Breakpoint Mapping and Array CGH in Translocations: Comparison of a Phenotypically Normal and an Abnormal Cohort

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We report the analyses of breakpoints in 31 phenotypically normal and 14 abnormal carriers of balanced translocations. Our study assesses the differences between balanced translocations in normal carriers and those in abnormal carriers, focusing on the presence of genomic imbalances at the breakpoints or elsewhere in the genome, presence of cryptic chromosome rearrangements, and gene disruption. Our hypothesis is that all four features will be associated with phenotypic abnormalities and absent or much less frequent in a normal population. In the normal cohort, we identified neither genomic imbalances at the breakpoints or elsewhere in the genome nor cryptic chromosome rearrangements. In contrast, we identified candidate disease-causing imbalances in 4/14 abnormal patients. These were three breakpoint associated deletions and three deletions unrelated to the breakpoints. All six de novo deletions originated on the paternally inherited chromosome. Additional complexity was also present in one of these cases. Gene disruption by the breakpoints was present in 16/31 phenotypically normal individuals and in 5/14 phenotypically abnormal patients. Our results show that translocations in phenotypically abnormal patients are molecularly distinct from those in normal individuals: the former are more likely to be associated with genomic imbalances at the breakpoints or elsewhere and with chromosomal complexity, whereas the frequency of gene disruption is similar in both normal and abnormal translocation carriers.

Introduction

Apparently balanced reciprocal translocations are a common type of chromosome rearrangement found both in patients with phenotypic abnormalities and in clinically unaffected individuals. Most rearrangements are inherited, but approximately one in five is a de novo event.[1] These de novo rearrangements represent a challenge in prenatal genetic counseling, given that the risk of an abnormal phenotype is quoted as ~6.1%.[2] The study of such rearrangements might provide an understanding of the molecular features that account for the abnormal phenotypes observed. First, reports of such studies have shown the presence of cryptic imbalances at or near the breakpoint regions in a proportion of patients with abnormal phenotypes. Kumar et al.[3] found breakpoint-associated deletions in 2/3 patients, and Wirth et al.[4] reported deletions in 2/6 patients presenting with mental retardation. These reports have been followed by others that show that a proportion of apparently balanced rearrangements are in fact unbalanced.[5–10] Second, imbalances unrelated to the breakpoint regions have been reported by Gribble et al.,[9] who applied array CGH to the study of ten phenotypically abnormal carriers of apparently balanced translocations and found 3/10 to have imbalances in regions not involved in the translocations. In a similar study, De Gregori et al.[10] found 4/27 phenotypically abnormal carriers of apparently balanced reciprocal translocations to have imbalances unrelated to the breakpoints. Although these imbalances could be causal to the abnormal phenotypes, the interpretation of such cases is complicated by the presence of an abnormal karyotype. Third, apparently balanced translocations in phenotypically abnormal carriers have been shown, in some cases, to be complex chromosome rearrangements.[8–14] Complex rearrangements have at least three breakpoints and involve two or more chromosomes, and it can be assumed that the probability of an abnormal phenotype increases with the number of breakpoints involved.[15] Fourth, the direct breakpoint-mediated disruption of the expression pattern of dosage-sensitive genes can also account for phenotypic abnormalities,[16–22] as can breakpoint-mediated disruption of regulatory regions by position effect.[23] These findings have established the value of the molecular characterization of breakpoints for the discovery of disease-causative genes and historically have led to the unraveling of the genetic cause of several Mendelian disorders.[24]

In contrast to the reports on phenotypically abnormal translocation carriers, molecular studies of phenotypically normal carriers have not been conducted systematically and are mainly undertaken in the context of familial studies in order to aid the interpretation of chromosome rearrangements found in phenotypically abnormal patients. We previously reported a molecular study of 13 normal carriers of balanced translocations in which we detected neither cryptic chromosomal complexity nor imbalances at the breakpoints or unrelated to the breakpoints. However, we did observe breakpoint-mediated gene disruption.[25] To

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further evaluate the significance of our original observations, we have analyzed 18 additional individuals and extended the molecular analyses of the 13 previously reported individuals. We obtained detailed clinical information on all 18 additional cases to gauge the significance of our findings.

In the present study, we aimed to determine the frequency of the aforementioned four features in 31 phenotypically normal individuals with balanced rearrangements, using fluorescence in situ hybridization (FISH) and 1 Mb array CGH, and to compare the results to those of a cohort of 14 phenotypically abnormal carriers of apparently balanced rearrangements analyzed by array painting, FISH, and array CGH with the Sanger 30K Whole Genome Tilepath clone set (WGTP) platform. Our findings will contribute to the determination of the differences between balanced rearrangements in phenotypically normal individuals from those in phenotypically abnormal patients, which will be of value in a diagnostic setting, particularly in the interpretation of de novo rearrangements diagnosed prenatally.

