ORIGINAL ARTICLE

The complex nature of constitutional de novo apparently balanced translocations in patients presenting with abnormal phenotypes

S M Gribble*, E Prigmore*, D C Burford*, K M Porter, Bee Ling Ng, E J Douglas, H Fiegler, P Carr, D Kalaitzopoulos, S Clegg, R Sandstrom, I K Temple, S A Youings, N S Thomas, N R Dennis, P A Jacobs, J A Crolla, N P Carter

J Med Genet 2005;**42**:8–16. doi: 10.1136/jmg.2004.024141

See end of article for authors' affiliations

Correspondence to: Dr N P Carter, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK; npc@sanger.ac.uk

Revised version received 28 July 2004
Accepted for publication 5 August 2004

* These authors contributed equally to the study

Objective: To describe the systematic analysis of constitutional de novo apparently balanced translocations in patients presenting with abnormal phenotypes, characterise the structural chromosome rearrangements, map the translocation breakpoints, and report detectable genomic imbalances.
Methods: DNA microarrays were used with a resolution of 1 Mb for the detailed genome-wide analysis of the patients. Array CGH was used to screen for genomic imbalance and array painting to map chromosome breakpoints rapidly. These two methods facilitate rapid analysis of translocation breakpoints and screening for cryptic chromosome imbalance. Breakpoints of rearrangements were further refined (to the level of spanning clones) using fluorescence in situ hybridisation where appropriate.
Results: Unexpected additional complexity or genome imbalance was found in six of 10 patients studied. The patients could be grouped according to the general nature of the karyotype rearrangement as follows: (A) three cases with complex multiple rearrangements including deletions, inversions, and insertions at or near one or both breakpoints; (B) three cases in which, while the translocations appeared to be balanced, microarray analysis identified previously unrecognised imbalance on chromosomes unrelated to the translocation; (C) four cases in which the translocation breakpoints appeared simple and balanced at the resolution used.
Conclusions: This high level of unexpected rearrangement complexity, if generally confirmed in the study of further patients, will have an impact on current diagnostic investigations of this type and provides an argument for the more widespread adoption of microarray analysis or other high resolution genome-wide screens for chromosome imbalance and rearrangement.

Structural abnormalities involving human chromosomes are estimated to occur in around 0.5% of newborn infants, using a moderate level of resolution in conventional cytogenetic analysis.[1] In the vast majority of cases, apparently balanced structural chromosome abnormalities are not associated with abnormal phenotypes and may be transmitted through several generations without detection; however, carriers of apparently balanced translocations are at higher risk of reproductive failure.[2]

By contrast, the cytogenetic identification of an unbalanced constitutional de novo chromosome abnormality in patients is usually accepted as the underlying cause of their abnormal phenotype. Such imbalances are almost always resolvable by chromosome banding studies and involve cytogenetically visible deletions or duplications of several megabase pairs (Mb) of DNA, leading to potential imbalance of many genes which may contribute to the phenotype.

Antenatally ascertained balanced de novo structural rearrangements are associated with abnormal phenotypes in around 6% of cases.[3] Abnormal phenotypes in patients with apparently balanced de novo translocations are thought to reflect the disruption of a gene or genes at the breakpoints,[4–16] or small duplications or deletions beyond the resolution of the light microscope.[17–19] Alternatively, the relation between an abnormal phenotype and karyotype might be coincidental rather than causal.

To date, only small numbers of apparently balanced de novo translocations have been analysed beyond the level of the light microscope to the resolution of the breakpoint regions, and these preliminary studies have shown not only that breakpoints interrupt genes but also that cryptic deletions and duplications may be present.[17–21] With the sequencing of the human genome we now have available a resource of overlapping, mapped, and sequenced large insert clones which are ideal for high resolution molecular cytogenetic analyses. The use of these clones for fluorescence in situ hybridisation (FISH) and for the production of DNA microarrays is providing new more efficient tools for high resolution analysis of chromosome rearrangements. Recently, we have shown how comparative genomic hybridisation using DNA arrays (array CGH) and array painting can be used for rapid high resolution analysis of chromosome abnormalities.[22] With both these methods, the resolution at which the rearrangements can be determined is limited only by the clone size and density on the array. The combined use of both array CGH and array painting allows rapid scanning of the genome for cryptic imbalances and mapping of translocation breakpoints, and can be followed by FISH to identify breakpoint spanning clones, typically of 150 kb in size.

We set out to use these techniques for rapid mapping of a large number of de novo translocations in patients with an associated abnormal phenotype. However, we soon found that in a large proportion of patients' karyotypes that

Abbreviations: BAC, bacterial artificial chromosome; FISH, fluorescence in situ hybridisation

appeared balanced by chromosome banding were more complex using these high resolution molecular cytogenetic methods, and that analysis of these complex rearrangements required considerable additional investigation.

Here we describe the high resolution molecular cytogenetic analysis of 10 patients, all with an abnormal phenotype and carrying apparently balanced de novo translocations. All patients were analysed using array CGH and array painting followed by FISH analysis to identify breakpoint spanning clones where appropriate.

METHODS

Patients

Patients recruited into this study originally presented to the Wessex Regional Genetics Laboratory (WRGL) which provides a cytogenetic, molecular cytogenetic, and molecular genetic diagnostic service to a population of three million people in south central England. We selected patients with an abnormal phenotype, an apparently balanced de novo translocation, and blood available from which to establish a lymphoblastoid cell line.

Conventional cytogenetic analysis

The 10 patient samples were analysed using phytohaemagglutinin (PHA) stimulated peripheral blood metaphases following standard procedures. All chromosome preparations were G banded using the method described by Seabright[23] and analysed to a minimum resolution of 550 to 600 bands. Parental origins of the translocations were determined by the cytogenetic analysis of both parents at a minimum of 650 band resolution and these samples were not available for re-examination as part of the study. Paternity testing was not done. It should be noted that the cytogenetic band assignments and the FISH probe positions are not directly comparable, as the former represent subjective opinions and the latter are determined by evolving assemblies of the tiling paths from which the clones were derived. The cytogenetic breakpoints are those recorded at the original diagnosis, and the BAC physical locations are as recorded on NCBI Build 33 of the human genome.

Cell lines

Lymphoblastoid cell lines for the patients were established by the European Cell and Culture Collection (ECACC), Porton Down, and/or at the Cell Bank at the WRGL. Cell line reference numbers (DD = ECACC; LN = WRGL) are as follows: case A1, DD0127; case A2, DD0391; case A3, DD1260; case B1, DD3572; case B2, DD3235; case B3, LN1/3D62/63; case C1, DD0719; case C2, LN1/1D1/2/9; case C3, DD3606; case C4, DD3615. All cell lines were re-karyotyped with a focused analysis to confirm the presence of the translocations and their breakpoints before being submitted for molecular analysis.

Table 1 Microsatellites selected from the corresponding regions using ContigView

Case A1	(Chromosome 10)	D10S1665, D10S1647, D10S1743
Case A3	(Chromosome 11)	D11S1332, D11S4176, D11S919, D11S4182
Case B1	(Chromosome 3)	D3S525, D3S3630, D3S4538, D3S3050
Case B2	(Chromosome 6)	D6S447, D6S268
Case B3	(Chromosome 18)	D18S468, D18S34, D18S1136, D18S454, D18S65, D18S872, D18S1130

Molecular origin of imbalances in cases A1, A3, B1, B2, and B3

The origins and transmission of the additional imbalances were determined by amplifying DNA extracted from each proband and their parents at various microsatellite loci. The location of the individual large insert clones on the array, which defined the extent of each imbalance, were determined using CytoView from the Ensembl Human Genome Browser (http://www.ensembl.org/Homo_sapiens/cytoview). Table 1 shows the microsatellites that were selected from the corresponding regions using ContigView.

