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### ► To cite this version:

Conny M.A. van Ravenswaaij-Arts, Ilse Feenstra, Nicolien Hanemaaijer, Birgit Sikkema-Raddatz, Helger Yntema, et al.. Balanced into array: genome-wide array analysis in 54 patients with an apparently balanced de novo chromosome rearrangement and a meta-analysis. *European Journal of Human Genetics*, 2011, 10.1038/ejhg.2011.120 . hal-00655429

**HAL Id: hal-00655429**

**<https://hal.science/hal-00655429>**

Submitted on 29 Dec 2011

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**Balanced into array: genome-wide array analysis in 54 patients with an apparently  
balanced *de novo* chromosome rearrangement and a meta-analysis**

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## Abstract

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High resolution genome-wide array analysis enables [detailed screening for cryptic and submicroscopic imbalances](#) of microscopically balanced *de novo* rearrangements in patients with developmental delay and/or congenital abnormalities. In this report we added the results of genome-wide array analysis in 54 patients to data on 117 patients from seven other studies. A chromosome imbalance was detected in 37% of all patients with two-breakpoint rearrangements. In 49% of these patients the imbalances were located in one or both breakpoint regions. Imbalances were more frequently (90%) found in complex rearrangements, with the majority (81%) having deletions in the breakpoint regions. The size of our own cohort enabled us to relate the presence of an imbalance to the clinical features of the patients by using a scoring system, the De Vries criteria, that indicates the complexity of the phenotype. The median De Vries score was significantly higher ( $p=0.002$ ) in those patients with an imbalance (5, range 1-9) than in patients with a normal array result (3, range 0-7).

This study provides accurate percentages of cryptic imbalances that can be detected by genome-wide array analysis in simple and complex *de novo* microscopically balanced chromosome rearrangements and confirms that these imbalances are more likely to occur in patients with a complex phenotype.

**Key words:** Array analysis, *de novo* translocation, *de novo* inversion, complex chromosome rearrangement, microdeletion, microduplication

## **Introduction**

The estimated frequency of balanced chromosome rearrangements in a population of unselected newborns is 0.52%.<sup>1</sup> The majority of these translocations, insertions and inversions is transmitted from one of the parents and not associated with abnormal phenotypes.<sup>2</sup> In 1991, Warburton reported data on the frequency and outcome of cases with apparently balanced, *de novo*, rearrangements detected at amniocentesis in over 350,000 pregnancies.<sup>3</sup> She found that a microscopically balanced, *de novo*, reciprocal translocation was detected in one out of every 2,000 pregnancies. The frequency of congenital abnormalities in fetuses and newborns with *de novo*, reciprocal translocations or inversions has been estimated at 6.1% and 9.4%, respectively.<sup>3</sup> This is more than twice as high as the risk of 2-3% in the general population. The increased number of abnormal phenotypes can be caused by: (1) a microdeletion or microduplication at the translocation or inversion breakpoint(s) which is only detectable by high-resolution techniques, (2) disruption or modulation of the expression of gene(s) located at the breakpoint(s), (3) otherwise inactivation (position effect) of gene(s) at the breakpoint region(s). Thus, an apparently balanced, *de novo*, chromosome rearrangement can underlie an abnormal phenotype, but it may also be coincidental. The actual confirmation or rejection of causality by detecting a cryptic deletion or duplication at the assumed breakpoints or elsewhere in the genome is often lacking. The unbalanced nature of small rearrangements will most often escape detection, since the resolution of standard cytogenetic banding techniques is only 5-10 Mb. It has already been shown that the yield of chromosome abnormalities in patients with developmental delay (DD) and/or multiple congenital anomalies (MCA) increases considerably with the resolution of the technique used. A microscopically visible chromosome

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abnormality can be detected by routine karyotyping in 3-5% of all DD/MCA patients, excluding Down syndrome,<sup>4-6</sup> whereas genome-wide array-based techniques are able to detect a chromosome imbalance in up to 15-20% of such cases.<sup>6-8</sup>

Recent studies have reported on genome-wide array analysis used to identify cryptic imbalances in cohorts of DD/MCA patients with an apparently balanced, *de novo*, chromosome rearrangement (table 1).<sup>9-15</sup> A cryptic imbalance was detected by genome-wide array analysis in 33-100% of DD/MCA patients with a *de novo* chromosome rearrangement. In the majority of patients, the imbalance was detected at one or more breakpoints, although a large percentage of imbalances (15-40%) was found elsewhere in the genome. The frequency of detected imbalances is significantly higher in patients with a more complex chromosome rearrangement (CCR), involving more than two chromosomes and/or more than two breakpoints.<sup>9,10,14</sup> In all studies, the reported imbalances were assumed to cause the abnormal phenotype.

