Disease associated balanced chromosome rearrangements: a resource for large scale genotype-phenotype delineation in man

Merete Bugge, Gert Bruun-Petersen, Karen Brøndum-Nielsen, Ursula Friedrich, Jan Hansen, Grethe Jensen, Peter K A Jensen, Ulf Kristoffersson, Claes Lundsteen, Erik Niebuhr, Kim R Rasmussen, Kirsten Rasmussen, Niels Tommerup

Department of Medical Genetics, IMBG, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark
M Bugge
E Niebuhr
K R Rasmussen
N Tommerup

Department of Clinical Genetics, Vejle Hospital, Denmark
G Bruun-Petersen

John F Kennedy Institute, Glostrup, Denmark
K Brøndum-Nielsen

Institute of Human Genetics, University of Aarhus, Denmark
U Friedrich

Cytogenetic Registry, Department of Clinical Genetics, University Hospital, Aarhus, Denmark
J Hansen
P K A Jensen

Chromosome Laboratory, Institute of Pathology, Frederiksberg Hospital, Denmark
G Jensen

Department of Clinical Genetics, University Hospital, Aarhus, Denmark
P K A Jensen

Department of Clinical Genetics, University Hospital, Lund, Sweden
U Kristoffersson

Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark
G Jensen
C Lundsteen

Department of Clinical Genetics, University Hospital Odense, Denmark
K Rasmussen

Correspondence to:
Dr Tommerup,
tommerup@imbg.ku.dk

Abstract
Disease associated balanced chromosomal rearrangements (DBCRs), which truncate, delete, or otherwise inactivate specific genes, have been instrumental for positional cloning of many disease genes. A network of cytogenetic laboratories, Mendelian Cytogenetics Network (MCN), has been established to facilitate the identification and mapping of DBCRs. To get an estimate of the potential of this approach, we surveyed all cytogenetic archives in Denmark and southern Sweden, with a population of ~6.6 million. The nine laboratories have performed 71 739 postnatal cytogenetic tests. Excluding Robertsonian translocations and chromosome 9 inversions, we identified 216 DBCRs (~0.3%), including a minimum estimate of 114 de novo reciprocal translocations (0.16%) and eight de novo inversions (0.01%). Altogether, this is six times more frequent than in the general population, suggesting a causal relationship with the traits involved in most of these cases. Of the identified cases, only 25 (12%) have been published, including 12 cases with known syndromes and 13 cases with unspecified mental retardation/congenital malformations. The remaining DBCRs were associated with a plethora of traits including mental retardation, dysmorphic features, major congenital malformations, autism, and male and female infertility. Several of the unpublished DBCRs defined candidate breakpoints for nail-patella, Prader-Willi, and Schmidt syndromes, ataxia, and ulna aplasia. The implication of the survey is apparent when compared with MCN; altogether, the 292 participating laboratories have performed >2.5 million postnatal analyses, with an estimated ~7500 DBCRs stored in their archives, of which more than half might be causative mutations. In addition, an estimated 450-500 novel cases should be detected each year. Our data illustrate that DBCRs and MCN are resources for large scale establishment of phenotype-genotype relationships in man.
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Keywords: translocations; inversions; abnormal phenotypes; frequency

When the sequencing of the human genome is finished within the next one to three years, the presence and location of the estimated 60 000-100 000 human genes will be known. However, knowledge about the function and potential involvement in human morbidity will only have been established for a tiny fraction of these genes. Therefore, large scale strategies to establish genotype-phenotype relationships will be of major importance.

One of the classical approaches for this is based on molecular characterisation of chromosomal breakpoints associated with abnormal phenotypes. Thus, disease associated balanced chromosome rearrangements (DBCRs) that truncate, delete, or otherwise inactivate specific genes have been instrumental in the positional cloning of many disease genes.[1 2] Although most of these published DBCRs have been associated with well known disorders/syndromes, reports from population surveys,[3] prenatal diagnosis,[4] and surveys of mental retardation[5 6] suggest that they may be associated with a variety of different phenotypes and may be more frequent than usually anticipated.

Chromosome analysis is the most widespread routine genetic test in the world, offered by hundreds of diagnostic laboratories world wide. In order to systematise the identification of specific disease associated genes by mapping and cloning of breakpoints in DBCRs, the Mendelian Cytogenetic Network (MCN) has been established, at present involving 292 diagnostic cytogenetic laboratories world wide. The strategy, which involves submission of data on DBCRs into a central database (MCN*db*),[7] includes each laboratory identifying and reporting the karyotype and phenotype of the DBCRs that have been encountered both retrospectively and prospectively, in order to improve the chances of identifying disease specific breakpoints.