All primer sequences and polymerase chain reaction (PCR) conditions are available from the Genome database (www.gdb.org). One primer from each pair was fluorescently labelled and the products analysed on an ABI automated DNA sequencer. For case B2 (chromosome 6), fluorescent dosage PCR was used to distinguish between homozygosity and hemizygosity when only single alleles were observed.

DNA microarray analysis

The DNA microarrays used were constructed from a set of approximately 3500 large insert clones, as described previously.[24] Array CGH, chromosome sorting, and array painting were carried out as described.[22] The genomic localisation of clones spotted on the array was determined using NCBI Build 33 of the human genome. Identification of candidate genes was done using Ensembl, based on NCBI Build 34 of the human genome.

Metaphase fluorescence in situ hybridisation

Clone DNA, either prepared by conventional alkaline lysis from glycerol stocks or by DOP amplification (as used in array production), was labelled with biotin-16-dUTP (Roche, Mannheim, Germany) or digoxigenin-11-dUTP (Roche) by nick translation. Hybridisations were conducted following conventional methods using chromosomes prepared from cell lines derived from patient samples. Biotin labelled probes were detected using Avidin TexasRed (Molecular Probes, Eugene, Oregon, USA), while digoxigenin labelled probes were detected with a combination of mouse anti-digoxigenin (Vector Laboratories, Peterborough, Northants, UK) and goat anti-mouse FITC antibodies (Sigma-Aldrich, Poole, Dorset, UK).

RESULTS

Analysis of the patients using the molecular cytogenetic methods described in this study allowed us to place them into three groups: group A, complex rearrangements with loss, gain or inversion at or near the translocation breakpoint; group B, chromosomal imbalance apparently unrelated to the translocation; and group C, apparently uncomplicated balanced translocation with no detected chromosomal imbalance. To aid comparison, the results are presented by group, although individual cases were analysed in random order without knowledge of the phenotype. Cases A1 and A2, which showed complex rearrangement, are described in full to illustrate the methods employed, whereas results are summarised for the others. A summary of all results is shown in table 2. The revised karyotypes are given at the end of each section, and the BACs described are all from the RP11 library unless otherwise stated.

Case A1: 46,XX,t(6;10)(q13;q21.2) *de novo*

This patient was initially referred at the age of six months because of developmental delay and hypotonia. At the age of three years she had learning difficulties and a dysmorphic appearance.

Array CGH identified a small 10q deletion close to the cytogenetically reported 10q21.2 breakpoint involving a

Table 2 Summary of results

Case	Sex	Conventional karyotype*	Phenotype	Molecular imbalance	Imbalance size	Parental origin
A1	F	t(6:10)(q13;q21.2)	LD; Dys	Complex; del(10)(q21.2)	~5.5 Mb	del(10) de novo (paternal)
A2	M	inv(6)(p21.3;q22.2), t(6;17)(p21;q23)	LD; epilepsy; MED	Complex	None detected	
A3	F	t(11;12)(q21;p13.33)	LD; epilepsy	del and inv at 11q breakpoint	~6.2 Mb	del(11) de novo (paternal)
B1	M	t(2;7)(q37.3;p15.1)	Severe DD, Dys	dup(3)(p26.3); translocation balanced	1.1–2.9 Mb	Carried by father
B2	F	t(2;5)(q31.1;q23.2)	Severe SD; autism	del(6)(q21); translocation balanced	2.2–3.4 Mb	del(6) de novo (paternal)
B3	F	t(4;9)(q25;q22.3)	LD, Dys; epilepsy	del(18)(q12.3); translocation balanced	~6 Mb	del(18) unknown (paternal)
C1	M	t(17;22)(q21.1;q12.2)	LD, epilepsy, BP	Balanced	None detected	
C2	F	t(2;7)(q37.2;q36.3)	Eye abnorm; Reiger's syndrome	Balanced	None detected	
C3	F	t(3;11)(q21;q12)	MCA (twin)	Balanced	None detected	
C4	M	inv(11)pat, t(7;13)(q31.3;q21.3)	DD, autistic features, epilepsy	Balanced	None detected	

*All translocations are de novo.
Abnorm, abnormality; BP, behavioural problems; DD, developmental delay; Dys, dysmorphic features; LD, learning difficulties; MCA, multiple congenital abnormalities; MED, multiple epiphysial dysplasia; SD, speech delay.

region of approximately 5.5 Mb with the proximal breakpoint between RP11-176H12 and RP11-161L14 in 10q21.3 and the distal breakpoint between RP11-816P15 and RP11-367H5 in 10q22.1 (fig 1). The flow karyotype for this patient is shown in fig 2. The derivative chromosome 10 could be identified between chromosome 8 and the 9–12 cluster, while the derivative chromosome 6 was positioned just below and merging with the X chromosome. As well as signal from the contaminating chromosome X, array painting with the two sorted derivatives showed a clear breakpoint (abrupt transition between high and low ratios) in chromosome 6q14.4 between RP11-173D14 and RP1-159G19 (fig 3). A clear breakpoint for chromosome 10 in 10q21.2 was also identified between RP11-166B18 and RP11-809M12. As expected from the array CGH results, no signal was observed for the more distal clones reporting the deletion on chromosome 10 (RP11-161L14 to RP11-816P15). However, surprisingly, signal was not seen by array painting for clones RP11-13A2 (10q21.2) and RP11-176H12 (10q21.3), both of which reported normal copy numbers in array CGH. From this we concluded that the sequences represented by these two clones spanning approximately 1.9 Mb are not present on either the derivative 6 or 10.

These two clones were hybridised by FISH onto metaphase spreads of the patient and were found to map to one homologue of chromosome 4 identifying a derivative 4 chromosome containing a chromosome 10 insertion at 4q13. Further FISH analysis with tiling path clones identified RP11-531F24 as showing a split signal on both the derivative 4 and 6. Thus this clone spans the proximal, secondary breakpoint associated with the insertion of chromosome 10 material into derivative chromosome 4.

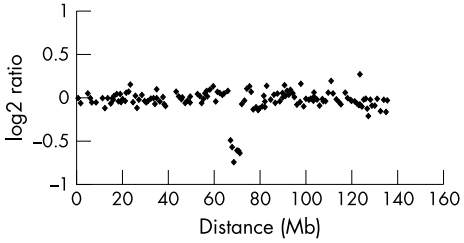

Figure 1 Array CGH analysis of chromosome 10 for case A1 showing microdeletion.

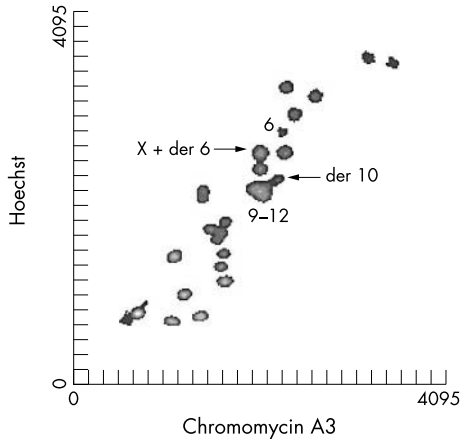

Figure 2 Flow karyotype of case A1.