In contrast to the studies performed in DD/MCA patients, Baptista and colleagues compared a cohort of 31 phenotypically normal individuals carrying a balanced chromosome rearrangement with a cohort of 14 DD/MCA patients.<sup>12,16</sup> No genomic imbalances at the breakpoints, or elsewhere in the genome, were detected in the 31 normal carriers, whereas a disease-causing imbalance was detected in four out of 14 DD/MCA patients. The authors concluded that translocations in patients with a clinically abnormal phenotype are molecularly distinct from those in normal individuals. An unexpected finding was that the frequency of gene disruption due to a chromosome rearrangement did not differ between phenotypically abnormal patients and the normal study population.<sup>12</sup> However, the percentage of disrupted genes that play a role in the nervous system was higher in the phenotypically abnormal patients.

Since there is limited data on patients with apparently balanced chromosome rearrangements, we decided to evaluate the results obtained from genome-wide array analysis in a cohort of 54 DD/MCA patients and a cytogenetically balanced, *de novo*, chromosome rearrangement. Since this is the largest postnatal cohort of DD/MCA patients with *de novo* balanced rearrangements reported thus far, we were able to improve the estimated percentage of submicroscopic imbalances detected by genome-wide array analysis in *de novo* chromosome rearrangements. The size of the cohort also enabled us to relate the probability of finding an imbalance to the clinical phenotype of the patient by using the De Vries scoring system.<sup>17</sup>

## **Methods**

### Patient selection

Clinical data and samples were collected from 54 patients with an apparently balanced, *de novo*, structural chromosome rearrangement. All patients had been referred for karyotyping because of DD and/or MCA and were enrolled in the study for diagnostic purposes. All chromosome rearrangements were detected by routine cytogenetic analysis at a minimum band level of 500: 46 patients carried a two-breakpoint rearrangement; 40 patients had a reciprocal translocation, while six patients carried an inversion. Eight patients had a CCR with at least three breakpoints.

All patients, parents or legal representatives gave informed consent for this study, according to local guidelines.

### Collection of clinical data

Clinical data were derived from medical records using a standardized form. Additional information was requested from the referring clinicians whenever necessary. All

patients were scored according to adapted De Vries criteria, which provided a checklist for patients with submicroscopic subtelomeric rearrangements (table 2).<sup>17</sup> Family history was replaced by developmental delay in this scoring system, because a positive family history, either compatible or non-compatible with Mendelian inheritance, does not enhance the chance of finding imbalances in the breakpoint regions in patients with a *de novo* chromosome rearrangement. In contrast, the De Vries criteria were developed for patients with an intellectual disability, while not all the patients in our study had a developmental delay. Therefore, one and two points were given for mild to moderate and severe developmental delay, respectively. Severe developmental delay was defined as a Developmental Quotient below 30, while mild to moderate developmental delay was a Developmental Quotient between 30 and 70. In this way the maximum number of points that could be scored remained 10 (table 2).

#### Genome-wide array analysis

Array analysis with an average genome-wide resolution of approximately 200 kb was performed using either an Agilent 105k or 244k oligo array, a 32k BAC array as previously described,<sup>18</sup> or the Affymetrix 250k SNP array platform,<sup>19</sup> following the protocols provided by the manufacturers (Agilent Technologies and Affymetrix Inc., Santa Clara, CA, USA).

For the Agilent array reference DNA, a mixture of 40 male or female DNA samples of the same gender was used as control. The data were processed using Feature Extraction V.9.1 and CGH analytics V. 3.4.27 provided by the manufacturer. For the SNP array experiments, copy number estimates were determined using the updated version 2.0 of the CNAG (Copy Number Analyzer for Affymetrix GeneChip mapping) software package.<sup>20</sup> The normalized ratios were then analyzed for genomic imbalances

by a standard Hidden Markov Model (HMM), essentially as described before.<sup>18</sup> The SNP array data obtained from patient DNA were compared to SNP array data from ten healthy, sex-matched individuals.

Regardless of the array platform employed, genome-wide data analysis was performed using previously determined criteria which provide 95% confidence of representing a true copy number variation (CNV).<sup>21</sup> A CNV was considered significant if five or more consecutive probes showed a single copy-number loss (N=1), or at least seven consecutive SNPs showed a single copy-number gain (N=3) for the Affymetrix array, or four or more consecutive probes showed gains or losses for the Agilent array. For interpretation purposes, various public web sources were consulted, including the Online Mendelian Inheritance of Man ([www.ncbi.nlm.nih.gov/Omim](http://www.ncbi.nlm.nih.gov/Omim)), the DECIPHER database ([decipher.sanger.ac.uk](http://decipher.sanger.ac.uk)), and ECARUCA ([www.ecaruca.net](http://www.ecaruca.net)). A CNV was considered a normal genomic variant if it had been detected in at least three control individuals as reported in the Database of Genomic Variants (<http://projects.tcag.ca/variants>), and/or been encountered in at least three in-house control samples. Data analyses were based on the NCBI36/hg18 build of the human genome.