Since the potential of this approach will rely on the actual number and types of DBCRs that can be expected within MCN, we performed a systematic search in all cytogenetics archives in Denmark and the South Swedish Health Care Region (comprising four counties in southern Sweden (SSHCR)), and from this we estimated the frequency and types of DBCRs among postnatal chromosome samples.

Materials and methods
Cytogenetic analysis in Denmark and SSHCR started in the 1960s. In Denmark, analyses

Table 1 Disease associated balanced chromosomal rearrangements (DBCR) in Denmark and southern Sweden

Laboratories*	Total analysis (No)	DBCR BT† (No)	DBCR BI‡ (No)	Total	DBCR % of total analysis
1	996	1	2	3	0.30
2	5 276	11	8	19	0.36
3	19 484	58	6	64	0.33
4	3 336	6	0	6	0.18
5	8 277	19	4	23	0.28
6	10 549	20	6	26	0.25
7	9 864	24	10	34	0.34
8	644	2	0	2	0.31
9	13 313	28	11	39	0.29
Total	71 739	169	47	216	0.30

*1. Chromosome Laboratory, Frederiksberg Hospital, DK. 2. Department of Clinical Genetics, Vejle Hospital, DK. 3. John F Kennedy Institute, Glostrup, DK. 4. Institute of Medical Genetics, University of Copenhagen, DK. 5. Cytogenetic Laboratory, Aarhus Psychiatric Hospital, DK. 6. Department of Clinical Genetics, Rigshospitalet, Copenhagen, DK. 7. Department of Clinical Genetics, Aarhus University Hospital, DK. 7. Department of Clinical Genetics, Odense University Hospital, DK. 9. Department of Clinical Genetics, University Hospital, Lund, Sweden.
†Reciprocal balanced translocations and insertions.
‡Balanced inversions.

have been offered by eight cytogenetics laboratories and the SSHCR has been covered by one laboratory.

The search included the period from the inception of the individual laboratory until the end of 1997. The registration of cases and results of analysis, which initially were performed manually in six of the nine laboratories, have now been computerised, but at very different times in each laboratory. Thus, manual files cover 20 to 30 years of diagnosis. The Danish Cytogenetic Central Registry (DCCR), established in 1968,[8] is an electronic registry comprising all subjects with a chromosome analysis in Denmark, including those diagnosed before the registry was started. DCCR made a search on name, date of birth, date of analysis, and karyotype for five of the laboratories, listing all cases with translocations, insertions, and inversions, excluding Robertsonian translocations and the common chromosome 9 inversions. In one laboratory, the archive search was exclusively manual. Three laboratories, which have been computerised since the start, made a similar local search.

Table 2 Origin of disease associated balanced chromosome rearrangements (DBCR) in Denmark and southern Sweden

Laboratories*	Translocations				Inversions			
	De novo	Mat†	Pat‡	Unknown	De novo	Mat†	Pat‡	Unknown
1	0	1	0	0	0	0	1	1
2	2	6	1	2	1	2	1	4
3	41	4	1	12	2	2	0	2
4	4	0	1	1	0	0	0	0
5	2	5	2	10	0	2	1	1
6	13	1	1	5	2	1	1	2
7	9	3	3	9	1	4	5	0
8	0	0	0	2	0	0	0	0
9	7	3	5	13	0	2	6	3
Total	78	23	14	54	06	13	15	13

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†Mat: maternal origin.
‡Pat: paternal origin.

Each laboratory stores records of all the patients analysed in the period, including the referral diagnosis and often with some clinical data. Once identified, all the records were scrutinised in the individual laboratories by one of us (MB). In some cases, the clinical information was so sparse that it was necessary to contact the clinical department(s) involved and request additional information. Included in the study were all patients, postnatally karyotyped, with cytogenetically balanced reciprocal translocations, insertions, or inversions, which have a recorded pathological phenotype. Excluded were patients referred for repeated spontaneous abortions, when the carrier had an otherwise normal phenotype. In families where a pathological phenotype segregates with the rearrangement, only one member was included.

Results

Denmark has a population of ~5.2 million and the area in southern Sweden ~1.4 million. Thus the investigation covers a population of ~6.6 million persons. In the area, 71 739 postnatal chromosome analyses were performed in the study period, 58 426 in Denmark and 13 313 in the laboratory in Lund, Sweden.