The more distal chromosome 10 clone, RP11-165K20, showed a reduced signal on the derivative chromosome 4 which we interpreted as the clone spanning the distal insertion breakpoint into the deleted region. This suggests that the distal insertion breakpoint and the proximal deletion breakpoint are shared.

Other breakpoint spanning clones were found by FISH to be as follows: RP11-343J3 partially deleted, thus spanning the distal deletion breakpoint; RP11-97A17 spanning the chromosome 10 translocation breakpoint; and RP1-232L24 spanning the chromosome 6 translocation breakpoint. Both parents were heterozygous at three microsatellites within the deleted region, confirming that the rearrangement is de novo. Two loci (D10S1665 and D10S1647) showed the deletion was paternal in origin.

In conclusion, the molecular cytogenetic analyses suggest that two rearrangement events have occurred in this case. One rearrangement involved a simple translocation between chromosomes 6 and 10 and a second deletion/insertion event involving chromosome 10 material and chromosome 4. The order in which these rearrangements took place cannot of course be determined. The rearrangements are summarised in fig 4A.

In summary, a de novo deletion of approximately 5.5 Mb distal to the 10q21.2 translocation breakpoint was found on further analysis to share one breakpoint with a small

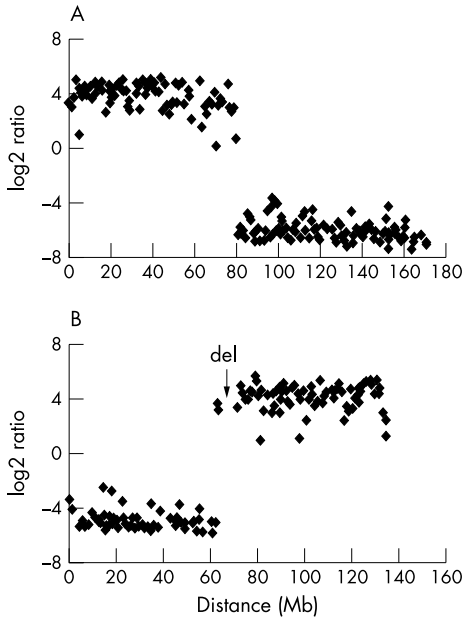

Figure 3 Array painting analysis of case A1. (A) Chromosome 6. (B) Chromosome 10. The microdeleted region on chromosome 10 is indicated by the arrow.

insertion of approximately 1.9 Mb of chromosome 10 into chromosome 4q13. The 6q13 and 10q21.2 breakpoints appear balanced. As the proximal insertion breakpoint from chromosome 10 was found to be just distal to the translocation breakpoint, it would appear that the insertion/deletion event and the translocation event were independent.

The revised karyotype is: 46,XX,t(6;10)(q13;q21.2) *de novo*.ish del(10)(q21.3q22.1)(161L14-,816P15-),t(6;10)(RP1-232L24sp;97A17sp),dirins(4;10)(q13;q?21.3q?21.3)(13A2+,176H12+;13A2-,176H12-).

Case A2: 46,XY,inv(6)(p21.3;q22.2) *de novo*,t(6;17)(p21;q23) *de novo*

This patient was referred at birth with cleft palate, micrognathia, low set ears, single palmar crease, and overriding fifth toe. At the age of 12 years, he had learning difficulties and attended a special school. He had epilepsy, multiple epiphysial dysplasia (which required him to use a wheelchair for significant distances), and conductive hearing loss.

No genomic imbalances were found using array CGH. A partial flow karyotype is shown in fig 5. The derivative 17 was clearly separated from the surrounding normal chromosomes 15 and 16, while the derivative 6 could not be separated from the normal chromosome 7. The peak containing the derivative 17 and the peak containing the derivative 6 and normal chromosomes 7 were flow sorted for array painting analysis. Array painting unexpectedly indicated the presence of chromosome 11 material in the derivative 17. To determine whether chromosome 11 was involved in a rearrangement with chromosome 17, we flow sorted the mixed peak of chromosomes 9, 10, 11, and 12, presumably containing a derivative chromosome 11. Array painting analysis was then carried out with derivative chromosome 6 against the mixed peak and the derivative 17 versus the mixed peak.

In the array painting of the derivative 6 versus the mixed peak, a clear transition from high ratios to low ratios was found at chromosome 6p22.3 between RP1-13D10 (derivative 11) and RP11-33I5 (derivative 6). However, the six clones (between RP11-68J15 and RP11-204E9 spanning approximately 3.9 Mb) which map between these flanking clones

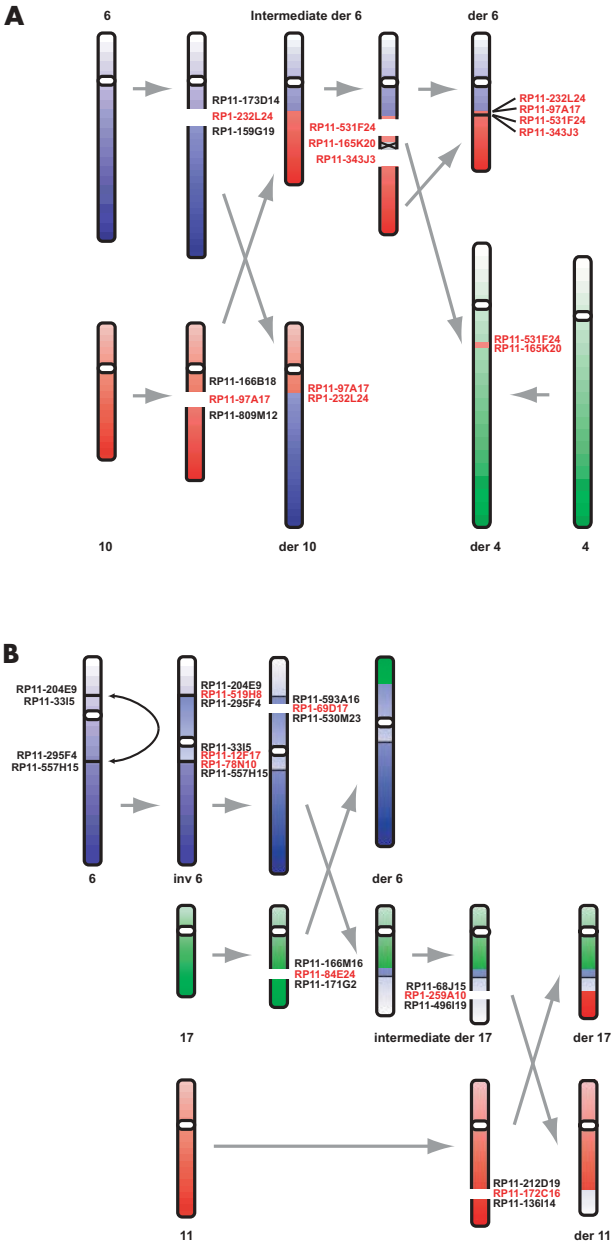

Figure 4 Summary diagrams of (A) case A1, and (B) case A2. Clone names in black are present on the 1 Mb resolution array, clone names in red are breakpoint spanning.

showed only background fluorescence, suggesting that this region is not present on either the derivative 6 or the derivative 11. The derivative chromosome 6 also contained the region of chromosome 17 from RP11-171G2 (17q24.3) to qter (approximately 9.6 Mb). In the array painting of the derivative 17 versus the mixed peak, we found that the derivative 11 contained the region of chromosome 6 from pter to RP1-13D10 (p22.3) while two regions of chromosome 6 were present on the derivative 17—that is, between RP11-68J15 and RP11-204E9 (the approximately 3.9 Mb of 6p22.3 not found on the derivative 6 or the derivative 11 by array painting) and between RP11-593A16 and RP11-295F4 (approximately 2.8 Mb of 6q22.33). The derivative 17 also contained the region of chromosome 11 from RP11-136I14 to 11qter and chromosome 17 material from pter to RP11-116M16.