#### FISH analysis

To validate the gains or losses identified by genome-wide array analysis, region-specific fluorescent in-situ hybridization (FISH) was performed following routine protocols. Bacterial Artificial Chromosome (BAC) clones were selected from the human library RPCI-11 according to the UCSC Human Genome Assembly (freeze March 2006) and kindly provided by the Wellcome Trust Sanger Institute (<http://www.sanger.ac.uk>) or obtained from the 32k set of BAC DNAs in the Nijmegen laboratory. BAC DNA was



indirectly labeled with biotin- or digoxigenin-11-dUTP using Nick translation. Slides were hybridized overnight at 37°C and fluorescently labeled with FITC or Texas Red.

#### Multiplex Ligation-dependent Probe Amplification

To validate the gains identified by array analysis, region-specific multiplex ligation-dependent probe amplification (MLPA) was performed. For each region, two uniquely sized probes were developed in accordance with a protocol provided by MRC Holland (Amsterdam, the Netherlands). Ten probes were combined in one MLPA assay together with a DNA quantity and a DNA denaturation control mix (EK-1 kit, MRC Holland).

The procedure was further carried out as described by De Vries et al.<sup>18</sup>

### **Results**

In this study, 54 patients with an apparently balanced, *de novo* chromosome rearrangement and an abnormal clinical phenotype were analyzed for submicroscopic chromosome imbalances by genome-wide array analysis. Forty-six patients had a two-breakpoint rearrangement upon routine karyotyping. In eight patients, a more complex aberration was found. All patients had facial dysmorphisms and/or congenital malformations and 46 out of 52 patients (88%) showed developmental delay, varying from mild psychomotor retardation (PMR) and speech delay to severe DD.

Development could not be assessed in two patients because they died at the age of one day and two months, respectively (patients 12 and 43). A detailed description of all the phenotypes is presented in Table 3.

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The total number of CNVs, including well known benign CNVs, detected by the platforms used ranged from 2 to 12 with an average of 5.6 per patient (Table 3). All the

potentially causative, copy number alterations detected by array could be confirmed by FISH (losses), MLPA (gains) or an independent array platform.

#### CNVs at or near the breakpoint regions

In 11 out of 54 patients (20%), the apparently balanced rearrangement was found to be unbalanced at the breakpoint region(s). We found no gains but 16 losses in these 11 patients in total (Table 4A). The size of the losses varied from 0.1 to 15.3 Mb. Seven patients had a single loss, two patients (6 and 53) had losses at multiple breakpoints, and two patients (49 and 51) had multiple, non-overlapping losses in one breakpoint region. Patient 51 also had a loss elsewhere in the genome (table 4B). In patient 6, with a loss at both breakpoints (1p22.1 and 6q15), the 1.1 Mb loss of chromosome 1 appeared to contain no known genes. The 4.25 Mb deletion in patient 42 contained the *FBNI* gene, explaining the observed Marfan phenotype.<sup>22</sup>

Five out of 46 (11%) patients with a two-breakpoint chromosome rearrangement had a cryptic imbalance related to their reciprocal translocation. No imbalances were found related to inversions (n=6). Six out of eight (75%) patients with a CCR (more than two breakpoints) appeared to have an abnormal genome profile upon array analysis. All of these six patients had losses at the breakpoint regions.

#### Imbalances elsewhere in the genome

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Copy number changes elsewhere in the genome were present in seven patients: six had a reciprocal translocation and one patient a CCR (table 4B). Six losses and three gains not related to the breakpoints were detected in total in these seven patients. Only the patient with a CCR (51) had additional copy number alterations at one of the breakpoint regions (table 4A). In this and two other patients (30 and 32), the respective CNV was

inherited from a healthy parent. Patient (29) had three imbalances: two losses were *de novo* (on the paternal allele) and one loss was also observed in his healthy father.

In an adult patient (10) with a translocation (1;17)(p36.1;q11), a 650 kb gain in 1p34.1 was found. Unfortunately, this patient's parents were not available for further investigation. A partially overlapping, *de novo* 650 kb gain was found in a clinically more severely affected boy (18). Both gains overlap a 450 kb region in 1p34.1.