A total of 216 DBCRs were identified, 165 reciprocal balanced translocations, two balanced direct insertions, two inverted insertions, and 47 balanced inversions. The 216 DBCRs represent an average of 0.3% of all the analysed samples (table 1). Of the translocations, 78 were de novo, 23 were of maternal, 14 of paternal, and 54 of unknown origin, whereas the corresponding figures for the inversions were six de novo, 13 of maternal, 15 of paternal, and 13 of unknown origin (table 2). The four insertions were included in the translocation group for simplicity.

DBCRs were discovered in 53 subjects (24% of the total) with 38 well known syndromes/phenotypes (appendix, 1-4). The most common clinical feature was mental retardation (MR) with or without dysmorphism and/or congenital malformations and/or additional neurological symptoms. MR was associated with 88 translocations and 20 inversions representing 50% of the total number (appendix, 14). The next most common groups were male infertility with 14 translocations and five inversions (appendix, 12) and female infertility with 13 translocations and two inversions (appendix, 13).

Discussion

Autosomal reciprocal balanced translocations and inversions occur in approximately 0.1% of newborns[3] and the estimated frequency of de novo balanced rearrangements in the general population, as determined from newborn screening series and from prenatal diagnosis, is 0.0283%.[4] The systematic search disclosed 216 DBCRs, which represents an average of 0.3% of all the analyses. Moreover, the 0.3%, which was remarkably similar in all but one of the Danish laboratories, was also observed in the Swedish laboratory.

The de novo cases will probably be those cases with the highest probability of detecting disease specific breakpoints. However, we included all DBCRs irrespective of their type (de novo, paternal or maternal origin, with or without concordant traits, or unknown origin) since we did not want to exclude anything, for example, potentially de novo cases among the 67 DBCRs of unknown origin.

Of the 115 translocations where both parents had been analysed, 2/3 of the cases (78/115) were de novo rearrangements. If we take the conservative view that 2/3 of the 54 translocations of unknown status are also of de novo origin, the estimated number of de novo translocations in our survey would be 78+2/3*54=114 (~0.16%). In fact, since the mother had been excluded from carrying the rearrangement in six of the unknown cases, this must actually be a minimum estimate. Only 6/34 of the inversions where both parents had been analysed were of de novo origin, so the total estimated numbers of de novo inversions would be 6+ 6/34*13 ~8 (~0.01%). Combined, the estimated minimal frequency of de novo reciprocal translocations and inversions associated with abnormal phenotypes in our survey was ~0.17%, or a six-fold increase compared to the general population, suggesting that in the majority of these DBCRs the rearrangement will be causatively linked to the observed trait(s).

The population frequency of familial DBCRs with discordance for an abnormal phenotype is unknown, but it is conceivable that many if not most of these cases in our survey may be coincidental findings. However, we included these cases since they might be the ones associated with autosomal recessive disorders[9] and, as illustrated by the familial, discordant DBCRs associated with Beckwith-Wiedemann syndrome,[10] associated with sex dependent imprinting.

Of the 52 cases with well known syndromes/phenotypes, which in most cases can be accessed as specific entities in OMIM (appendix, 1-4), only 13 or 6% of the 216 DBCRs have been published previously in relation to the abnormal phenotype.[10–22] One immediate consequence of the present study was the first fine mapping of a balanced chromosomal breakpoint within the Prader-Willi syndrome (PWS) chromosome region in a patient with PWS stigmata.[23] Since then, additional translocation breakpoints involving the PWS imprinted region have been characterised.[24–26] Furthermore, FISH mapping of an identified t(1;9) associated with nail-patella syndrome (NPS1) has narrowed the 9q34 breakpoints in this and in another NPS1 associated translocation[27] to a single PAC clone, which has been shown to contain the NPS1 lim-homeobox gene *LMX1B*.[28]

In the PWS case, the karyotype was performed in 1979, and although microdeletions at 15q11-13 were not described before 1981,[29] numerous reports had suggested an involvement of chromosome 15 in PWS.[29 30] In the NPS1 case, which was also karyotyped in 1979, the balanced translocation was not considered to be associated with the phenotype despite the fact that *NPS1* was mapped to 9q34 in 1976.[31] These two cases illustrate that cytogenetic archives may contain DBCRs associated with well known disorders with known or suspected chromosomal locations, which may only be disclosed by systematic re-examination.

The survey uncovered several other DBCRs associated with specific genetic entities which are now under investigation: a familial t(6;14) segregating with Schmidt syndrome (MIM 269200); a de novo t(2;10) associated with ulnar aplasia; and a de novo inv(3) associated with radial aplasia. The cause of Cornelia de Lange syndrome (MIM 122470) is unknown, but two patients with different inversions have concordant involvement of 12p13. In addition, a t(8;20) segregating with atypical cerebellar ataxia in a large family suggests a putative locus for early onset mild ataxia at either 8p21 or 20q12 (Hertz *et al*, in preparation).