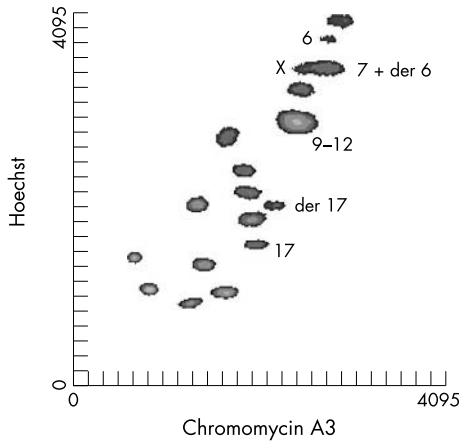

Figure 5 Partial flow karyotype of case A2.

From this analysis we can infer that it is the inverted chromosome 6 that is the translocation partner with chromosomes 11 and 17. These rearrangements are summarised in fig 4B. We further mapped the inversion breakpoints on chromosome 6 by FISH and found that the distal clone RP11-519H8 and the two proximal overlapping clones (RP11-12F17 and RP1-78N10) spanned the breakpoints. Finally, we identified the translocation breakpoint spanning clones by FISH as RP1-69D17 (first chromosome 6 breakpoint), RP1-259A10 (second chromosome 6 breakpoint), RP11-84E24 (chromosome 17), and RP11-172C16 (chromosome 11).

In summary, a complex rearrangement was found involving an inverted chromosome 6, chromosome 11, and chromosome 17 (fig 4B). All six chromosome breakpoints appear to be balanced at cytogenetic and molecular resolutions.

The revised karyotype is: 46,XY,inv(6)(p21.3;q22.2)t(6;17)(p21;q23) *de novo*.ish inv(6)(204E9st,519H8sp,295F4mv,33F5mv,12F17sp,RP1-48N10sp,357H15st)t(6;17;11)(RP1-69D17sp,RP1-259A10sp;84E24sp;172C16sp).

Case A3: 46,XX,t(11;12)(q21;p13.33) *de novo*

This case has been described previously.[22] Briefly, the patient was first referred at age seven years with short stature and triangular facies. She developed seizures at the age of 18, and two years later was described as having mild cognitive difficulty and temporal lobe epilepsy.

Array painting, array CGH, and identification of breakpoint spanning clones for this patient have been described previously.[22] In brief, array CGH identified an approximately 6.2 Mb deletion of chromosome 11 at q14.3-q22.1. Both parents were heterozygous at four microsatellites within the deleted region, confirming that the rearrangement is de novo. One microsatellite (D11S1332) showed that the deletion was paternal in origin; the other loci were not informative. Array painting for chromosome 11 showed a complex pattern, with a transition from low to high ratios between clones RP11-90K17 (87.1 Mb) and RP11-320L11 (87.5 Mb) immediately followed by a transition from a high to a low ratio between clones RP11-137O10 (88.9 Mb) and RP11-156E23 (89.1 Mb). The next set of clones encompassing RP11-325I16 (92.9 Mb) to RP11-379J13 (99.1 Mb) showed only background fluorescence, confirming the deletion. High ratios were then observed from the next clone RP11-49M9 (101.5 Mb) to the qter. We interpreted these results to indicate the presence of an inversion/deletion event on chromosome 11 close to but not involving the chromosome 11 breakpoint. Further FISH analysis with tiling path clones showed that RP11-876F8 spanned the chromosome 11 translocation breakpoint. The pattern for chromosome 12 appeared balanced, with an intermediate ratio reported for RP11-359B12 (1 Mb from pter), suggesting that this clone would span the breakpoint. This was confirmed by FISH.

In summary, a complex rearrangement was identified involving a deletion/inversion of approximately 6.2 Mb in chromosome 11q14.3-q22.1, but with a simple breakpoint on chromosome 12 close to pter. The deletion was confirmed by FISH.

The revised karyotype is: 46,XX,t(11;12)(q21;p13.33) *de novo*.ish del(11)inv(11) (90K17st,320L11mv,137O10mv,156E23mv,325I16-,99C10-,49M9st),t(11;12)(876F8sp;359B12sp).

Case B1: 46,XY,t(2;7)(q37.3;p15.1) *de novo*

This patient was referred at the age of eight months with mild generalised developmental delay, a slightly beaked nose, and adducted thumbs. At the age of two years and eight months his development was clearly delayed: he was able to sit but unable to walk unaided and he had no intelligible words. He had a dysmorphic appearance with brachycephaly, blepharophimosis, medially flared eyebrows, a broad nasal tip, short philtrum, thin upper lip, and prominent lower jaw.

Array CGH revealed a small duplication towards the pter of chromosome 3 involving three clones on the array (RP11-299N3, RP11-95E11, and RP11-10H6) spanning between 1.1 and 2.9 Mb of 3p26.2–26.3. The duplication of the sequences represented by these three clones was confirmed by FISH. Molecular analyses revealed three different alleles in the proband at the locus D3S3630 and the ratio of alleles at the other loci was consistent with a duplication. Three different alleles were also observed in the father, at the loci D3S3630 and D3S3525, indicating that the duplication was familial. The mother appeared normal at all loci tested. The translocation was found to be balanced, with RP11-680O16 spanning the chromosome 2 breakpoint and CTA-471E18 spanning the chromosome 7 breakpoint.

In summary, this was an apparently balanced translocation at the 2q37.3 and 7p15.1 breakpoints but with an additional unsuspected 3p26.2–26.3 duplication 1.1–2.9 Mb in size which had been inherited from the proband's phenotypically normal father.

The revised karyotype is: 46,XY,t(2;7)(q37.3;p15.1) *de novo*.ish t(2;7)(680O16sp;CTA-471E18sp), dup(3)(p36.3p36.3)(299N3++,95E11++,10H6++).

Case B2: 46,XX,t(2;5)(q31.1;q23.2) *de novo*

This patient was referred at the age of 26 months because of developmental and severe speech delay, unusual pinnae, dry skin, and absent tears. At eight years she was found to fulfil ICD10 criteria for autism, with impaired social interaction and communication and some evidence of repetitive stereotyped patterns of behaviour.

Array CGH revealed a deletion on chromosome 6 involving clones RP3-454N4, RP3-448K1, and RP11-35D6 spanning between 2.2 and 3.4 Mb of 6q21. Molecular analyses of the 6q21 deletion showed that the proband was hemizygous at both loci tested. Each parent was heterozygous at one locus and homozygous at the other. D6S268 was not informative for parental origin but at D6S447 the proband could only have inherited his single allele from his mother. Therefore the deletion is de novo and originated on the paternally inherited chromosome.

