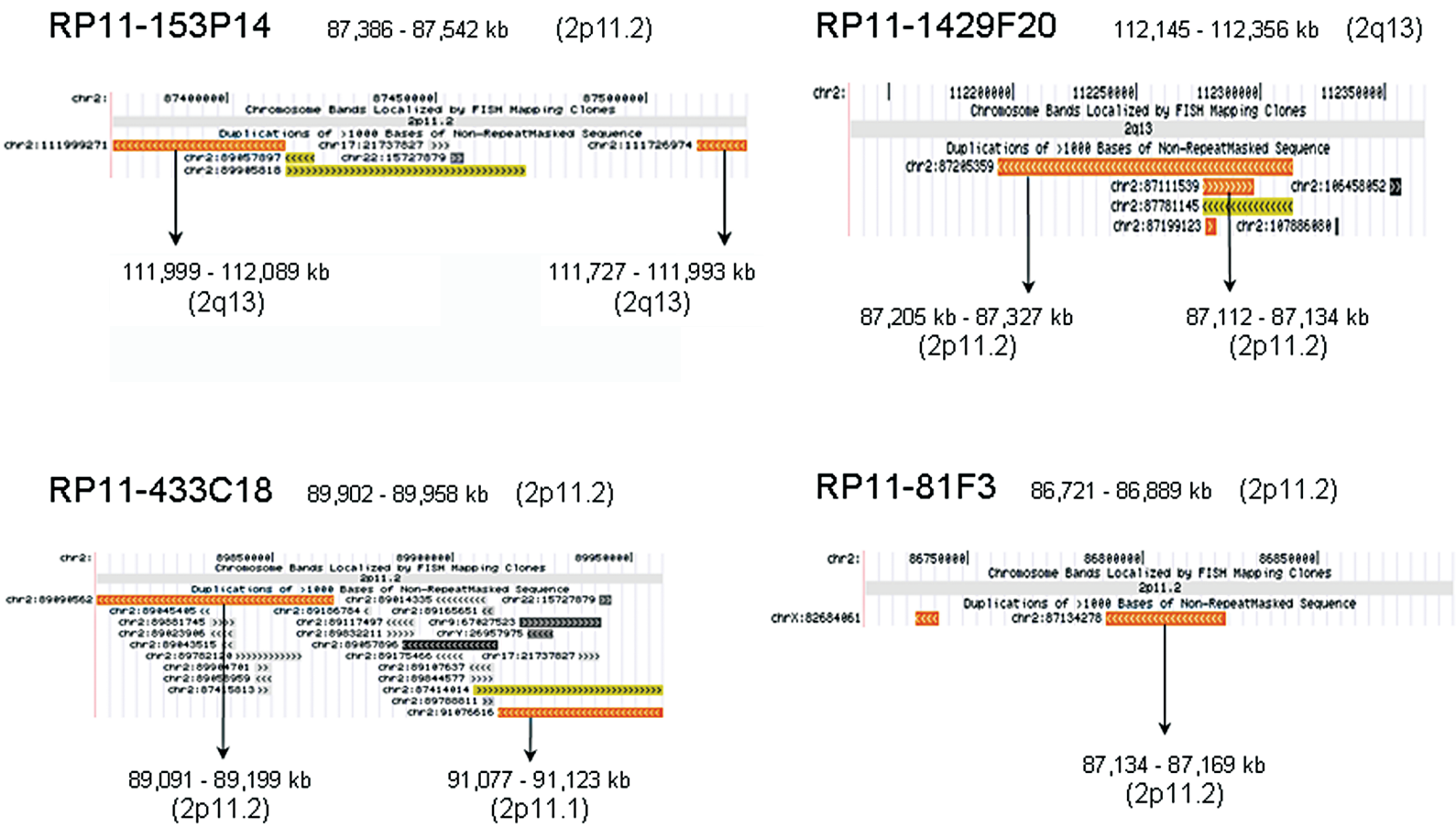

Figure 2. Segmental duplication content of breakpoint-spanning BACs. Each image shows the SD content of one BAC. The name of each BAC, its physical location in kilobases, and the corresponding cytogenetic band are given above the image. Images are taken from the UCSC Genome Browser. SDs are shown in blocks of chevrons. The color indicates the degree of sequence homology: orange, >99% similarity; yellow, 98%–99%; gray, 90%–98%. For simplicity, most of the SDs with only 90%–98% similarity (*gray*) have been removed. The location to which each SD with >99% similarity (*orange*) has sequence homology is shown below the image.