Mental retardation (MR) is one of the most common abnormal traits in man and the single most important indication for a chromosome analysis. In line with this, non-syndromal and syndromal MR, with or without other neurological symptoms, was the most common trait associated with DBCRs, seen in 108 (50%) of the 216 cases. The fact that almost any visible chromosomal imbalance will result in mental impairment probably indicates that haploinsufficiency of numerous genes may lead to abnormal mental development. Still, relatively few specific genetic causes of MR have been characterised so far, including some forms of X linked MR, where specific DBCRs have aided in the characterisation of the genetic defects.[32–34]

Some of the apparently balanced rearrangements associated with MR may be unbalanced at the molecular level. Recent mapping of six DBCRs with mental retardation with breakpoints on chromosome 13 showed submicroscopic deletions in two cases, one of which originated from this survey.[35] If we can extrapolate, a substantial fraction of DBCRs associated with MR may be associated with microdeletions, and as such may help specify novel microdeletion syndromes.

Another strategy to identify candidate breakpoints and genes associated with MR is to identify common breakpoints associated with MR. In this study we observed two to four cases with mental retardation and the following common breakpoints: 1p36, 1q31, 1q44, 2q23, 4p16, 4q22, 4q35, 5q13, 6q27, 7q22, 7q36, 9p24, 9q34, 12q15, 13q34, and 14q32. Interestingly, half of these 16 clusters involve telomeric cytogenetic bands (appendix, 13), and altogether 29 of the 238 breakpoints detected in association with mental retardation occurred in telomeric cytogenetic bands. Haploinsufficiency of subtelomeric regions are frequently detected in idiopathic MR,[36 37] indicating that these regions must contain MR related genes. Therefore, DBCRs with terminal breakpoints may be good candidates for defining some of these genes, for example, by the presence of subtle microdeletions, truncating events, or other inactivation mechanisms. However, the observed clustering outside the telomeric

regions suggests that DBCRs in general may be instrumental in the delineation of autosomal genes, which cause MR by haploinsufficiency.

Of the 108 cases with MR, only 12 cases have been published in relation to their phenotype, and none has been published since 1982 (Skovby and Niebuhr[38] (two cases), Kristoffersen *et al*[39] (two cases), Rasmussen *et al*[40] (five cases)).[41] [42] Most of the DBCRs associated with MR were diagnosed in the newborn period and later clinical examinations have not been reported. Some of these conditions may represent specific syndromes or may later have manifested more specific or additional symptoms and a systematic clinical follow up would be of great value. Although this is very time consuming and has only been done in a limited number of patients, we plan to do this.

Infertility is the second largest group that was identified. Among the cases with female infertility, eight involve X;autosomal translocations, all of which have a breakpoint at Xq21-23 within the known critical region on the X chromosome.[43] However, almost as frequent (seven cases) are rearrangements only involving autosomes. Since very little is known about autosomal genes in female infertility, such DBCR may become important for genetic exploration of female infertility. In the group of male infertility, chromosome 1 is involved in four rearrangements and in three of them the breakpoint is at 1q21, whereas 2q21 is a common breakpoint in two other cases.

It is at present speculative whether reciprocal translocations can cause infertility by disrupting genes involved in spermatogenesis/oogenesis, or whether the infertility is associated with a mechanical disruption of the segregation of chromosomes. However, in the latter case it would be expected that chromosomes 1 and 2, which are of almost similar size, would be affected to a similar degree. Although we observed clustering of breakpoints in male infertility on both chromosomes in the present study, chromosome 1 has significantly more breakpoints than chromosome 2 (van Assche *et al*, in preparation), suggesting that the location of the breakpoint is important too.

Among the 14 DBCRs associated with well known disorders with a mapped locus (appendix, 1, 2, and 3), only three DBCRs involved breakpoints outside the proven disease region: an inv(8) associated with Williams syndrome (with proven deletion of the *ELN* gene on chromosome 7 (data not shown)), a t(13;18) associated with adrenogenital syndrome (with proven mutation of *CYP21* on chromosome 6 (data not shown)), and an inv(2) associated with Rett syndrome with a proven mutation of the X linked *MECP2* gene (Nielsen *et al*, in preparation) (appendix, 3). This is also consistent with the notion that the majority of DBCRs detected in the present study are causally related to the associated abnormal phenotypes.