RP11-681B22 was found to span the chromosome 2 breakpoint. The results for the chromosome 5 breakpoint were more difficult to interpret. While a very small RP11-436H11 signal could be seen on the derivative 2, suggesting that this clone spans the breakpoint, RP11-48C14, which

maps distal to RP11-436H11, was shown to be retained on the derivative chromosome 5 by both FISH analysis and array painting. Further FISH studies using normal chromosomes confirmed that the map had not been misassembled. As the region involved is so small (< 200 kb) we were unable to resolve this discrepancy using FISH but this might suggest the possibility of a small inversion at the chromosome 5 breakpoint.

In summary, this was an apparently balanced translocation at cytogenetic and molecular resolutions (with a possible small inversion at the chromosome 5 breakpoint) but with additional, unsuspected de novo cryptic 2.2–3.4 Mb deletion in chromosome 6q21 of paternal origin.

The revised karyotype is: 46,XX,t(2;5)(q31.1;q23.2) *de novo*.ish t(2;5)(681B22sp;436H11sp), del(6)(q21q21)(RP3-454N4-,35D6-).

Case B3: 46,XX,t(4;9)(q25;q22.3) *de novo*

This patient was originally referred aged 11 months because of generalised hypotonia. At the age of 18 she was dysmorphic with a high arched palate, downslanting palpebral fissures, and ptosis. Her head circumference was 57 cm and her vision and hearing were normal, but she had no speech and communicated by noises and singing. At the age of 22 she was reported to have severe learning difficulties and epilepsy.

Array CGH revealed a deletion on chromosome 18 involving clones RP11-25C13, RP11-11F23, RP11-164M8, RP11-486C18, and RP11-463D17 and spanning approximately 6.0 Mb of 18q12.3. DNA was only available from the proband and her mother. At all seven loci tested within the 18q12.3 deletion interval, the proband's DNA contained one of the alleles for which the mother was heterozygous, so that the proband was hemizygous with no paternal alleles. We were, however, unable to obtain paternal DNA so cannot distinguish between a de novo or paternally transmitted 18q deletion. Further FISH analysis indicated that the proximal deletion breakpoint mapped between RP11-482F20 and RP11-1I2 (when selected and hybridised, the intermediate tiling clone RP11-244M2 mismapped to chromosome 18p and was not used in this study) and the distal deletion breakpoint within RP11-366J11. The translocation was found to be balanced with RP11-242D9 spanning the chromosome 4 breakpoint and RP11-64C9 spanning the chromosome 9 breakpoint.

In summary, this was an apparently balanced translocation at cytogenetic and molecular resolutions but with additional and unsuspected ~6.0 Mb deletion in chromosome 18q12.3.

The revised karyotype is: 46,XX,t(4;9)(q25;q22.3) *de novo*.ish t(4;9)(242D9sp;64C9sp),del(18)(q12.3q12.3)(25C13-,463D17-).

Case C1: 46,XY,t(17;22)(q21.1;q12.2) *de novo*

This case has been described previously.[22] The patient was seen by a paediatrician at the age of 2.5 years because of delayed speech development following an uneventful early history. He made developmental progress with speech delay as his only problem until the age of four years when he became withdrawn and unresponsive. At four years and eight months he showed severe autistic behaviour which has persisted to the age of 13.

Array painting, array CGH, and identification of breakpoint spanning clones for this patient have been described previously.[22] Briefly, a simple balanced translocation was found mapping to within the spanning clones RP11-46E17 (chromosome 22q12.1) and RP11-749I16 (chromosome 17q21.1).

In summary, this was an apparently balanced translocation at cytogenetic and molecular resolutions.

The revised karyotype is: 46,XY,t(17;22)(q21.1;q12.2) *de novo*.ish t(17;22)(749I16sp;46E17sp).

Case C2: 46,XX,t(2;7)(q37.2;q36.3) *de novo*

Details of this patient have been reported previously.[25] Briefly, she initially sought genetic advice at the age of 29 years because of a combination of bilateral syndactyly of the hands and feet, bilateral Rieger anomaly of the anterior chamber of the eyes with secondary glaucoma, a congenital skin defect of the neck, folds of redundant skin over the trunk, hirsutism, and polycystic ovaries.

Array CGH, array painting, and metaphase FISH showed a balanced reciprocal translocation with RP11-263G22 spanning the chromosome 2q37.1 breakpoint and RP11-69O3 spanning the chromosome 7q36.3 breakpoint.

In summary, this was an apparently balanced translocation at cytogenetic and molecular resolutions.

The revised karyotype is: 46,XX,t(2;7)(q37.2;q36.3) *de novo*.ish t(2;7)(263G22sp;69O3sp).

Case C3: 46,XX,t(3;11)(q21;q12) *de novo*

This patient is one of phenotypically discordant, monochorionic, monoamniotic twins born at 29 weeks' gestation. She had a congenital duodenal obstruction requiring surgery, complex congenital heart disease, and facial dysmorphism. Her twin sister who carries the same apparently balanced t(3;11) is clinically normal.

Array CGH, array painting, and metaphase FISH showed a balanced reciprocal translocation with RP11-529F4 (mapping to 3q21.3) spanning the chromosome 3 breakpoint and RP11-855O10 (mapping to 11q12.2) spanning the chromosome 11 breakpoint.

In summary, this was an apparently balanced translocation at cytogenetic and molecular resolutions.

The revised karyotype is: 46,XX,t(3;11)(q21;q12) *de novo*. ish t(3;11)(529F4sp;855O10sp).

Case C4: 46,XY,t(7;13)(q31.3;q21.3) *de novo*, inv(11)(p15.3;p15.5)pat

This patient was referred at the age of six years because of developmental delay and autistic features. By the age of seven years, he had epilepsy, learning difficulties, disordered speech and language, and an autism spectrum disorder.

Array CGH, array painting, and metaphase FISH showed a balanced reciprocal translocation with RP11-384A20 spanning the chromosome 7 breakpoint at 7q31.32, and RP11-360I23 spanning the chromosome 13 breakpoint at 13q21.33. In addition, the patient had a familial inv(11) identified at the original diagnosis.

In summary, this was an apparently balanced translocation at cytogenetic and molecular resolutions.

The revised karyotype is: 46,XY,inv(11)pat,t(7;13)(q31.3;q21.3), *de novo*.ish t(7;13)(384A20sp;360I23sp).

DISCUSSION

We have used high resolution molecular cytogenetic methods to analyse in detail 10 phenotypically abnormal individuals, nine of whom had an apparently balanced, reciprocal de novo chromosome translocation at the level of the light microscope. The remaining case had a de novo balanced translocation involving two chromosomes and a de novo inversion which by conventional methods involved one of the breakpoints of the translocation. Our results led us to classify these 10 patients into three groups.

Group A: Additional breakpoint complexity revealed by molecular cytogenetics

Three of the 10 patients showed additional complexity at or near the breakpoints.

Case A1 had a complex rearrangement involving three chromosomes with six breakpoints and a deletion of ~5 Mb very close to one of the breakpoints. Case A2 had a complex rearrangement involving three chromosomes and six different breakpoints, although no imbalances were detected. Case A3 had an inversion and a deletion of ~6 Mb close to one of the translocation breakpoints. Subsequent molecular studies showed that the deletions in cases A1 and A3 were both de novo and paternal in origin.