Subjects and Methods

Subjects

(1) Phenotypically Normal Study Population

This study included individuals presenting with what was assumed to be a clinically normal phenotype who were found to be carriers of a de novo apparently balanced reciprocal translocation following referral for cytogenetic analysis. Individuals were classified as having a normal phenotype if no clinical abnormalities were mentioned in the original referral and if the reason for referral included recurrent miscarriages or routine diagnostic testing for family members of known carriers of cytogenetic abnormalities. Individuals meeting these criteria were selected from the Salisbury Treasury of Interesting Chromosomes (STOIC), which contains records, dating from 1967 to the present, on all cytogenetic abnormalities diagnosed at the Wessex Regional Genetics Laboratory. Ethical approval for this study was given by a Multi-center Research Ethics Committee (UK). A blood sample was obtained by a clinical geneticist, genomic DNA was extracted, and PHA-stimulated cultures were prepared. Additionally, lymphoblastoid cell lines (LCLs) were established by Epstein-Barr virus transformation according to standard procedures and stored for future investigations. We also carried out further analyses in the 13 individuals previously reported[25] and from whom detailed clinical information is not available. A summary of the karyotypes and mode of ascertainment is given in Table 1.

(2) Phenotypically Abnormal Study Population

This study included 14 patients with a clinically abnormal phenotype who were found by conventional microscopy to have an apparently balanced chromosome rearrangement. All patients had confirmed de novo rearrangements, except for case 50, of which the maternal karyotype was normal but a paternal sample was unavailable. Peripheral blood samples were collected from the patients and their parents after fully informed consent was obtained, and genomic DNA- and PHA-stimulated cultures were prepared from the blood samples. In addition, LCLs were established from each patient. Karyotypes of all patients and their modes of ascertainment are given in Table 2.

(3) Genotype-Phenotype Correlations

A detailed medical examination by a clinical geneticist (C.M.) was carried out, and this phenotypic information is presented in Tables S1 and S2. Patient examination was performed without prior knowledge of the molecular findings. The molecular results were subsequently collated with the clinical information in an attempt to establish genotype-phenotype correlations, and a reexamination of the patient was undertaken when necessary. Details of the translocation breakpoints and array-cgh results have been added to the DECIPHER database and will appear in Ensembl when consent for this has been specifically obtained.

Methods

Normal Cohort

Breakpoint Mapping Studies. FISH studies were carried out by standard methods, with probes derived from BACs (Bacterial Artificial Chromosomes) or PACs (P1-derived Artificial Chromosomes) mapped to the bands of the cytogenetically assigned breakpoints and selected from the Ensembl Human Genome Browser. The gene content of the breakpoint regions was determined with the Ensembl and the UCSC genome browsers based on NCBI Build 36. For breakpoints located in regions harboring genes, further fine mapping was undertaken by FISH with fosmid clones or by array-painting analysis[26] with fosmid clones and customized PCR products.

Array CGH. Array CGH experiments were conducted via the Sanger 1 Mb array platform, as described by Fiegler et al.[27] Patients' genomic DNA samples were competitively hybridized with a reference DNA from multiple donors (Promega) in a sex-mismatch experiment. The results were analyzed with the BlueFuse for microarrays software (BlueGnome, UK). Copy-number gains and losses were called manually for replicate clones showing a $\log_2$ ratio of ± 0.5. Results were compared to data from studies on normal subjects recorded in the database of genomic variants (DGV) and in the human genome browsers Ensembl and UCSC.

Abnormal Cohort

Whole-genome array-CGH studies were performed with the Sanger 30K Whole Genome TilePath (WGTP) arrays as described by Fiegler et al.[28] Briefly, genomic patient- and reference-DNA samples were differentially labeled and hybridized to the arrays in duplicate via dye-reversal experiments. Custom Perl scripts were used to combine the dye-swap results and to detect regions of copy-number changes. All copy-number changes observed were compared to copy-number variants (CNVs) reported in previous studies of normal populations and available from the database of genomic variants (DGV) and the Ensembl and ucsc browsers. Regions not previously reported as CNVs and including at least two clones were singled out for further investigations, which included confirmatory studies by FISH and parent-of-origin analysis by microsatellite analysis with genomic DNA samples from the proband and each parent according to standard methods. Table 3 lists the markers used.

Array Painting. The rearrangement breakpoints were mapped by array painting, as described by Fiegler et al.[26] Derivative chromosomes were flow sorted from LCLs and amplified via DOP PCR or via the GenomePlex Complete Whole Genome Amplification kit (Sigma). The derivative chromosomes from each rearrangement were differentially labeled in Cy3 and Cy5 and hybridized onto Sanger 30K WGTP arrays. Data analysis was undertaken with the BlueFuse for microarrays software (BlueGnome, UK), and the array-painting results were confirmed by FISH.

FISH Experiments. Large-insert clones in target regions were selected from the Ensembl browser. FISH experiments were