In conclusion, we have shown in a survey of our population, which is as complete as it can be, that ~0.3% of all postnatal cytogenetic analyses are DBCRs; this includes DBCRs associated with both mapped and unmapped but well known disorders/syndromes. More than half of the DBCRs were associated with mental retardation; some of these may be associated with microdeletions at the molecular level, and a remarkable number of MR associated breakpoints were clustered in specific cytogenetic regions. This clustering may indicate the location of specific MR genes. Although the geographical area covered by the study is rather limited, both with respect to population (6.6 million) and number of postnatal analyses performed (71 739), the importance of these figures is evident if we extrapolate to MCN. Altogether, the 292 participating laboratories have performed more than 2.5 million postnatal analyses, suggesting that their archives contain information on more than 7500 DBCRs, of which only a small fraction will have been published. In addition, the 292 participating laboratories perform more than 150 000 postnatal analyses annually, suggesting that they will encounter 450-500 novel DBCRs each year. It will be no trivial matter to uncover these cases, especially in larger laboratories, but the present study illustrates that it will be worthwhile to do it in a systematic fashion and that pooling of such data within MCN can become a significant resource for large scale establishment of phenotype-genotype relationships in man.

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Appendix   Karyotype and phenotype of 216 disease associated balanced chromosome rearrangements (DBCR)

MIM No	Phenotype	Karyotype
(1) Disorders with proven candidate regions/genes (indicated in bold)		
175700	Greig cephalopolysyndactyly syndrome	46,XY,t(3;7)(p21.1;**p13**)pat[15]
161200	Nail-patella syndrome	46,XY,t(1;**9**)(q41;**q34**)unknown[28]
190350	Trichorhinophalangeal syndrome type I	46,XX,t(7;13;**8**)(p21;q21;**q24.1**)de novo[22]
130650	Beckwith-Wiedemann syndrome	46,XX,t(2;**11**)(q14;**p15.4**)mat
130650	Beckwith-Wiedemann syndrome	46,XX,t(9;**11**)(p11.2;**p15.4**)mat[10]
176270	Prader-Willi syndrome	46,XY,t(9;**15**)(q21.2;**q13.1**)de novo[23]
176270	Prader-Willi syndrome	46,XX,t(**15**;18)(**q13**;q23)de novo
205900	Blackfan-Diamond anaemia	46,X,t(X;**19**)(p11;**q11**)de novo[21]
11010	BPES	46,XY,t(2;**3**)(q22;**q24**)de novo[20]
310200	Duchenne muscular dystrophy	46,X,t(**X**;9)(**p21**;q13)de novo[16]
310200	Duchenne muscular dystrophy	46,X,t(**X**;11)(**p21**;q23)de novo
(2) Disorders with suspected candidate regions/genes (indicated in bold)		
163950	Noonan syndrome	46,XX,t(4;**12**)(q26;**21.3**)unknown
269200	Smidt syndrome	46,XX,t(**6**;14)(**p22.3**;q12)mat (concordant)
	Aplasia of ulna	46,XX,t(2;10)(**q24.3**;q23.3)de novo
	Atypical cerebellar ataxia	46,XX,t(**8;20**)(**p22;q13**)mat (concordant)
	Polydactyly and syndactyli	46,XX,inv(**7**)(p14.3**p22**)de novo
(3) Phenotypes considered not to be associated with the cytogenetic aberration		
104050	Williams syndrome	46,XX,inv(8)(p12q23)unknown
201910	Adrenogenital syndrome	46,XX,t(13;18)(q31;p11.2)de novo[14]
312750	Rett syndrome	46,XX,inv(2)(p21q11)de novo (Nielsen *et al*, in preparation)
(4) Miscellaneous		
122470	Cornelia de Lange syndrome	46,XX,inv(12)(p13q13)unknown
122470	Cornelia de Lange syndrome	46,XY,inv(12)(p11.23p13.33)mat
157900	Moebius syndrome	46,XY,t(7;8;11;13)(q21.1;q21.3;p14.3;q21.2)de novo[35]
248770	Marfanoid syndrome	46,XX,t(12;21)(q22;q22.1)de novo