the long and short arms; in groups B and E, the haplotype extends beyond the inverted region on the long arm only; and, in group A, the haplotype diverges immediately beyond the inversion. Variation in the extent of haplotype conservation among groups probably reflects the number of meioses through which the inversion has passed since the founder mutation.

Genome architecture is known to confer a generalized susceptibility to structural rearrangements,[20] and the pericentric region of chromosome 2 contains numerous duplicated and repetitive sequence elements that could mediate the formation of the recurrent class i inversion. According to the Ensembl database, the BACs RP11-153P14 and RP11-1429F20 are 135 kb and 212 kb in size, respectively, and both contain only a single gene: *KV3J,* the immunoglobin (Ig) kappa chain V-III region VH precursor (fragment) gene on 2p11.2, and *ANAPC1* (MIM 608473), anaphase-promoting complex subunit 1, a mitotic checkpoint regulator, on 2q13.

The ancestral fusion site located at 2q14 is ~2 Mb away from the class i breakpoint, and only the rare class iv inversion has a breakpoint close to this site. Multiple genes encoding Ig kappa chain proteins also map to proximal 2p and proximal 2q, and these have extensive sequence homology.[21,22] On 2p, both the class ii and class iv breakpoints are within the large ancestral Ig kappa gene cluster, and the single gene within RP11-153P14 spanning the class i breakpoint, *KV3J,* is an Ig kappa chain gene. However, the Ig kappa genes on the long arm are a considerable distance proximal to all the inversion breakpoints, with the exception of a single gene at 113.9 Mb, close to the class iv breakpoint. Therefore, neither the fusion site nor the Ig kappa loci on chromosome 2 appear to play a major role in the formation of pericentric inversions.

Goidts et al.[13] proposed that the SDs mapping to the breakpoint regions could predispose to the formation of the inv(2)(p11.2q13). Therefore, we have examined the number and position of SDs within each spanning BAC by use of the UCSC Genome Browser. BACs RP11-153P14 and RP11-1429F20 are composed almost entirely of SDs, and, moreover, large SDs with >99% similarity from within RP11-153P14 map to 2q13 and from within RP11-1429F20 map to 2p11.2. (fig. 2, top). This is consistent with the dual hybridization signals observed by FISH on 2p and 2q on both the normal and inverted homologues. Regions of copy-number variation occur within[12,13] and flanking the BACs spanning the class i breakpoint, and variation between individuals could alter the risk of inv(2)(p11.2q13) formation. In contrast, spanning BACs involved in inversions other than the common class i have a lower SD content and/or contain SDs that map exclusively to the same chromosome arm (fig. 2, bottom).

Therefore, the class i inversion appears to be mediated by SDs common to both 2p11.2 and 2q13. However, according to current genome browsers and sequence information, the SDs from one breakpoint map close to the opposite breakpoint but not actually within the spanning BAC. The complexity of the sequence makes it difficult to unequivocally define the inversion breakpoints. For example, two BACs in 2p11.2 (RP11-316G9 and RP11-433C18) span the class iv breakpoint but are not overlapping and are separated by 100 kb. The physical mapping data and the hybridization characteristics of each BAC may be influenced by copy-number variation.[12,13] BAC end sequences could be informative, but we have found on chromosome 9 that, although BACs map to a single location in genome browsers, in practice they actually hybridize to more than one location.[23] Interestingly, the pericentric inv(9) may also be mediated by large tracts of SDs that flank the centromeric heterochromatin.[24]

The extensive sequence homology among the SDs at the breakpoint regions would suggest that the underlying mechanism is nonallelic homologous recombination (NAHR).[25] NAHR mediates recombination between low-copy repeats (LCRs) resulting in the loss, gain, or inversion of the intervening sequence—for example, in the formation of microdeletion syndromes. Despite the high level of sequence homology throughout LCRs, there is generally a preference for recombination at a specific site within each LCR in the formation of microdeletions[26] and in the formation of the pericentric inv(9).[27] Thus, the variant inv(2) breakpoints may also display site preference within a subdomain of each SD, rather than occurring at random.