Table 1. Karyotypes and Mode of Ascertainment of the Normal Study Population

Case[1]	Karyotype	Ascertainment	Previous Description
1A	46,XY,t(2;14)(p21;q13)de novo	Parent of a 46,XX,t(2;14)(p21;q13) miscarriage	
1B	46,XX,t(3;9)(p26.2;p22.3)de novo	Parent of a 46,XY,add(3)(p25.3) clinically affected child	
1C	46,X,t(X;7)(?q27;q22)de novo	Parent of a 46,X,Xq+ clinically affected child	
1D	46,XX,t(10;18)(q24.3;q12.2)mat	Relative of 46,XY,t(10;18)(q24.3;q12.2) ascertained prenatally because of high serum screen risk	case 12 in Baptista et al.(2005)
2A	46,XX,t(4;16)(q35.1;p13.13)de novo	Parent of a clinically affected child presenting a rea(4)	
2B	46,XX,t(1;13)(q32.3;q32.3)de novo	Parent of a 46,XY,13q+ clinically affected child	
2C	46,XY,t(2;18)(q35;q21.3)de novo	Recurrent miscarriages	
2D	46,XX,t(2;9)(q21.3;p13)de novo	Parent of a 46,XX,t(2;9)(q21.3;p13) amniocentesis because of advanced maternal age	
2E	46,XX,t(7;17)(q36.1;q25.1)de novo	Recurrent miscarriages	
2F	46,XY,t(8;15)(p11.2;q24)de novo	Parent of a 46,XY,t(8;15)(p11.2;q24) amniocentesis because of advanced maternal age	
2G	46,XY,t(1;13)(p22;q32)de novo	Parent of a 46,XY,t(1;13)(p22;q32) clinically affected child	
2H	46,XY,t(11;21)(p15.4;p12)de novo	Parent of a clinically affected child presenting a paternal dup(11)	
2I	46,XX,t(2;7)(p23.3;p22.3)de novo	Prenatal for advanced maternal age	case 1 in Baptista et al.(2005)
2J	46,XX,t(11;17)(p13;p13.1)mat	Parent of a 46,XY,t(11;17)(p13;p13.1) clinically affected child	case 2 in Baptista et al.(2005)
2K	46,XX,t(7;16)(p15;q22)mat	Recurrent miscarriages	case 4 in Baptista et al.(2005)
2L	46,XY,t(8;16)(q22.1;q13)pat	Sibling of a 46,XX,t(8;16) (q22.1;q13) girl ascertained because of delayed puberty	case 5 in Baptista et al.(2005)
2M	46,XX,t(16;18)(q24;q21.1)mat	Recurrent miscarriages	case 7 in Baptista et al.(2005)
2N	46,XX,t(5;18)(p13;q11)pat	Relative of 46,XX,t(5;18)(p13;q11)pat amniocentesis because of family history of Down syndrome.	case 9 in Baptista et al.(2005)
2O	46,XY,t(1;11)(q42.3;q21)pat	Parent of 46,XX, t(1;11)(q42.3;q21) amniocentesis because of family history of Down syndrome.	case 10 in Baptista et al.(2005)
2P	46,XX,t(3;10)(p23;q21.2)pat	Relative of a 46,XY,t(3;10)(p23;q21.2) clinically affected child	case 11 in Baptista et al.(2005)
3A	46,XX,t(4;6)(q27;p25)de novo	Recurrent miscarriages	
3B	46,XX,t(8;12)(p23.1;p13.1)de novo	Parent of a 46,XY, add(8)(p23.1) clinically affected child	
3C	46,XX,t(6;9)(q22.2;p22.3)de novo	Recurrent miscarriages	
3D	46,XY,t(2;4)(p23;p12)de novo	Recurrent miscarriages	
3E	46,X,t(X;22)(p11.23;q13.1)de novo	Parent of a 46,X,t(X;22)(p11.23;q13.1) amnio because of family history of Down syndrome	
3F	46,XX,t(11;15)(q23;q22)de novo	Parent of a 46,XY,t(11;15)(q23;q22) amnio because of advanced maternal age	
3G	46,XX,t(2;6)(q32.2;p23)de novo	Recurrent miscarriages	
3H	46,XX,t(6;22)(p21.3;q13)pat	Parent of a 46,XX,del(15)(q11q12) clinically affected child	case 3 in Baptista et al.(2005)
3I	46,XX,t(1;19)(q42.13;p13.2)mat	Recurrent miscarriages	case 6 in Baptista et al.(2005)
3J	46,XX,t(9;20)(p24.1;p11.2?3)mat	Parent of a 46,XY,der(9)t(9;20)(p24.1;p11.2?3) clinically affected child	case 8 in Baptista et al.(2005)
3K	46,XY,t(2;3)(p23.1;q29)mat	Relative of a 46,XX,der(3)t(2;3)(p23.1;q29) clinically affected child	case 13 in Baptista et al.(2005)

[1] Prefix 1 refers to cases with no genes at the breakpoints. Prefix 2 indicates cases with obligatory breakpoint-mediated gene disruption. Prefix 3 refers to cases with potential breakpoint-mediated gene disruption.

undertaken on cell suspensions in 3:1 methanol:acetic acid prepared either from LCLs or from peripheral blood samples according to standard methods. The gene content of regions of interest was determined with the Ensembl browser and/or the UCSC browsers based on NCBI build 36. For regions containing putative candidate genes, further refining of the breakpoints was undertaken with fosmid clones selected from the UCSC browser.

Results

We present the results first for the clinically normal cohort and second for the clinically abnormal cohort. For each cohort, we consider the four features that are the focus of this study; namely, breakpoint-associated imbalances, genomic imbalances unrelated to the breakpoints, additional chromosomal complexity, and gene disruption.