Appendix   Continued

MIM No	Phenotype	Karyotype
261800	Pierre Robin syndrome	46,XY,t(7;17)(p11.1;q21)de novo
	Radial aplasia	46,XY,inv(3)(p22q27)de novo
119600	Cleidocranial dysplasia	46,XX,t(4;18)(q28;q23)de novo[13]
255310	Congenital myopathy with fibre type disproportion	46,XX,t(10;17)(p11.21;q25.1)mat[19]
206500	Anencephaly	46,XY(6;19)(p21;q12)pat
146450	Hypospadias	46,XY,t(1;16)(q12;q12)mat[41 42]
142340	Diaphragmatic hernia	46,XY,t(5;13)(p15;q14.1)pat
600057	Extrophy of bladder	46,XX,inv(5)(p11q12)pat
164750	Omphalocele	46,XY,t(17;22)()unknown
121000	Congenital heart disease	46,XY,inv(1)(q25q44)mat
121000	Congenital heart disease	46,XY,t,(2;13)(q22;q34)mat
121000	Congenital heart disease	46,XY,inv(10)(p11q21)pat
312865	Short stature	46,XX,t(1;11)(p36;q12)mat
312865	Short stature	46,XX,t(2;6)(p21;q21)de novo
312865	Short stature	46,XX,t(2;8;14)(q13;p21.2;q23.2)de novo
312865	Short stature	46,XX,inv(3)(p13q27)unknown
318265	Short stature	46,XX,t(6;17)(q14;q25)pat
318265	Short stature	46,XX,inv(9)(p24q13)pat
318265	Short stature	46,XX,inv(12)(q21.3q24.2)mat
218000	Agenesia of corpus callosum	46,XX,t(1;18)(p32.1;q12.2)de novo
218000	Agenesia of corpus callosum	46,XY,inv(3)(p12q13.3)mat
218000	Agenesia of corpus callosum	46,XX,dir ins(22;15)(q12;q15q22)de novo
182940	Neural tube defect	46,XY,t(2;3)(q37;q13.3)de novo
119540	Cleft palate	46,XY,t(2;12)(p13;q13.1)de novo
	Cleft palate and glaucoma	46,XY,t(1;6)(q21.2;q25.2)de novo[12]
	XY sex reversal	46,XY,t(11;18)(p15.2;q21.1)de novo
	Short limbs	46,XX,inv(12)(p11.23q14.3)pat
	Syndactyly	46,XX,t(7;19)(p11;q12)unknown
	Ambiguous genitalia	46,XX,t(5;13)(p13;q14)unknown
	Kyphoscoliosis, vertebral anomalies, ureteric stenosis	46,XY,t(3;7)(p23;q11)mat
(5) Eye disorders		
	Retinal cone dystrophy, +	46,XY,t(1;6)(q44;q27.2)de novo[17]
	Coloboma of iridis, +	46,XY,inv(5)(p13p15)mat[18]
	Microphthalmia, coloboma +	46,XX,t(10;16)(p11;q21)de novo
	Coloboma of iridis, microphthalmia sin	46,XX,inv(11)(q21q23)pat
(6) Malignancies		
	Leukaemia	46,XY,t(2;11)(q21;p15)unknown
		46,X,inv(Y)(p1q1)unknown
	Cancer of the larynx	46,XY,t(2;7)(p21;q22)unknown
	Histiocytoma in colon	46,XY,inv(2)(p11q13)pat
	Myeloid leukaemia	46,XX,inv(10)(q11q24)unknown
(7) Obesity		
		46,XX,t(1;13)(q11;p12)de novo
		46,XY,t(5;8)(q22;q11)unknown
(8) Muscular disorder		
		46,XX,inv(10)(p11.1q21.1)pat
(9) Behavioural disorder		
	Psychosis	46,XX,t(9;18)(p13.3;q12.3)mat
	Behavioural problems	46,XY,t(4;10)(q25;q24)pat
	Behavioural problems, alcoholism, arsonism	46,XY,t(1;18)(p21;p11)unknown
	Schizophrenia	46,XY,t(10;17)(p14;p13)unknown
	Psychosis, autism, +	46,XY,t(7;12)(q21.4;q15)de novo
(10) Autism		
		46,XX,t(5;18)(q34;q12)de novo
		46,XX,t(9;18)(p22;q21)pat,inv(10)(p11q21)mat
		46,XY,t(14;16)(q32.2;q22)de novo
(11) Others		
	Haemolytic uraemic syndrome	46,XX,inv(Y)(p11q11)pat
(12) Male infertility		
Cases with cytogenetically matching breakpoints		
1q21		46,XY,t(1;21)(q21;p11)unknown
		46,XY,inv(1)(q21.2q42)unknown
		46,XY,ins(1;9)(q21q42;q13)unknown
		46,X,t(Y;1)(q12;q21.2)de novo
2q21		46,XY,t(2;21)(q21;p11)unknown
		46,XY,t(2;6)(q21;q25)mat
Cases without cytogenetically matching breakpoints		
		46,XY,t(1;8)(p32;q11)unknown
		46,XY,t(2;5)(p21;q12.2)de novo
		46,XY,t(2;19)(p11.2;p13.3)unknown
		46,XY,t(3;15)(q11;p12)unknown
		46,XY,inv(5)(p15.2p13)unknown
		46,XY,inv(6)(p21.1q13)mat
		46,XY,t(7;22)(p10;p10)unknown
		46,XY,t(9;19)(q22;q12)unknown
		46,XY,t(9;22)(p24;q12)unknown
		46,XY,inv(12)(p12q13)unknown
		46,XY,t(12;15)(q24;24)unknown
		46,XY,t(13;20)(q11;q13)unknown
		46,XY,t(4;21)(q34;q11.1)unknown
(13) Female infertility		
Cases with breakpoints within the critical region Xq13-q26		
		46,X,t(**X**;2)(**q21**;q22)de novo
		46,X,t(**X**;5)(**q22**;p15.2)unknown
		46,X,t(**X**;5)(**q22**;p14)de novo
		46,X,t(**X**;9(**q21.4**;q21)unknown
		46,X,t(**X**;10)(**q22**;q24)unknown
		46,X,t(**X**;16)(**q22.1**;p11.2)unknown
		46,X,t(**X**;12)(**q23**;p13)unknown
		46,X,t(**X**;13)(**q21**;p11)de novo