The high resolution molecular cytogenetic analysis allows us to suggest the most likely events which have led to the complex karyotype in these three cases by identifying the smallest number of rearrangements consistent with the chromosome constitution.

In case A1, we found a complex rearrangement involving a balanced translocation between chromosomes 6 and 10 but with an additional insertion/deletion event involving a small piece of chromosome 10 inserted into chromosome 4. The segment of chromosome 10 involved in the insertion/deletion was located just distal to but not involving the chromosome 10 translocation breakpoint. A possible chain of events leading to this karyotype is shown in fig 4A. The chromosome 6 breakpoint, in contrast, appeared balanced.

In case A2 we found chromosome 6 material in a previously undetected derivative chromosome 11 and chromosome 11 material in the derivative 17, suggesting that at least two translocation events had taken place. This rearrangement could have occurred as follows: after inversion of a chromosome 6, there was a translocation between the inverted 6 and chromosome 17 which was then followed by translocation of this derivative 17 with chromosome 11. This is summarised in fig 4B. Of course we cannot rule out alternative routes of rearrangement, each proceeding in a different order.

A model for the possible events leading to the karyotype of case A3 has been described previously.[22]

It seems reasonable to suppose that the deletion of ~5 Mb of chromosome 10q21.2 in case A1 and the deletion of ~6 Mb of chromosome 11q14.3–22.1 in case A3 make a major contribution to the phenotypic abnormalities of these patients, as neither deletion was present in the phenotypically normal parents. Various cases are reported in the Schinzel catalogue[26] with interstitial deletions of differing sizes which include 10q21.2. Of these, the one reported by Davis *et al*,[27] with a del(10q21-q22), was ascertained with congenital heart defect, mental retardation, and hypotonia and therefore most closely resembles the phenotype described in case A1 of the present study. By comparison, 19 cases are described in Schinzel with interstitial 11q deletions and breakpoints that include part or all of the 11q segment deleted in case A3 of the present study. Cases quoted by Schinzel with smaller visible 11q deletions appear to have milder phenotypes with mild to moderate developmental delay and mental retardation, with or without congenital abnormalities. These features do not suggest a pattern. In case A2 there were no obvious deletions or duplications, but the complex rearrangement involved no fewer than six breakpoints and it seems plausible that one or more of these is either associated with a very small duplication/deletion or interrupts a gene which contributes to the phenotype.

There is also the risk that at least some of the rearrangements described above may have resulted from the unbalanced segregation of a cryptic balanced parental rearrangement. Further targeted studies of the parental karyotypes will therefore be carried out as part of follow up clinical genetic/molecular cytogenetic investigations of these families.

Group B: Balanced translocation breakpoints and additional genomic imbalances

The most surprising finding in this study was that in three patients the translocation appeared balanced but the 1 Mb microarray uncovered unsuspected cryptic abnormalities involving chromosomes that were not involved in the rearrangements. Case B1 had a ~2 Mb duplication at distal 3pter which subsequent molecular studies confirmed was inherited from a phenotypically normal father. Case B2 had a de novo ~3 Mb deletion of paternal origin at 6q21, and case B3 had a ~6 Mb deletion on chromosome 18q12.3 which was confirmed molecularly and by FISH, but in which we were unable to determine whether the deletion was carried by the father or was a de novo event. The deletion in 6q21 in case B2 contains a large number of both predicted and known genes and haploinsufficiency of one or any number of these genes may be contributing to the patient's phenotype. By contrast, the deletion segment defined in 18q12.3 in case B3 contains only seven known genes (plus one of unknown function, *Q9H8C8*): *RIT2* (RAS-like without CAAX 2; RIC-like, expressed in neurones (*Drosophila*)); *PIK3C3* (phosphoinositide-3-kinase); *SYT4* (synaptotagmin IV); *SETBP1* (set binding protein 1); *SLC14A2* (solute carrier family 14, member 2); *SLC14A1* (solute carrier family, member 1); and *PSTPIP2* (proline-serine-threonine phosphatase-interacting protein). Nine patients with cytogenetically visible 18q12.3 deletions have been described (summarised in Schinzel[26]), and some of the features seen in case B3 of the current study—particularly muscular hypotonia, ptosis, severe mental retardation, and a history of seizures—have been reported in other del(18q12.3) patients. Finally, we have not studied the translocation breakpoints in detail and so cannot rule out either the possibility of smaller deletions within the breakpoint spanning clones or the fact that the breakpoints have disrupted significant genes which may also have contributed to the phenotypes observed.

Group C: Balanced translocation breakpoints without additional abnormalities

The remaining four patients showed simple balanced translocations on higher resolution molecular cytogenetics. The further analysis of these four patients allowed us to identify candidate genes which might be responsible for the phenotype.

In case C1 the translocation breakpoint spanning clone on chromosome 22 lies in a region devoid of genes but the chromosome 17 breakpoint spanning clone encompasses four genes. However, we have subsequently sequenced across both chromosome breakpoints and have found that no genes are disrupted by either breakpoint (data not shown). It remains to be determined whether the translocation might involve disruption of promoter or enhancer elements as yet unknown in this region, or be coincidental to the patient's phenotype.[28]

In case C2 the chromosome 2 breakpoint spanning clone encompasses a single gene, *SPP2* (secreted phosphoprotein 2). *SPP2* shares sequence homology with thiol protease inhibitors of the cystatin family and is expressed in bone.[29] Thiol proteases are involved in bone turnover and it is possible that *SPP2* may have an inhibitory function. The chromosome 7 breakpoint spanning clone encompasses two predicted genes and also SHH (sonic hedgehog protein precursor). SHH is a secreted signalling protein which has an important role in vertebrate development.[30] Mutations and haploinsufficiency in SHH cause varying degrees of holoprosencephaly, which was not detected in the patient.

In case C3, the chromosome 3 breakpoint spanning clone encompasses seven genes, none of which have functions that strongly suggest association with the phenotype. The chromosome 11 breakpoint spanning clone encompasses

RPLP0P2 (ribosomal protein, large, P0 pseudogene 2), disruption of which is also unlikely to be associated with the phenotype. The apparent absence of effect of the translocation seems consistent with the clinical normality of the monozygotic twin sister of this patient. We can only postulate that this patient carries a phenotype associated mutation elsewhere in the genome which could not be identified at the resolution of the methods used.

In case C4 the chromosome 7 breakpoint spanning clone encompasses one gene, *PTPRZ1* (protein tyrosine phosphatase, receptor-type, Z polypeptide 1), which may be involved in the development of the central nervous system and so is a good candidate gene for the patient's phenotype. The chromosome 13 breakpoint spanning clone encompasses a single gene, *DACH* (homologue of the dachshund gene in *Drosophila*). Homologues of *DACH* are expressed in the leg and eye primordia (*Drosophila*) and predominantly in the periphery of limb, neural retina, and the telencephalon including the olfactory bulbs (mouse). *DACH* is also a possible candidate gene for the patient's phenotype.