Phenotypically Normal Cohort

(1) FISH Investigation of the Presence of Imbalances at the Breakpoint Regions

We initially characterized the breakpoint regions by FISH with BAC- or PAC-derived probes (resolution of ~150 kb), and for a subset of breakpoints found to map in the vicinity of genes we conducted higher-resolution mapping with fosmid clones (resolution of ~40 kb) or PCR products (resolution up to ~10 kb). At this level of resolution, none of

Table 2. Karyotypes and Mode of Ascertainment of the Abnormal Study Population

Case	Karyotype	Ascertainment
16	46,XX,t(10;22)(q24.3;q13.31)dn	DD, epilepsy
20	46,XX,t(2;5)(q33;q12)dn	DD, mild mental retardation
43	46,XY,t(4;17)(q35.1;q25.1)dn	Truncus arteriosus, NAA
45	46,X,t(X;19)(q21;p13.11)dn	Premature ovarian failure, NAA
48	46,XY,t(4;6)(q33;q22.2)dn	DD, dysmorphic features
49	46,XX,t(2;10)(q33;q21.2)dn	DD, cleft palate, behavioral abnormalities
50	46,X,t(X;8)(q22.1;q24.13)nk	Premature ovarian failure, NAA
51	47,XX,t(4;20)(p15.2;p11.23)dn,+mar mat	DD, autistic spectrum disorder
52	46,XX inv ins (11;4)(q22.2;q13.2q21.3)dn	DD, LD, short stature, scoliosis
53	46,XX,t(4;8)(q21.1;p12)dn	DD, regressive skills
54	46,XY,t(14;15)(q23;q26.3)dn	dysmorphic features, coarctation of aorta
55	46,XY,t(19;20)(q13.43;q11.1)dn	Severe oligospermia, NAA
56	46,XY,t(6;21)(q16.2;q11.2)dn	Severe oligospermia, NAA
57	46,XY,t(2;5)(p23;q11.2)dn,t(18;22)(q11.2;p13)dn	DD, short stature, macrocephaly, epilepsy

DD denotes developmental delay.
LD denotes learning difficulties.
NAA denotes no additional abnormalities.
nk denotes parental origin unknown.

the breakpoint regions analyzed were associated with deletions or duplications of DNA segments.

(2) Whole-Genome Analysis by Array CGH

Using array CGH at 1 Mb resolution we identified an average of 8.4 regions of copy number change in each genome studied. All regions of imbalance found in this normal cohort were totally or partially overlapping with regions previously reported as harbouring normal CNVs documented in the DGV, UCSC and Ensembl browsers.

(3) Assessment of the Presence of Cryptic Chromosomal Complexity

Using FISH analysis, we observed that all the translocations analyzed involved only two chromosomes with a maximum of two breakpoints per rearrangement, a finding that is consistent with the absence of chromosomal complexity in the rearrangements studied. However, for case 3H, 46,XX,t(6;22)(p21.3;q13)pat, we found that the breakpoint-mapping results were inconsistent with the assembly order of the fosmid clones used. This was because the fosmid WI2-1419L13, which mapped centromeric to the breakpoint-spanning fosmid (WI2-2878H11), did not hybridize to chromosome 22 and derivative 22 as expected, but instead hybridized to chromosome 22 and derivative 6. This could be explained by the presence of a small inversion at the breakpoint, but we were unable to determine this by FISH due to the close proximity of these fosmids. Alternative explanations for this result are an error in the assembly or an error in the assigned identity of the clones used.

Table 3. Microsatellites Selected for Parental-Origin Studies

Case	Chromosome	Markers
20	5q12.1-q12.3	D5S1474, D5S76, D5S1718, D5S427, D5S1956, D5S1359
52	4q13.3	D4S2641, D4S2389
52	4q21.23	D4S1534, D4S2691
53	4q13.3-q21.1	D4S3249, D4S2958, D4S1558
53	10p14	D10S1649, D10S465
57	4q32.1	D4S3016, D4S1556, D4S1498

(4) Analysis of the Gene Content at the Breakpoint Regions

Table 4 details the breakpoint-mapping results. Based on the status of gene disruption by the breakpoints, we have subdivided the individuals into three groups: 1, 2, and 3. In group 1, no known genes were mapped to the breakpoint regions. In group 2, for at least one of the translocation breakpoints, the breakpoint region was fully mapped within a gene and consequently caused interruption of the sequence of that gene. Because we have not carried out gene-function analysis, we refer to gene disruption when the sequence of a gene is interrupted by a breakpoint and acknowledge that the interruption of a gene's sequence might not always correlate with impairment of its function caused by complex gene-expression-regulatory mechanisms in humans. Individuals in group 3 had potential gene disruption by at least one of the breakpoints due to the presence of a gene(s) in part of the breakpoint-containing region. We present estimates of the likelihood of gene disruption based on the gene size within the total area of the breakpoint-containing interval. This measurement offers a good indication of the probability of gene disruption, although a more accurate estimate would need to take into account not only the size but also the relative position of the gene(s) within the breakpoint region. In summary, Table 4 shows that 16 breakpoints mapped to regions containing no known genes, 18 were fully contained within a gene, and 23 were mapped to an interval partly occupied by a gene. The remaining three breakpoints mapped to regions for which Ensembl and UCSC showed gene-mapping discrepancies.

Phenotypically Abnormal Cohort

The results of array-CGH analysis are summarized in Table 5, and full details of the breakpoint-mapping results are given in Table 6.

(1) Rearrangements with Breakpoint-Associated Imbalances

Case 20: 46,XX,t(2;5)(q33;q12)de novo. This patient had a 2.5 Mb deletion at the 5q breakpoint that encompasses 6 known genes (Table S3). FISH studies showed the deletion to be de novo and microsatellite analysis showed that it originated on the paternal chromosome.