The last imbalance detected elsewhere was a 270 kb deletion at 5p13.1 in a patient with a translocation (2;10) (patient 16). Unfortunately, this patient's parents were not available for further investigation.

#### Clinical criteria

All but three patients (n=51) could be scored according to the adapted clinical De Vries criteria (tables 2 and 3).<sup>17</sup> Patient 43 died one day post partum, patient 12 died at the age of two months, and patient 52 had Sotos syndrome due to an *NSD1* mutation interfering with the phenotype. The distribution of the scores is shown in supplementary figure 1.

All patients with a chromosome imbalance in the breakpoint region (n=11) had a score of at least 3 with a median score of 5 (range 3-9). The highest score was found in the patient with imbalances both at a breakpoint and elsewhere (score 9 in patient 51).

Patients with a chromosome imbalance restricted to elsewhere in the genome (n=6) tended to have a lower score (median 4.5, range 1-6). One of the imbalances in this group was considered not clinically relevant (see discussion and table 4B). Correction for this patient 32 led to a median score of 5 (range 1-6). The median score in the total group with a possibly clinically relevant CNV (n=16) was 5 (range 1-9), while the median score in the group without a significant CNV (n=35) was 3 (range 0-7). The difference between these two groups is significant (p=0.002, Mann Whitney U test).

## Discussion

In this study, 54 patients with an apparently balanced, *de novo* chromosome rearrangement were examined by high resolution genome-wide array analysis. The mean number of CNVs, including well-known recurrent copy number polymorphisms, that was detected was 5.6 per patient (range 2 – 12). In general, the number of CNVs detected per patient depends on the platform and detection thresholds used, but the number found in our study does not differ substantially from patients without apparently balanced rearrangements.<sup>18,23</sup>

Out of 46 patients with a two-breakpoint chromosome rearrangement, 11 (25%) appeared to have an abnormal genome profile encompassing six losses, each at one of the breakpoints in five patients, and five losses and three gains elsewhere in the genome in six patients. From analysis of their parents, two of the latter category could be specified as rare, inherited CNVs. Six out of eight patients with a CCR were found to have one or more clinically significant losses at one of the breakpoints. In addition, one of these six patients had a paternally inherited imbalance elsewhere in the genome. Although the overall percentage of patients with a cryptic or submicroscopic, clinically significant imbalance in this cohort is 31%, there is a remarkable difference between patients with a two-breakpoint chromosome rearrangement (24%) and those with a more complex rearrangement (75%).

The number of imbalances seen in our patient cohort is similar to the studies of Sismani and Baptista,<sup>11,12</sup> but lower than the studies of others (table 1).<sup>9,10,13-15</sup> This might be due to differences in patient selection (reflected in the high number of aberrations found elsewhere in the genome in the studies of Gribble, Higgins and

Gijsbers) and to the higher number of complex rearrangements studied by De Gregori. Compiling the data of the previous and present studies, we conclude that in almost half of the patients with a *de novo* chromosome rearrangement, a genomic imbalance can be detected by genome-wide array analysis. We confirmed that, in complex rearrangements, the chance of finding copy number alterations at the breakpoints is very high: 75% and 72%, in our study and the combined studies, respectively.

#### Imbalances are not always located at breakpoints

In most patients (20%) with clinically relevant copy number alterations, the imbalance is detected in or near the breakpoints of the chromosomes involved (table 4A).

However, in 13% an imbalance is found elsewhere in the genome (table 4B). As shown here and in previous studies, this was especially true for two-breakpoint *de novo* aberrations. In 19% of all patients with a two-breakpoint rearrangement, imbalances are found elsewhere. Especially in these cases, the clinical significance of the detected CNVs should be determined by parental analysis, amongst other investigations. The observed percentage of 19% is in agreement with the general figure of 17% of imbalances that is found in the DD/MCA population.<sup>7,8</sup> These results underline the importance of a genome-wide approach in patients with an apparently balanced, *de novo* chromosome rearrangement. If imbalances are found independent of the rearrangement breakpoints, this may have implications for the recurrence risk and warrants studies in the parents to exclude cryptic balanced translocations and insertions. Furthermore, it is crucial to critically examine an apparently balanced rearrangement after initial detection, because they are often more complex than they appear at first.

#### Losses are more frequent than gains at breakpoints

The clinically significant imbalances at the breakpoint regions found in this study were all deletions. Breakpoint deletions are more frequent in patients with a CCR than in patients with a two-breakpoint rearrangement. In the present study, we detected deletions in six out of eight CCR patients (75%) deletions. This is comparable to the results of De Gregori and Schluth-Bolard, who detected deletions in 69% and 80% of patients with a *de novo* CCR, respectively.<sup>10,14</sup> Thus deletions may be the main cause of phenotypic abnormalities in patients with a CCR.