In summary, our data have produced two significant findings regarding the origin of the variant inv(2)(p11.2q3): the inversion is a genuinely recurrent rearrangement with a large number of separate mutations but displays some breakpoint heterogeneity. Thus, the frequent identification of the inversion can be explained by three mechanisms: the majority have arisen independently from different founders, while a minority either have been transmitted IBD from a common founder or have different breakpoints at the molecular cytogenetic level.

Acknowledgments

This work was supported in part by the Dr. Robert Pfleger-Stiftung, the Ernst-Abbe-Stiftung, the Bundesministerium fur Bildung und Forschung (BMBF), and the Wellcome Trust. We are grateful to Dr. James Lespinasse, Dr. Merete Bugge, Dr. Carl Birger van der Hagen, Dr. Nick Dennis, and Barbara O'Prey for supplying patient material; to Professor Patricia Jacobs for helpful discussions on this project; and to the cytogenetic staff members of all contributing laboratories.

Web Resources

The URLs for data presented herein are as follows:

Ensembl Cytoview, http://www.ensembl.org/Homo_sapiens/cytoview
Genome Database, http://www.gdb.org/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *ANAPC1*)
UCSC Genome Browser, http://www.genome.ucsc.edu/index.html

References

1. Kaiser P (1984) Pericentric inversions: problems and significance for clinical genetics. Hum Genet 68:1–47
2. Youings S, Ellis K, Ennis S, Barber J, Jacobs P (2005) A study of reciprocal translocations and inversions detected by light microscopy with special reference to origin, segregation, and recurrent abnormalities. Am J Med Genet 126A:46–60
3. Gardner RJM, Sutherland GT (1996) Chromosome abnormalities and genetic counselling, 2nd ed. Oxford University Press, Oxford, pp 115–182
4. Boue J, Boue A (1978) Prenatal diagnosis in 100 structural rearrangements of the chromosomes. Cytogenet Cell Genet 20:213–225
5. Groupe de Cytogeneticiens Francais (1986) Pericentric inversions in man. Ann Genet 29:129–168
6. MacDonald IM, Cox DM (1985) Inversion of chromosome 2(p11q13): frequency and implications for genetic counseling. Hum Genet 69:281–283
7. Vejerslev LO, Friedrich U (1984) Experiences with unexpected structural aberrations in prenatal diagnosis in a Danish series. Prenat Diagn 4:181–186
8. Hysert M, Bruyere H, Cote GB, Dawson AJ, Dolling J-A, Fetni R, Hrynchak M, Lavoie J, McGowan-Jordan J, Tihy F, et al (2006) Prenatal cytogenetic assessment and inv(2)(p11.2q13). Prenat Diagn 26:810–813
9. Horvath JE, Gulden CL, Vallente RU, Eichler MY, Ventura M, McPherson JD, Graves TA, Wilson RK, Schwartz S, Rocchi M, et al (2005) Punctuated duplication seeding events during evolution of human chromosome 2p11. Genome Res 15:914–927
10. Ijdo JW, Baldini A, Ward DC, Reeders ST, Wells RA (1991) Origin of human chromosome 2: an ancestral telomere-telomere fusion. Proc Natl Acad Sci 88:9051–9055
11. Fan Y, Linardopoulou E, Friedman C, Williams E, Trask BJ (2002) Genomic structure and evolution of the ancestral chromosome fusion site in 2q13-2q14.1 and paralogous regions on other human chromosomes. Genome Res 12: 1651–1662
12. Sebat J, Kakshmi B, Troge J, Alexander J, Young J, Lundin P, Maner S, Massa H, Walker M, Chi M, et al (2004) Large-scale copy number variation polymorphism in the human genome. Science 305:525–528