Case 53: 46,XX,t(4;8)(q21.1;p12)de novo. This patient had a 2.1 Mb deletion at the 4q breakpoint, which encompasses 21 known genes (Table S3). Microsatellite analysis showed this to be a de novo deletion of paternal origin. In addition, array CGH identified a 1.1 Mb deletion on 10p14, which encompasses the predicted gene *LOC389936* (unknown function). This was found by microsatellite analysis to be de novo and paternal in origin.

(2) Rearrangements with Genomic Imbalances Unrelated to the Breakpoints

Case 50: 46,X,t(X;8)(q22.1;q24.13). This patient had a balanced translocation, but array CGH identified a ~200 kb deletion on 2p13.2. This deletion included part of the *EXOC6B* (exocyst complex component 6B) gene and was shown by FISH to be present in the patient's phenotypically normal mother. This deletion is therefore likely to be an example of a novel asymptomatic CNV, but it is included here because it has not been reported previously.

Case 57: 46,XY,t(2;5)(p23;q11.2)de novo, t(18;22)(q11.2;p13)de novo. This patient had two independent balanced translocations. In addition, array CGH identified a 2 Mb deletion unrelated to the translocations, on 4q32.1, which encompasses nine known genes (Table S3). Microsatellite analysis showed this deletion to have occurred de novo on the paternally derived chromosome.

(3) Rearrangements with Additional Chromosomal Complexity

Case 52: 46,XX inv ins (11;4)(q22.2;q13.2q21.3)de novo. This patient had a ~170 kb deletion at the 4q21.23 breakpoint, which contains no known genes. In addition, array CGH identified a 1.8 Mb deletion ~8.4 Mb distal to the 4q13.1 translocation breakpoint and ~12 Mb proximal to the 4q21.23 breakpoint. This encompasses six known genes (Table S3). Both deletions were confirmed by FISH to have a de novo origin, and microsatellite marker analysis showed that these originated on the paternal chromosome. In addition, FISH results near the 4q21.23 breakpoint were consistent with a potential inversion at this location.

(4) Analysis of the Gene Content in Rearrangements with no Detected Chromosomal Imbalance

The remaining rearrangements in this study were all found to be balanced translocations with no genomic imbalances detected (other than reported CNVs). We further analyzed the gene content of the breakpoint-containing regions in these cases. Table 6 shows that eight breakpoints mapped to regions containing no known genes, whereas five were definitely associated with gene disruption and eleven were potentially associated with gene disruption. Two other breakpoints mapped to regions for which Ensembl and UCSC showed gene-mapping discrepancies. The remaining two breakpoints were mapped to regions containing genes which were deleted.

Discussion

Imbalances at the Translocation Breakpoints

We have analyzed 31 translocations in phenotypically normal carriers and have not identified any breakpoint-associated imbalances. In contrast, three of the 14 clinically abnormal patients had a breakpoint-associated imbalance. All the imbalances were de novo deletions of 170 kb to 2.5 Mb, and all had arisen on the paternal chromosome. These findings provide further evidence that genomic imbalances are an important cause of phenotypic abnormalities in carriers of apparently balanced rearrangements.[3–11,29]

Genomic Imbalances Unrelated to the Breakpoints

We have detected genomic imbalances unrelated to the breakpoints in all of the 31 normal individuals, but these were previously reported CNVs. In contrast, when we applied tiling-path array CGH to the 14 patients in the abnormal cohort we found previously reported CNVs in all patients, and four patients also had previously unreported deletions unrelated to the breakpoints. The deletions in cases 52, 53, and 57 were de novo with origins on the paternally derived chromosomes, whereas case 50 had a maternally inherited deletion, representing a novel CNV. Genomic imbalances unrelated to the breakpoints have also been reported in other studies,[9,10] and the increasing utilization of array CGH will help define the overall frequency of such imbalances. Furthermore, these observations have important implications for the establishment of genotype-phenotype correlations because they imply that the contribution of novel unbalanced regions independent of the breakpoints needs to be considered. For example, Hayashi et al.[30] described a 1 Mb de novo deletion at 1q25 in a girl with Cornelia de Lange syndrome [MIM 122470] and a t(5;13)(p13.1;q12.1), and it appeared that both the deletion and the disruption of the *NIPBL* [MIM 608677] gene by the 5p translocation breakpoint contributed to the phenotype.

Additional Chromosomal Complexity in Apparently Balanced Rearrangements

Patsalis et al.[13] reported cryptic chromosomal complexity in 3/20 phenotypically abnormal balanced translocation carriers analyzed by FISH. In other studies, with FISH and/or array CGH, cryptic chromosomal complexity was uncovered in 4/4 patients,[11] in 3/10 patients,[9] and in 5/27 patients[10] with phenotypic abnormalities. In our series, additional chromosomal complexity was not detected in any of the normal individuals, and only one of the clinically abnormal patients had a complex rearrangement: case 52 had a rearrangement with three breakpoints, which on further analysis was found to have a minimum of six breakpoints.