The preponderance of deletions is similar to the results of others (table 1).<sup>9,10,12-14</sup>

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Recently, Howarth *et al* showed that in breast cancer cell lines reciprocal translocations arising during mitosis may result in both deletions (up to 31 Mb) and duplications (up to 200 kb) at the breakpoint regions.<sup>24</sup> They demonstrated that the underlying mechanism most likely is stalled replication bubbles during the interchromosomal exchange. *De novo* constitutional translocations have their origin during meiosis. Nonetheless, the same mechanism may cause imbalances during meiotic interchromosomal exchanges. That we and others did not find breakpoint duplications in DD/MCA patients might be explained by their size (often under the detection threshold) and the fact that small duplications rarely result in a phenotype.

#### Clinical significance of the detected imbalances

The size of the deletions and gains in our patients ranged from 100 kb to 15.3 Mb and from 240 kb to 650 kb, respectively. In patient 6 with deletions at both breakpoints, the abnormal phenotype was considered to be a consequence of the 9.2 Mb deletion at chromosome 6, because the small deletion at chromosome 1 did not contain any known genes. All other breakpoint deletions were considered pathogenic based on the criteria mentioned in the methods.

In four of the seven patients with an imbalance elsewhere in the genome, the imbalance was found to be inherited from a clinically unaffected parent. The deletion 16p13.11 (patient 30) and deletion 1q21.1 (patient 51) are known microdeletion syndromes with variable phenotypes.<sup>25-27</sup> Patient 51 also carries two significant losses at a breakpoint region, but we cannot exclude that the 1q21.1 deletion also contributes to the phenotype. The maternally inherited gain in 1q23.3 (patient 32) was considered unlikely to be clinically relevant because a larger gain has been detected in two control individuals from one study in the Database of Genomic Variants.<sup>23</sup> The paternally inherited loss in patient 29 is in a gene-less region of 12p11.22 and therefore likely to be benign. Of the two *de novo* losses in the same patient (29), the 4.9 Mb loss in 2q33.3q34 is most likely to be clinically relevant. The 9q21.12q21.1 loss has not been detected before and thus its clinical significance remains uncertain, although a contribution to the clinical phenotype of patient 29 cannot be excluded.

The 650 kb gain in 1p34 in patient 10 is not a known polymorphism according to the Database of Genomic Variants, and is only partially overlapping gains that have been found in normal individuals (Nijmegen and Groningen in-house control data). Patient 18 had a similarly sized duplication, of which 450 kb overlapped with the gain of patient 10. The distal 200 kb, non-overlapping region, contains several genes, including *POMGNT1*. The phenotype of patient 18 is similar to previously published patients with larger overlapping duplications that included this gene.<sup>28</sup>

The 270 kb loss in 5p13.1 (patient 16) is not a known polymorphism but only contains the *LIFR* gene involved in autosomal recessive Stuve-Wiedemann syndrome, although the patient's clinical features do not resemble this syndrome. Unfortunately, the parents were unavailable for further studies and the clinical significance of the

deletion remains unclear, since no similar microdeletion has been found in controls or other patients so far.

Thus, in at least four of the seven patients with imbalances elsewhere, the detected imbalance was considered to contribute to the abnormal phenotype.

#### Clinical features pointing to an imbalance

All 16 patients with a potentially clinically relevant CNV showed developmental delay, ranging from mild psychomotor or speech delay (in 5 patients) to severe DD (in 7 patients). As discussed above, the gain in patient 32 with severe DD was, in retrospect, considered very unlikely to be causative for the phenotype. If we had only analyzed patients with an adapted De Vries score above 3, we would not have missed any clinically relevant chromosome imbalances at the breakpoint regions (supplementary

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figure 1). [This is in line with the results of the original study using de De Vries criteria: all patients with a subtelomeric aberration had a De Vries score of at least 3.<sup>17</sup>](#)

Two out of six patients with an aberration elsewhere in the genome had a score below 3. This concerned the maternally inherited 1q23 gain in patient 32 (score 2) that was considered unlikely to be clinically relevant, and one 1p34 gain in patient 10 of uncertain clinical relevance (score 1). The median De Vries score of all 14 patients with a certainly clinically relevant CNV (table 4) was 5 (range 3-9), while in the 35 patients without a relevant CNV the median score was 3 (range 0-7). Three patients could not be scored (see results), and two patients had an imbalance of uncertain clinical relevance.