13. Goidts V, Armengol L, Schempp W, Conroy J, Nowak N, Muller S, Cooper DN, Estervill X, Enard W, Szamalek JM, et al (2006) Identification of large-scale human-specific copy number differences by inter-species array comparative genomic hybridization. Hum Genet 119:185–198
14. Bailey JA, Eichler EE (2006) Primate segmental duplications: crucibles of evolution, diversity and disease. Nat Rev Genet 7:552–564
15. Gilling M, Dullinger JS, Gesk S, Metzke-Heidemann S, Siebert R, Meyer T, Brondum-Nielsen K, Tommerup N, Ropers HH, Tumer Z, et al (2006) Breakpoint cloning and haplotype analysis indicate a single origin of the common inv(10)(p11.2q21.2) among Northern Europeans. Am J Hum Genet 78:878–883
16. Liehr T, Wiese A, Heller A, Starke H, Mrasek K, Kuechler A, Weier HU, Claussen U (2002) Multicolor chromosome banding (MCB) with YAC/BAC-based probes and region-specific microdissection DNA libraries. Cytogenet Genome Res 97:43–50
17. Warburton D (1991) De novo balanced chromosome rearrangements and extra marker chromosome identified at prenatal diagnosis: clinical significance and distribution of breakpoints. Am J Hum Genet 49:995–1013
18. Lacbawan FL, White BJ, Anguiano A, Rigdon DT, Ball KD, Bromage GB, Yang X, DiFazio MP, Levin SW (1999) Rare interstitial deletion (2)(p11.2p13) in a child with pericentric inversion (2)(p11.2q13) of paternal origin. Am J Med Genet 87:139–142
19. Magee AC, Humphreys MW, McKee S, Stewart M, Nevin NC (1998) De novo duplication 2(p12→p21) with paternally inherited pericentric inversion 2p11.22q12.2. Clin Genet 54: 65–69
20. Stankiewicz P, Lupski JR (2002) Genome architecture, rearrangements and genomic disorders. Trends Genet 18:74–82
21. Huber C, Thiebe R, Hameister H, Barbi G, Zachau HG (1990) Mapping immunoglobulin kappa orphon without sequence defects may be the product of a pericentric inversion. Nucleics Acids Res 18:3475–3478
22. Zimmer F-G, Hameister H, Schek H, Zachau HG (1990) Transposition of human immunoglobulin Vk genes within the same chromosome and the mechanism of their amplification. EMBO J 9:1535–1542
23. Willatt LR, Barber JC, Clarkson A, Simonic I, Raymond FL, Docherty Z, Ogilvie CM (2007) Novel deletion variants of 9q13-q21.12 and classical euchromatic variants of 9q12/ch involve deletion, duplication and triplication of large tracts of segmentally duplicated pericentromeric euchromatin. Eur J Hum Genet 15:45–52
24. Park JP, Wojiski SA, Spellman RA, Rhodes CH, Mohandas TK (1998) Human chromosome 9 pericentric homologies: implications for chromosome 9 heteromorphisms. Cytogenet Cell Genet 82:192–194
25. Shaw CJ, Lupski JR (2004) Implications of human genome architecture for rearrangement-based disorders: the genomic basis of disease. Hum Mol Genet 13:R57–R64
26. Bi W, Park S-S, Shaw CJ, Withers MA, Patel PI, Lupski JR (2003) Reciprocal crossovers and a positional preference for strand exchange in recombination events resulting in deletion or duplication of chromosome 17p11.2. Am J Hum Genet 73: 1302–1315
27. Starke H, Seidel J, Henn W, Reichardt S, Volleth M, Stumm M, Behrend C, Sandig KR, Kelbova C, Senger G, et al (2002) Homologous sequences at human chromosome 9 bands p12 and q13-q21.1 are involved in different patterns of pericentric rearrangements. Eur J Hum Genet 10:790–800