Table 4. Summary of the Breakpoint-Mapping Results in the Normal Cohort

Case	BCI Genomic Position	Size of the BCI (bps)	Gene Content within the BCI	Likelihood of Gene Disruption[1]
1A	chr2:42,992,855-43,173,584	180,729	—	—
	chr14:40,212,036-40,381,064	169,028	—	—
1B	chr3:581,645-958,276	376,631	—	—
	chr9:11,302,383-11,764,217	461,834	—	—
1C	chrX:148,867,102-149,050,276	183,174	—	—
	chr7:108,572,249-108,768,779	196,530	—	—
1D	chr10:110,225,647-110,411,173	185,526	—	—
	chr18:37,199,353-37,363,565	164,212	—	—
2A	chr4:187,776,924-187,818,201	41,277	*FAT*	100
	chr16:8,687,646-8,767,351	79,705	*ABAT*	100
2B	chr1:206,420,427-206,463,044	42,617	*PLXNA2*	100
	chr13:95,145,761-95,229,198	83,437	*DNAJC3*	100
2C	chr2:214,463,710-214,641,904	178,194	*SPAG16*	100
	chr18:53,150,966-53,228,407	77,441	*ST8SIA3*	15
2D	chr2:138,955,571-139,047,575	92,004	*SPOPL*	75
	chr9:35,316,944-35,352,859	35,915	*UNC13B*	100
2E	chr7:132,668,430-132,886,439	218,009	*EXOC4*	100
	chr17:53,768,215-53,854,109	85,894	*RNF43*	80
2F	chr8:42,400,367-42,558,693	158,326	*SLC20A2, C8orf40*	80
	chr15:71,562,896-71,677,866	114,970	*NPTN, LOC283677*	100
2G	chr1:101,729,637-101,888,180	158,543	—	—
	chr13:91,284,473-91,451,991	167,518	*GPC5*	100
2H	chr11:8,789,238-8,828,410	39,172	*ST5*	70-100
	chr21: satellite stalk	nd	nd	nd
2I	chr2:36,974,205-36,984,164	9,959	*STRN*	100
	chr7:11,125,470-11,131,360	5,890	*PHF14*	100
2J	chr11:37,799,191-37,800,372	1,181	—	—
	chr17:12,739,251-12,759,331	20,080	*QRICH2*	100
2K	chr7:24,352,315-24,556,482	204,167	—	—
	chr16:68,412,623-68,499,123	86,500	*WWP2*	100
2L	chr8:100,008,198-100,013,461	5,263	*STK3*	0-100
	chr16:59,138187-59,146,748	8,561	—	—
2M	chr16:82,997,304-83,005,803	8,499	*ATP2C2*	100
	chr18:42,902,560-42,915,062	12,502	*HDHD2*	100
2N	chr5:38,450,546-38,489,171	38,625	*EGFLAM*	100
	chr18:16,935,079-16,977,780	42,701	*ROCK1*	25
2O	chr1:235,542,200-235,725,748	183,548	*RYR2*	100
	chr11:98,312,650-98,487,722	175,072	*CNTN5*	0-50
2P	chr3:25,187,406-25,225,486	38,080	—	—
	chr10:71,325,230-71,369,369	44,139	*COL13A1*	100
3A	chr4:125,648,232-125,829,198	180,966	*ANKRD50*	15
	chr6:7,324,263-7,409,496	85,233	*CAGE1, RIOK1*	45
3B	chr8:6,719,131-7,053,466	334,335	*DEFB1, DEFA6, DEFA4, DEFA1, DEFA3, DEFA5*	10
	chr12:7,987,953-8,310,582	322,629	*Q9UCR6, FOXJ2, C3AR, NECAP1, CLEC4A, Q9BZV8, ZNF705A, Q4G0H1, FAM90A1*	25-75
3C	chr6:119,215,818-119,302,998	87,180	*C6orf61, ASF1, MCM9*	40-65
	chr9:14,904,963-15,184,142	279,179	*C9orf52*	10
3D	chr2:26,218,578-26,257,187	38,609	*FAM59B*	20
	chr4:48,429,074-48,516,206	87,132	*FRYL*	55
3E	chrX:57,142,101-57,184,980	42,879	*SPIN2B, SPIN2A*	10
	chr22:19,026,823-19,108,026	81,203	*USP41, ZNF74*	35-75
3F	chr11:115,668,958-115,830,806	161,848	—	—
	chr15:48,315,253-48,399,500	84,247	*HDC, GABPB2*	75
3G	chr2:200,490,832-200,529,742	38,910	*FLJ38973, FLJ37953*	95
	chr6:6,601,851-6,706,437	104,586	—	—
3H	chr6:40,838,172-40,840,190	2,018	—	—
	chr22:30,870,135-30,908,432	38,297	*LOC150297*	25
3I	chr1:223,565,344-223,691,038	125,694	*LBR*	20
	chr19:13,999,383-14,039,028	39,645	*RLN3, IL27RA*	60
3J	chr9:6,427,961-6,601,726	173,765	*UHRF2, GLDC*	85
	chr20:23,000,436-23,041,115	40,679	*CD93*	15
3K	chr2:27,620,439-27,812,447	192,008	*C2orf16, ZNF512, CCDC121, XAB1, SUPT7L, SLC4A1AP*	65

Table 5. Summary of the Array-CGH Results in the Abnormal Cohort

Case	Breakpoint-Associated Imbalance (Genomic Position)	Other Genomic Imbalance (Genomic Position)
20	del(5)(q12.1q12.3)de novo(61,052,997-63,598,644)	—
50	—	del(2)(p13.2p13.2)mat(72,376,684-72,574,196)
52	del(4)(q21.23q21.23)de novo(86,384,696-86,558,920)	del(4)(q13.3q13.3)de novo(72,636,161-74,421,392)
53	del(4)(q13.3q21.1)de novo(74,932,950-77,060,297)	del(10)(p14p14)de novo(8,423,513-9,583,339)
57	—	del(4)(q32.1q32.1)de novo(156,373,857-158,388,330)