#### Other mechanisms causing DD/MCA in balanced rearrangements

A truly balanced, *de novo* chromosome rearrangement may still contribute to an abnormal clinical phenotype due to disruption of a gene or due to a position effect. An example of the former was seen in patient 45 who appeared to have a disruption of the



*TCF4* gene at 18q21.1, as described in a previous study.<sup>29</sup> Conventional methods for mapping chromosome breakpoints, such as fluorescent in situ hybridisation, are laborious, and often fail to identify the disrupted gene. Combining DNA array hybridisation with chromosome sorting, improves the efficiency of breakpoint mapping, but can only be applied when the physical properties of the derivative chromosomes allow them to be flow sorted. Nowadays more efficient and accurate breakpoint identification can be performed by next-generation paired-end sequencing.<sup>30</sup>

A position effect was most likely responsible for the split-hand-feet syndrome (SHFM) in patient 37 with an inversion breakpoint in 7q near the SHFM1 locus and the candidate genes *DSS1*, *DLX5* and *DLX6*.<sup>31</sup>

### **Final conclusions**

The combined results of our study and previous reports show that in 79/171 (46%) of DD/MCA patients with a *de novo* chromosome rearrangement, a genomic imbalance could be detected by genome-wide array analysis. In patients with a rearrangement involving more than two breakpoints, there is a high chance of detecting an imbalance at one of the breakpoints (21/29; 72%). In two-breakpoint rearrangements, an imbalance located at a breakpoint was detected in 26/142 (18%) patients. However, a substantial number of imbalances were also detected outside the breakpoint regions: in 33/171 (19%) patients an imbalance was found elsewhere in the genome, which is comparable to the general DD/MCA population. In conclusion, diagnostic studies should not only focus on the rearrangement breakpoints, but a genome-wide approach should be used to investigate patients with apparently balanced, *de novo* chromosome rearrangements.

## **Acknowledgements**

We are grateful to all the patients and their parents for their kind cooperation. We would also like to thank the requesting physicians, Bregje van Bon, Ineke van de Burgt, Ton van Essen, Ben Hamel, Marjolijn Jongmans, Tjitske Kleefstra, Carlo Marcelis, Sonja de Munnik, Gretel Oudesluijs, C. Nur Semerci, Liesbeth Spruijt, Irene Stolte-Dijkstra, Peter van Tintelen, Joep Tuerlings, and Michèl Willemsen, for their contribution.

Special thanks to the Array Diagnostics teams of Groningen and Nijmegen, to Hanneke Mieloo for extensive FISH analyses, to Marian Bakker for statistical assistance and to Jackie Senior for editorial support. This work was supported by grants from the Netherlands Organization for Health Research and Development (ZonMW 917-86-319 to B.d.V.) and the Brain Foundation of the Netherlands (Hersenstichting) (B.d.V.)

## **Conflict of interest**

The authors declare no conflict of interest.

## **Web resources**

The URLs for data presented here are as follows:

Database of Genomic Variants (DGV), <http://projects.tcag.ca/variation/>

DECIPHER database, <http://decipher.sanger.ac.uk/>

European Cytogeneticists Association Register for Unbalanced Chromosome Aberrations (ECARUCA), <http://ecaruca.net>

Ensembl Human Genome Browser, [http://www.ensembl.org/Homo\\_sapiens/](http://www.ensembl.org/Homo_sapiens/)

Online Mendelian Inheritance in Man, <http://www.ncbi.nlm.nih.gov/Omim>

University of California-Santa Cruz Human Genome Browser, <http://genome.ucsc.edu/>

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### **Supplementary Figure 1**

Distribution of the adjusted De Vries score (table 2) in patients without clinically relevant imbalance (□), patients with a clinically relevant imbalance at the breakpoint region (■), patients with a clinically relevant imbalance elsewhere in the genome (▨), and patients with a potentially clinically relevant imbalance elsewhere in the genome (▩). \*Patient 51 who had imbalances at a breakpoint region and elsewhere in the genome.