Appendix   Continued

Phenotype	Karyotype
Cases with autosomal breakpoints only	
	46,XX,inv(1)(p23q21)de novo
	46,XX,t(1;11)(q44;p13)unknown
	46,XX,t(3;20)(q25;q13)mat
	46,XX,t(4;11)(q31;q24)mat
	46,XX,inv(5)(p13q13)pat
	46,XX,t(5;7)(q22;q119unknown
	46,XX,t(7;10)p21;q21)unknown
(14) Mental retardation with or without multiple congenital malformations	
Cases with cytogenetically matching breakpoints	
1q31	46, XX,t(1;4)(q31;q21),t(2;13)(p14.1;q33)de novo
	46,XY,t(1;16)(q31q13)pat
1q44	46,XX,t(1;19)(q44;q13.2)pat
	46,XY,t(1;4)(q44;q22)de novo[38]★
	46,XY,t(1;22)(q44;q11.9)unknown
	46,XX,t(1;2)(p36;q23)unknown[40]
2q23	46,XX,t(2;5)(q23.1;p13.1)pat
	46,XY,inv(2)(p23q23.1)de novo
	46,XY,t(2;4)(q23;q35)mat†
	46,XX,t(2;16)(q23.2;22.1)de novo
	46,XX,inv ins(2)(p23q37q23)de novo
4p16	46,XY,inv(4)(p16q11)pat
	46,XX,inv(4)(p16q21.1)mat,inv(4)(p15.1q13)pat
4q22	46,XX,t(4;9)(q22;p24)pat[38]
	46,XY,t(1;4)(q44;q22)de novo[38]★
4q35	46,XY,t(2;4)(q23;q35)mat†
	46,XX,t(4;5)(q35;p12)de novo[39]
5q13	46,XX,t(5;7)(q13;p13)unknown
	46,XX,t(5;9)(q13;q22)pat
	46,XY,t(5;8)(q13;q11)de novo
	46,XY,t(5;15)(q13;q25)mat[42]
6q27	46,XX,inv(6)(p12q27)mat
	46,XX,t(6;13)(q27;q14)unknown
7q22	46,XX,t(2;7)(q31;q22)pat
	46,XX,t(6;7)(p25;q22)mat
	46,XX,t(7;13)(q22;q34)de novo‡
7q36	46,XX,t(1;7)(p35;q36)unknown[40]
	46,XX,t(1;7)(p22;q36)inv(9)mat
9p24	46,XX,inv(9)(p13p24)unknown
	46,XX,t(9;12)(p24;q21.2)de novo
9q34	46,XY,t(9;12)(q34;q13)de novo
	46,XY,t(9;17)(q34.1;q25.3)de novo
12q15	46,XY,t(2;12)(p15;q15)de novo
	46,XY,t(7;12)(q11;q15)mat
	46,XX,inv(12)p13q15)mat
13q34	46,XXt(7;13)(q22;q34)de novo‡
	46,XX,t(2;13)(p24;q34)mat
14q32	46,XX,t(12,13;14;20)(p11;q14;q32;p13),dir ins (9;12)(q21;p?)de novo
	46,XY,t(14;18)(q32.33;q21.31)de novo