Gene Disruption at the Breakpoints

Unexpectedly, we observed that in both the clinically normal and the abnormal cohorts, the great majority of the breakpoints were located in the vicinity of genes (typically within less than 200 kb, see Tables 4 and 6). Furthermore, for breakpoint regions with no known genes (in both cohorts), all but one mapped within 1.2 Mb of a gene, i.e., within the current maximum known range of position effects.[31] In the normal cohort, 27% of the breakpoints mapped within regions that harbor no genes, 32% were associated with obligatory gene breakage, and the remaining 41% were associated with potential gene disruption. Similarly, in the 14 phenotypically abnormal carriers, 33% of the breakpoints located to regions containing no genes, 21% disrupted genes, and 46% were potentially associated with gene disruption. The proportion of the human genome occupied by annotated genes has been estimated as ~38.5% (37.2% of introns and 1.3% of exons)[32] or ~34.8% (Ensembl database version 46.36h). We observed that a minimum of 21% and a maximum of 70% of the breakpoints disrupt genes. The lower figure of 21% is within the expected values for a random localization of breakpoints, whereas the higher figure of 70% suggests a preferential location of the breakpoints to within genes. However, at the resolution of the techniques used here we cannot draw further conclusions.

Genes Disrupted by the Breakpoints and Genotype-Phenotype Correlations

We conducted extensive literature searches on all genes mapped within breakpoint regions in both cohorts using data from the GO database, OMIM, the Human Gene Mutation database, UniProt, GeneCards, and the DGV. Surprisingly, we found that genes implicated in transcription and signal transduction were the most common types of genes in the breakpoints of both cohorts. The most striking difference between the cohorts was that genes with a role in the nervous system were present in 5/14 (36%) abnormal patients but in only 2/31 (6.5%) of the normal individuals, suggesting that this type of gene is an important cause of clinical abnormalities. Further analysis showed that, in both cohorts, as many as 50% of the genes mapped to the breakpoints were located in regions previously reported to have normal copy-number variation. Moreover, many genes were largely uncharacterized, but a minority have been implicated in phenotypic abnormalities and are discussed below.

Gene Disruption in Phenotypically Normal Individuals

Among the 31 phenotypically normal patients, we found breakpoint-mediated disruption of 18 genes (Table 4). Of these, *RYR2* (ryanodine receptor 2 cardiac [MIM 180902]), *FAT* (FAT tumor-suppressor homolog 1, *Drosophila* [MIM 600976]), *ABAT* (4-aminobutyrate aminotransferase [MIM 137150]), and *EXOC4* (exocyst complex component 4) are of particular interest. Mutations in *RYR2* (case 2O) are a cause of familial arrhythmogenic right-ventricular dysplasia 2 [MIM 600996] and of stress-induced polymorphic ventricular tachycardia [MIM 604772]. Unfortunately, the clinical history of case 2O is unknown, and therefore we are unable to establish genotype-phenotype correlations. Case 2A had disruption of both the *FAT* and the *ABAT* genes. *FAT* encodes a tumor-suppressor protein in *Drosophila* but is largely uncharacterized in humans. Interestingly, case 2A developed a prolactinoma at 37 years of age (Table S1), suggesting that further studies would be of interest to ascertain whether *FAT* disruption causes this type of tumor. Mutations in *ABAT* are associated with GABA-AT deficiency [MIM 137150]. Case 2A did not present any of the features of this autosomal-recessive disorder, which is consistent with the presence of a normal functioning allele on the nontranslocated chromosome. Finally, *EXOC4* was disrupted in case 2E. Disruption of this gene and the formation of a truncated protein have been previously reported in a patient with microcephaly, developmental delay, and a t(7;10)(q33;q23) de novo.[29]

Gene Disruption in Phenotypically Abnormal Individuals

Among the clinically abnormal patients, we have attempted to identify disease-candidate genes at the breakpoint regions. For case 49, we identified disruption of the *SATB2* (SATB homeobox 2 [MIM 608148]) gene. Haploinsufficiency of *SATB2* is known to cause cleft palate,[18] and

BCI denotes breakpoint-containing interval.
nd denotes not determined.
[1] The probability of gene disruption (as a percentage) was calculated as the physical size of a given gene in relation to the size of the breakpoint-containing interval. Two estimates are given in the cases in which gene-mapping discrepancies were found between the Ensembl and the UCSC genome browsers (these are due to the use of different algorithms for gene annotation).