**Table 1. Overview of genome-wide array studies in patients with apparently balanced, *de novo* chromosome rearrangements and abnormal phenotypes**

Study	Gribble 2005 <sup>8</sup>	De Gregori 2007 <sup>9</sup>	Sismani 2008 <sup>10,*</sup>	Baptista 2008 <sup>11,*#</sup>	Higgins 2008 <sup>12,*</sup>	Schluth-Bolard 2009 <sup>13,*</sup>	Gijsbers 2010 <sup>14,*</sup>	Present study	Total
Array platform	1 Mb BAC	44B or 244A oligo Agilent	1Mb Cytochip Bluegenome	Sanger 30k Whole Genome Tilepath	2600 BAC Spectral Genomics or 244k oligo Agilent	44k or 244k oligo Agilent	Affymetrix GeneChip 262k Nspl	32k BAC, 105k or 244k oligo Agilent or 250k SNP Affymetrix	
Patients with <b>2bp</b> <sup>§</sup> rearrangement	8	27	6	12	10	28	5	46	142
Patients (percentage) with imbalance at breakpoint	0 (0%)	7 (26%)	0 (0%)	3 (25%)	2 (20%)	6 (21%)	3 (60%)	5 (11%)	26 (18%)
Patients (percentage) with genomic imbalance elsewhere	3 (38%)	4 (15%)	2 (33%)	1 (8%)	3 (30%)	6 (21%)	2 (40%)	6 (13%)	27 (19%)
Patients with <b>CCR</b> <sup>§</sup>	2	13	0	1	0	5	0	8	29
Patients (percentage) with imbalance at breakpoint	2 (100%)	9 (69%)	0 (0%)	0 (0%)	0 (0%)	4 (80%)	0 (0%)	6 <sup>&amp;</sup> (75%)	21 (72%)
Patients (percentage) with genomic imbalance elsewhere	0 (0%)	3 (23%)	0 (0%)	1 (100%)	0 (0%)	1 (20%)	0 (0%)	1 <sup>&amp;</sup> (13%)	6 (21%)
<b>Total number of patients</b>	<b>10</b>	<b>40</b>	<b>6</b>	<b>13</b>	<b>10</b>	<b>33</b>	<b>5</b>	<b>54</b>	<b>171</b>
Patients (percentage) with imbalance at breakpoint	2 (20%)	16 (40%)	0 (0%)	3 (23%)	2 (20%)	10 (30%)	3 (60%)	11 (20%)	47 (27%)
Patients (percentage) with genomic imbalance elsewhere	3 (30%)	7 (18%)	2 (33%)	2 (15%)	3 (30%)	7 (21%)	2 (40%)	7 (13%)	33 (19%)
<b>Total number (percentage) of patients with an imbalance</b>	<b>5 (50%)</b>	<b>23 (58%)</b>	<b>2 (33%)</b>	<b>5 (38%)</b>	<b>5 (50%)</b>	<b>17 (52%)</b>	<b>5 (100%)</b>	<b>17<sup>&amp;</sup> (31%)</b>	<b>79<sup>&amp;</sup> (46%)</b>

\* Only patients with a *de novo* cytogenetically balanced rearrangement, studied by array CGH have been included in this table

# Including two patients with premature ovarian failure and two patients with severe oligospermia and no additional known abnormalities

§ **2bp** = Two-breakpoint rearrangement at routine karyotyping; **CCR** = complex chromosomal rearrangement (three or more breakpoints) at routine karyotyping

& One patient with a CCR had imbalances at a breakpoint and elsewhere, therefore the total of imbalances at breakpoints and elsewhere equals -1.

**Table 2. De Vries score and adjusted De Vries score for assessing clinical phenotypes**

Original De Vries Score <sup>17</sup>		De Vries Score, adjusted for this study	
Trait (points)	Score	Trait (points)	Score
Family history of MR:		Developmental delay	
Compatible with Mendelian inheritance (1)		Mild - moderate developmental delay	
Incompatible with Mendelian inheritance (2)	1-2	(1)	1-2
		Severe developmental delay (2)	
Prenatal-onset growth retardation	2	Prenatal-onset growth retardation	2
Postnatal growth abnormalities:		Postnatal growth abnormalities:	
Microcephaly (1)		Microcephaly (1)	
Short stature (1)		Short stature (1)	
Macrocephaly (1)	max 2	Macrocephaly (1)	max 2
Tall stature (1)		Tall stature (1)	
≥2 Facial dysmorphic features <sup>#</sup>	2	≥2 Facial dysmorphic features <sup>#</sup>	2
Non-facial dysmorphism and congenital abnormalities <sup>§</sup>	1-2	Non-facial dysmorphism and congenital abnormalities <sup>§</sup>	1-2
Total maximum	10	Total maximum	10

\* Including discordant phenotypes.

# Notably, hypertelorism, nasal anomalies, and ear anomalies.

§ Notably, hand anomaly, heart anomaly, hypospadias with/without undescended testis; assign 1 point for each, with a maximum score of 2 points.