General conclusions

Overall, we found that one third of our patients had genomic imbalances which do not appear to be directly related to their de novo translocations. Recent 1 Mb resolution microarray CGH studies, using methods comparable with those used in the present study and applied to groups of chromosomally normal patients with either mental retardation or dysmorphic features or both,[31 32] detected 16 of 70 patients (23%) with either cryptic duplications or deletions. Molecular follow up showed that seven of these 16 cases were familial (five of six duplications and two of 10 deletions), while the remaining nine cases were de novo (one duplication and eight deletions). The patients in the present study with ''non-translocation'' imbalances are comparable with those found in the studies quoted above and presumably reflect the rate at which these types of chromosome abnormalities will be found in groups of patients with mental retardation and dysmorphic phenotypes. Significant imbalances were also found at or near the translocation breakpoints in two of the 10 cases and these imbalances may have contributed to the clinical abnormalities observed. A further case had a complex rearrangement involving six breakpoints but with no imbalances detected. However, we cannot rule out the possibility that the imbalances or complexity reflect the ascertainment bias in the patients recruited into this study. Comparable studies on the breakpoints of a group of patients with de novo translocations and normal phenotypes are therefore needed to determine whether such translocation breakpoints are simpler and not associated with detectable imbalances, in contrast to a proportion of those tested in this study.

There have been previous reports of balanced translocations that interrupt genes and are causal to the patient's phenotype,[33–35] and balanced translocations where the breakpoints are in gene-free areas and thus are likely to be coincidental to the patient's phenotype, or possibly to exert a long range position effect.[28] There have also been previous reports of additional complexity of balanced translocation breakpoints. For example Wirth *et al*,[17] using FISH with YACs, found cryptic deletions ranging in size from 3 to 5 Mb at the breakpoints in two of six de novo translocations studied in patients with dysmorphism, congenital abnormalities, and mental retardation. One of the translocations had deletions at both breakpoints. Similarly, Kumar *et al*[36]—in a study of three de novo structural abnormalities (two translocations and an inversion) in three phenotypically abnormal patients, using FISH and microsatellite markers—found cryptic deletions at the breakpoints in two of the abnormalities studied. In a recent study by Astbury *et al*,[37] deletions were also detected at the breakpoints in de novo chromosome rearrangements in a selected group of patients with phenotypic anomalies. However, these previously reported examinations of the breakpoints of balanced translocations have used FISH analysis or sequencing directly focused on the breakpoints. In none of these studies was the whole genome or indeed the derivative chromosomes themselves screened in a systematic way at high resolution for genomic imbalance, and so the frequency at which additional complexity in apparently balanced translocations occurs could not be assessed accurately. Using a genome-wide approach in our study with a 1 Mb microarray, we found that 60% of our patients with apparently balanced translocations also had additional rearrangements and karyotype complexity. It is clear, therefore, that a genome-wide scan—either using a high resolution analysis such as array CGH with 1 Mb or higher resolution tiling path clone sets[38] followed by FISH and parental origin studies—is essential in order to fully elucidate the range and complexity of genomic rearrangements in patients with apparently balanced structural rearrangements and clinically abnormal phenotypes.

Conventional FISH analysis of patients with simple balanced translocations allows the identification of breakpoint spanning clones and the selection of candidate genes; indeed detailed and lengthy FISH mapping can be used to analyse complex rearrangements. However, our results show that in over half the cases we studied, more complex rearrangements, often involving many megabases of DNA, would have remained undetected were it not for a comprehensive analysis using advanced molecular cytogenetic techniques. In particular, a genome-wide screen for copy number imbalance is required when the translocation appears balanced. While these additional studies can identify further candidate genes, more detailed molecular analyses—involving techniques such as higher resolution breakpoint mapping and sequencing, mutational analysis, and gene expression and transcript analysis—are clearly required to help identify how, in each case, chromosome translocation alone or with further cryptic rearrangements can lead to the abnormal phenotype.

ACKNOWLEDGEMENTS

We would like to thank Dr J Tyrrell and Dr C Spratt for clinical information, Berendine van Zyl for help with microsatellite analysis, and the Microarray Core Facility of the Wellcome Trust Sanger Institute for printing the arrays. This work was supported by the Wellcome Trust. RS was in part supported by a grant from Peacehealth, Longview, WA.

Authors' affiliations

S M Gribble, E Prigmore, D C Burford, K M Porter, Bee Ling Ng, E J Douglas, H Fiegler, P Carr, D Kalaitzopoulos, S Clegg, N P Carter, The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK
R Sandstrom, Lower Columbia Pathologists, 1217 14th Avenue, Longview, Washington, USA
I K Temple, S A Youings, N S Thomas, N R Dennis, P A Jacobs, J A Crolla, Division of Human Genetics, University of Southampton Medical School, Southampton

Competing interests: none declared

REFERENCES

1 **Jacobs PA**, Browne C, Gregson N, Joyce C, White H. Estimates of the frequency of chromosome abnormalities detectable in unselected newborns using moderate levels of banding. *J Med Genet* 1992;**29**:103–8.
2 **Clifford K**, Rai R, Watson H, Regan L. An informative protocol for the investigation of recurrent miscarriage: preliminary experience of 500 consecutive cases. *Hum Reprod* 1994;**9**:1328–32.