Table 6. Summary of the Breakpoint-Mapping Results in the Abnormal Cohort

Case	BCI Genomic Position	Size of the BCI (bps)	Gene Content within the BCI	Likelihood of Gene Disruption[1]
16	chr10:102,470,424-102,509,479	39,055	*PAX2*	40
16	chr22:42,975,258-43,015,630	40,372	*KIAA1644*	100
20	chr2:203,884,002-203,963,572	79,570	*ABI2*	80
20	chr5:60,919,174-61,052,997	nd	associated with a deletion	nd
43	chr4:181,557,356-181,712,629	155,273	—	—
43	chr17:62,750,206-62,824,693	74,487	*PSMD12, PITPNC1*	60
45	chrX:78,833,496-78,996,054	162,558	—	—
45	chr19: centromeric	nd	nd	nd
48	chr4:172,112,115-172,314,669	202,554	—	—
48	chr6:111,251,547-111,344,802	93,255	*AMD1*	23
49	chr2:199,874,411-199,911,683	37,272	*SATB2*	100
49	chr10:64,314,352-64,495,348	180,996	—	—
50	chrX:115,042,292-115,233,384	191,092	*AGTR2*	3
50	chr8:142,582,086-142,790,550	208,464	—	—
51	chr4:17,806,803-18,134,168	327,365	—	—
51	chr20:10,200,284-10,241,852	41,568	*SNAP25*	85
52	chr4:64,164,472-64,348,036	183,564	—	—
52	chr4:87,196,675-87,397,572	200,897	*MAPK10*	100
52	chr11:99,613,168-99,929,275	316,107	*CNTN5*	40
53	chr4:74,827,036-75,000,632	nd	associated with a deletion	nd
53	chr8:35,542,879-35,705,808	162,929	*UNC5D*	100
54	chr14:55,276,675-55,382,178	105,503	*Q6NVV1_HUMAN*	0-2
54	chr15:94,659,356-94,695,383	36,027	*NR2F2, AK000872*	30-65
55	chr19:63,177,283-63,220,873	43,590	*ZNF606, Q8N9G5_HUMAN*	70
55	chr20:28,033,231-28,197,751	164,520	*Q6ZS48_HUMAN*	0-5
56	chr6:97,826,527-97,908,737	82,210	*C6orf167, AK091365*	14-68
56	chr21: centromeric	nd	nd	nd
57	chr2:24,640,005-24,682,052	42,047	*NCOA1*	50
57	chr5:57,413,898-57,782,197	368,299	—	—
57	chr18:20,893,727-21,067,881	174,154	*ZNF521*	100
57	chr22: satellite stalk	nd	nd	nd

"BCI" denotes "breakpoint-containing interval."
"nd" denotes "not determined."
[1] The probability of gene disruption (as a percentage) was calculated as the physical size of a given gene in relation to the size of the breakpoint-containing interval. Two estimates are given in the cases in which gene-mapping discrepancies were found between the Ensembl and the UCSC genome browsers (these are due to the use of different algorithms for gene annotation).

thus *SATB2* disruption must explain the presence of this malformation in case 49. Case 51 had potential disruption of the *SNAP25* (synaptosomal-associated protein, 25kDa [MIM 600322]) gene. This gene has a role in regulation of neurotransmitter release, and it could be involved in the synaptic function of specific neuronal systems. Furthermore, polymorphisms of *SNAP25* have been associated with behavioral traits such as hyperactivity/impulsivity and inattention,[33] suggesting that *SNAP25* is a good candidate for the Attention Deficit Hyperactivity Disorder (ADHD [MIM 143465]) in case 51. In case 48, *AMD1* (adenosylmethionine decarboxylase 1 [MIM 180980]) was potentially disrupted. This gene encodes an intermediate enzyme involved in polyamine biosynthesis. Polyamines might be involved in brain development and cognitive function,[34] suggesting that *AMD1* is a good candidate gene for the developmental delay in case 48. Case 54 had potential disruption of the *NR2F2* (nuclear receptor subfamily 2, group F, member 2 [MIM 107773]). Studies in mice indicate that *NR2F2* might be implicated in heart defects,[35] suggesting that this gene is a good candidate for the cardiac abnormality in case 54.

In summary, in the phenotypically normal cohort we have not identified (1) breakpoint-associated imbalances, (2) genomic imbalances unrelated to the breakpoints, or (3) chromosomal complexity. In contrast, in our phenotypically abnormal cohort, breakpoint-associated imbalances, both at the breakpoints and elsewhere in the genome, and chromosome complexity were present in 4/14 patients. These results, considered in combination with those of our previously studied cohort,[9] show that genomic imbalances detectable by array CGH could be the underlying cause of phenotypic abnormalities in a significant proportion of patients with an apparently balanced rearrangement. These observations agree with those reported by De Gregori et al.[10] Furthermore, it is of interest that all but one of the de novo imbalances identified in studies of apparently balanced rearrangements[9–11] were paternal in origin. This suggests that male gametogenesis is particularly susceptible to the factor(s) responsible for this class of chromosome abnormality.[10]

We identified gene disruption by the breakpoints in both phenotypically normal and abnormal individuals, the proportion being similar in both groups but with genes

implicated in biological processes of the nervous system being more frequent in the abnormal cohort. The presence of a normal phenotype is consistent with the disruption of genes that are not developmentally regulated dosage-sensitive genes, whereas the presence of an abnormal phenotype suggests the interruption of putative disease genes, which are good candidates for further study.

Supplemental Data

Supplemental Data include three tables and can be found with this article online at http://www.ajhg.org/.

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Web Resources

The URLs for data presented herein are as follows:

Database of Genomic Variants (DGV), http://projects.tcag.ca/variation/
DECIPHER database, http://www.sanger.ac.uk/PostGenomics/decipher/
Ensembl Human Genome Browser, http://www.ensembl.org/Homo_sapiens/
GeneCards, http://www.genecards.org/
Gene Ontology (GO), http://www.geneontology.org/
Human Gene Mutation Database, http://www.hgmd.cf.ac.uk/
Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/Omim
UniProt, http://www.ebi.uniprot.org/
University of California-Santa Cruz Human Genome Browser, http://genome.ucsc.edu/

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