★, †, and ‡: same cases with two different matching breakpoints
Cases without cytogenetically matching breakpoints

	46,XY,inv(1)(p13q12)mat
	46,XX,inv(1)(p21q41),inv(5)(q13q34)de novo
	46,XY,t(1;3)(p22;q27)pat
	46,XY,t(1;7)(p32;p11)unknown
	46,XX,t(1;11)(p13;p11)de novo[39]
	46,XY,t(1;15)(q12;q11)de novo
	46,XY,t(1;16)(q12;p13)unknown[40]
	46,XY,inv(2)(p11q14)pat
	46,XY,t(2;3)(q31.2;q11.1)unknown
	46,XY,t(2;3;7)(q14;q25;q34)unknown
	46,XX,t(2;4;6;9;10)(p12;q12;q23;q12;pter)unknown
	46,XX,t(2;5)(q22.3;q33.1)de novo
	46,XY,t(2;6)(p23;q15)unknown
	46,XX,t(2;9)(p23;q22)de novo
	46,XY,t(2;10)(q14.3;p12.3)de novo
	46,XY,t(2;12)(p23;q24.1)unknown
	46,XY,t(2;20)(q11;p11)de novo
	46,XY,t(2;22)(q11.2;q11.2)de novo
	46,XY,t(2;22)(q13;q11.1)de novo
	46,XX,t(3;4)(q24;q31.3)unknown
	46,XY,t(3;6)(p14;p22)de novo
	46,XX,t(3;7)(q21;p14)de novo
	46,XY,t(3;19)(p25;p13)de novo
	46,XY,t(4;7)(q34;q31)unknown[40]
	46,XX,t(4;7;14)(q13;q23;q24)unknown
	46,XY,t(4;14)(q12;q24.1)de novo
	46,XY,t(4;16)(q24;p13.2)unknown
	46,XX,t(4;17)(q21;q23)de novo
	46,XX,t(4;20)(q;p)de novo
	46,XX,t(4;22)(q31.3;q12.2)de novo
	46,XY,inv(5)(p13p15),inv(9)unknown[39]
	46,XX,t(5;12)(q13;q13)unknown
	46,XY,t(5;14)(q11;p12)de novo
	46,XX,t(5;20)(q12;p11)mat
	46,XY,t(5;16)(q14.1;p11.2)de novo
	46,XY,inv(6)(p22q21)pat
	46,XY,inv(6)(p24q23)mat
	46,XX,t(6;8),dir ins(11;12)(p15.1;q13q21.3)de novo
	46,XY,t(6;13)(p21.2;q33.3)de novo

Appendix   Continued

Phenotype	Karyotype
	46,XX,t(6;14)(q13;q32)pat[41]
	46,XY,t(6;22)(p22;q12)de novo
	46,XY,t(7;12)(q11;q13)mat
	46,XY,t(7;12)(q21.4;q15)de novo
	46,XX,t(7;13)(q21;q21)de novo
	46,XY,t(7;19)(q11.21;p13.1)de novo
	46,XX,t(7;22)(p15;q12)mat
	46,XX,inv(8)(p22q22)unknown
	46,XY,t(8;19)(q24;q12)unknown
	46,XX,t(9;10)(p12;q11.2)de novo
	46,XY,t(9;12)(p21;q24)de novo
	46,XX,inv(10)(p11q21)mat
	46,XX,inv(10)(p13q22)mat
	46,XY,t(10;12)(p15;p11)de novo
	46,XY,t(10;13)(q24;p11)unknown
	46,XX,t(10;15)(q22;q24)de novo
	46,XY,t(10;16)(q21;q11)de novo
	46,XX,t(10;18)(q11;q12)de novo
	46,XY,t(11;14)(q25;q24.3)de novo
	46,XX,inv(12)pat
	46,XY,inv(12)(p13q13)pat
	46,XY,t(12;14)(q14;q13)de novo
	46,XX,inv ins (15;5)(q15;p15.2p14.1)de novo
	46,XY,t(15;18)(p13;q11)unknown
	46,XY,t(16;17)(p11;q21)mat
	46,XX,inv(20)(p11q12)pat
	46,X,t(X;1)(q28;p25)unknown
	46,X,t(X;4;13)(p11.1;q12;q22.2)unknown
	46,X,t(X;7)(q22;p22)unknown
	46,X,t(X;10)(p11;q26)unknown
	46,X,t(X;15)(p22;q11)unknown
	46,X,t(X;17)(p11.2;p13.2)de novo
	46,X,inv(Y)de novo