3 **Warburton D**. De novo balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: clinical significance and distribution of breakpoints. *Am J Hum Genet* 1991;**49**:995–1013.
4 **Holmes SE**, Riazi MA, Gong W, McDermid HE, Sellinger BT, Hua A, Chen F, Wang Z, Zhang G, Roe B, Gonzalez I, McDonald-McGinn DM, Zackai E, Emanuel BS, Budarf ML. Disruption of the clathrin heavy chain-like gene (CLTCL) associated with features of DGS/VCFS: a balanced (21;22)(p12;q11) translocation. *Hum Mol Genet* 1997;**6**:357–67.
5 **Toriello HV**, Glover TW, Takahara K, Byers PH, Miller DE, Higgins JV, Greenspan DS. A translocation interrupts the COL5A1 gene in a patient with Ehlers-Danlos syndrome and hypomelanosis of Ito. *Nat Genet* 1996;**13**:361–5.
6 **Giacalone JP**, Francke U. Common sequence motifs at the rearrangement sites of a constitutional X/autosome translocation and associated deletion. *Am J Hum Genet* 1992;**50**:725–41.
7 **Hill AS**, MacCallum PK, Young BD, Lillington DM. Molecular cloning of a constitutional t(7;22) translocation associated with risk of hematological malignancy. *Genes Chromosomes Cancer* 2003;**38**:260–4.
8 **McMullan TW**, Crolla JA, Gregory SG, Carter NP, Cooper RA, Howell GR, Robinson DO. A candidate gene for congenital bilateral isolated ptosis identified by molecular analysis of a de novo balanced translocation. *Hum Genet* 2002;**110**:244–50.
9 **Millar JK**, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, Devon RS, Clair DM, Muir WJ, Blackwood DH, Porteous DJ. Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum Mol Genet* 2000;**9**:1415–23.
10 **Holder JL**, Butte NF, Zinn AR. Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. *Hum Mol Genet* 2000;**9**:101–8.
11 **Ikegawa S**, Masuno M, Kumano Y, Okawa A, Isomura M, Koyama K, Okui K, Makita Y, Sasaki M, Kohdera U, Okuda M, Koyama H, Ohashi H, Tajiri H, Imaizumi K, Nakamura Y. Cloning of translocation breakpoints associated with Shwachman syndrome and identification of a candidate gene. *Clin Genet* 1999;**55**:466–72.
12 **Draptchinskaia N**, Gustavsson P, Andersson B, Pettersson M, Willig TN, Dianzani I, Ball S, Tchernia G, Klar J, Matsson H, Tentler D, Mohandas N, Carlsson B, Dahl N. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. *Nat Genet* 1999;**21**:169–75.
13 **Yoshiura K**, Machida J, Daack-Hirsch S, Patil SR, Ashworth LK, Hecht JT, Murray JC. Characterization of a novel gene disrupted by a balanced chromosomal translocation t(2;19)(q11.2;q13.3) in a family with cleft lip and palate. *Genomics* 1998;**54**:231–40.
14 **Kurahashi H**, Sakamoto M, Ono J, Honda A, Okada S, Nakamura Y. Molecular cloning of the chromosomal breakpoint in the LIS1 gene of a patient with isolated lissencephaly and balanced t(8;17). *Hum Genet* 1998;**103**:189–92.
15 **Ishikawa-Brush Y**, Powell JF, Bolton P, Miller AP, Francis F, Willard HF, Lehrach H, Monaco AP. Autism and multiple exostoses associated with an X;8 translocation occurring within the GRPR gene and 3′ to the SDC2 gene. *Hum Mol Genet* 1997;**6**:1241–50.
16 **van Bakel I**, Holt S, Craig I, Boyd Y. Sequence analysis of the breakpoint regions of an X;5 translocation in a female with Duchenne muscular dystrophy. *Am J Hum Genet* 1995;**57**:329–36.
17 **Wirth J**, Nothwang HG, van der Maarel S, Menzel C, Borck G, Lopez-Pajares I, Brondum-Nielsen K, Tommerup N, Bugge M, Ropers HH, Haaf T. Systematic characterisation of disease associated balanced chromosome rearrangements by FISH: cytogenetically and genetically anchored YACs identify microdeletions and candidate regions for mental retardation genes. *J Med Genet* 1999;**36**:271–8.
18 **Cox JJ**, Holden ST, Dee S, Burbridge JI, Raymond FL. Identification of a 650 kb duplication at the X chromosome breakpoint in a patient with 46,X,t(X;8)(q28;q12) and non-syndromic mental retardation. *J Med Genet* 2003;**40**:169–74.
19 **Borg I**, Squire M, Menzel C, Stout K, Morgan D, Willatt L, O'Brien PC, Ferguson-Smith MA, Ropers HH, Tommerup N, Kalscheuer VM, Sargan DR. A cryptic deletion of 2q35 including part of the PAX3 gene detected by breakpoint mapping in a child with autism and a de novo 2;8 translocation. *J Med Genet* 2002;**39**:391–9.
20 **Kirchhoff M**, Rose H, Maahr J, Gerdes T, Bugge M, Tommerup N, Tumer Z, Lespinasse J, Jensen PK, Wirth J, Lundsteen C. High resolution comparative genomic hybridisation analysis reveals imbalances in dyschromosomal patients with normal or apparently balanced conventional karyotypes. *Eur J Hum Genet* 2000;**8**:661–8.
21 **Zhu G**, Gillessen-Kaesbach G, Wirth J, Passarge E, Bartsch O. Girl with phenotypic abnormalities and a de novo, apparently balanced translocation 46,XX,t(5;10)(q35.2q11.2). *Am J Med Genet* 2001;**98**:317–19.
22 **Fiegler H**, Gribble SM, Burford DC, Carr P, Prigmore E, Porter KM, Clegg S, Crolla JA, Dennis NR, Jacobs P, Carter NP. Array painting: a method for the rapid analysis of aberrant chromosomes using DNA microarrays. *J Med Genet* 2003;**40**:664–70.
23 **Seabright M**. A rapid banding technique for human chromosomes. *Lancet* 1971;ii:971–2.
24 **Fiegler H**, Carr P, Douglas EJ, Burford DC, Hunt S, Scott CE, Smith J, Vetrie D, Gorman P, Tomlinson IP, Carter NP. DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. *Genes Chromosomes Cancer* 2003;**36**:361–74.
25 **Temple IK**, Browne C, Hodgkins P. Anterior chamber eye anomalies, redundant skin and syndactyly – a new syndrome associated with breakpoints at 2q37.2 and 7q36.3. *Clin Dysmorphol* 1999;**8**:157–63.
26 **Schinzel A**. *Catalogue of unbalanced chromosome aberrations in man*, 2nd ed. Berlin: Walter de Gruyter, 2001.
27 **Davis DG**, Kardon NB, Selman JE. De novo 10q interstitial deletion [abstract]. *Am J Hum Genet* 1982;**34**:122A.
28 **Kleinjan DJ**, van Heyningen V. Position effect in human genetic disease. *Hum Mol Genet* 1998;**7**:1611–18.
29 **Hu B**, Coulson L, Moyer B, Price PA. Isolation and molecular cloning of a novel bone phosphoprotein related in sequence to the cystatin family of thiol protease inhibitors. *J Biol Chem* 1995;**270**:431–6.
30 **Ho KS**, Scott MP. Sonic hedgehog in the nervous system: functions, modifications and mechanisms. *Curr Opin Neurobiol* 2002;**12**:57–63.
31 **Shaw-Smith C**, Redon R, Rickman L, Rio M, Willatt L, Fiegler H, Firth H, Sanlaville D, Winter R, Colleaux L, Bobrow M, Carter NP. Microarray based comparative genomic hybridisation (array-CGH) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. *J Med Genet* 2004;**41**:241–8.
32 **Vissers LE**, de Vries BB, Osoegawa K, Janssen IM, Feuth T, Choy CO, Straatman H, van der Vliet W, Huys EH, van Rijk A, Smeets D, van Ravenswaaij-Arts CM, Knoers NV, van der Burgt I, de Jong PJ, Brunner HG, van Kessel AG, Schoenmakers EF, Veltman JA. Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. *Am J Hum Genet* 2003;**73**:1261–70.
33 **Nevin NC**, Hughes AE, Calwell M, Lim JH. Duchenne muscular dystrophy in a female with a translocation involving Xp21. *J Med Genet* 1986;**23**:171–3.
34 **Cockburn DJ**, Munro EA, Craig IW, Boyd Y. Mapping of X chromosome translocation breakpoints in females with Duchenne muscular dystrophy with respect to exons of the dystrophin gene. *Hum Genet* 1992;**90**:407–12.
35 **Praphanphoj V**, Goodman BK, Thomas GH, Niel KM, Toomes C, Dixon MJ, Geraghty MT. Molecular cytogenetic evaluation in a patient with a translocation (3;21) associated with blepharophimosis, ptosis, epicanthus inversus syndrome (BPES). *Genomics* 2000;**65**:67–9.
36 **Kumar A**, Becker LA, Depinet TW, Haren JM, Kurtz CL, Robin NH, Cassidy SB, Wolff DJ, Schwartz S. Molecular characterization and delineation of subtle deletions in de novo ''balanced'' chromosomal rearrangements. *Hum Genet* 1998;**103**:173–8.
37 **Astbury C**, Christ LA, Aughton DJ, Cassidy SB, Kumar A, Eichler EE, Schwartz S. Detection of deletions in de novo ''balanced'' chromosome rearrangements: further evidence for their role in phenotypic abnormalities. *Genet Med* 2004;**6**:81–9.
38 **Ishkanian AS**, Malloff CA, Watson SK, DeLeeuw RJ, Chi B, Coe BP, Snijders A, Albertson DG, Pinkel D, Marra MA, Ling V, MacAulay C, Lam WL. A tiling resolution DNA microarray with complete coverage of the human genome. *Nat Genet* 2004;**36**:299–303.