**Table 3. Overview of all patients giving karyotype, phenotype and DeVries score**

Patient	Karyotype*	Array Platform <sup>#</sup>	N <sup>o</sup> of CNVs <sup>¶</sup>	Phenotype <sup>§</sup>	Adjusted De Vries score <sup>&amp;</sup>
<b>Two-breakpoint aberrations</b>					
1	t(X;3)(p21.3;p25.1)	32k	3	Dolichocephaly, long face, strabism, full tip of the nose, prominent columella, dysmorphic ears, short philtrum, hyperthyroidism, severe DD, speech delay, convulsions, hypotonia	4
2	t(X;10)(p22.32;q22.2)	244k	4	Short stature, microcornea, iris coloboma, cataract, short and broad hand and feet, hirsutism, adipositas, secondary amenorrhoea, severe DD, blindness, hypotonia	4
3 <sup>32, pt A</sup>	t(X;19)(p11.4;q13.3)	32k	6	Autism, borderline DD (IQ 82), mild hypotonia	0
4	inv(X)(q22.1q28)	32k	7	Down-slant palpebral fissures, open mouth appearance, pes planus, convulsions, speech delay, autism	2
5	t(1;2)(p35;q33)	105k	4	Chondrodysplasia punctata, severe short stature, low-set dysplastic ears, flat nose, hemangioma, PMR, deafness, hypertonia	5
6	t(1;6)(p22.1;q15)	244k	7	Macrocephaly, cerebral atrophy, deep-set eyes, prominent fore head, midface hypoplasia, low nasal bridge, short philtrum, low-set ears, pectus excavatum, small hands with broad short phalanges of the thumbs, pes planus, severe DD	6
7	t(1;8)(p22.1;p23.3)	244k	6	Severe DD, absent tendon reflexes, autism, hypotonia	2
8	t(1;14)(q42.1;q31.1)	32k	7	Dysplastic ears, mild DD, obstipation	2
9	t(1;16)(q21;p11.2)	250k	6	Pre-auricular tag, DD (IQ 50), behavioral and sleep problems	2
10	t(1;17)(p36.1;q11)	244k	5	Mild DD, obstipation, recurrent airway infections	1
11	inv(1)(p22.3p34.1)	32k	4	Macrocephaly (+4.5 SD), dolichocephaly, mild ventriculomegaly, hypertelorism, upward slanted and narrow palpebral fissures, micrognathia, proximally placed thumbs, mild DD	5
12	t(2;9)(q34;p22)	244k	2	Broad tip of the nose, micrognathia, single palmar crease, convulsions, deceased at age 2 months	n.a
13	t(2;10)(p13;p14)	105k	3	Broad tip of the nose, moderate/severe DD, convulsions, hypotonia	2
14 <sup>33</sup>	t(2;10)(p23;q22.1)	32k	6	Birth weight >P98, macrocephaly, sparse hair, hypoplastic alae nasi, dysplastic ears, moderate DD, psychotic disorder, hypotonia, nasal speech, disturbed serine metabolism	4
15	t(2;10)(p25;q26)	250k	5	Epicanthus, club foot, hyperlaxity, mild DD (IQ 64), affective psychotic episodes	2
16	t(2;10)(q22;q22.3)	244k	10	Growth retardation, down slanting palpebral fissures, small nose, mild/moderate DD, convulsions, hypotonia, obstipation	4

17	t(2;10)(q23;p12)	244k	8	Narrow fore head, high narrow palate, mild retrognathia, mild DD, autism	3
18 <sup>28</sup>	t(2;14)(q37.3;q13)	105k	2	IUGR, microcephaly, iris coloboma, laryngomalacia, umbilical hernia, inguinal hernia, severe PMR	6
19	t(2;15)(p22.2;p11)	32k	3	Left-sided hemiparesis, upturned nose, 3 maxillary incisors, absent lower canine tooth, dilatation of aorta, scoliosis, arachnodactyly, mild DD, pubertas tarda, hyperlaxity	5
20	t(2;17)(p25;q23)	250k	3	No dysmorphisms, eczema, PMR, speech delay, IQ 50-60	1
21	t(2;18)(q23;q23)	250k	6	Macrosomia at birth, bulbous nose, high narrow palate, pointed chin, tibial bowing, obesity, mild DD	4
22	inv(2)(q11.2q33)	32k	5	High birth weight (>P98), deep set eyes, short palpebral fissures, high bridge of the nose, micrognathia, high palate, micropenis, large hands, mild DD, aggressive behavior	4
23	t(3;12)(p13;p13.3)	244k	4	Macrocephaly, macro-orchidism, nervus opticus atrophy, kyphosis, DD	4
24	t(4;8)(p16.1;p23.1)	244k	3	Short stature, preauricular tags, synophris, prognathia, epicanthus, broad nasal bridge, thin upper lip, wide spaced teeth, mild DD, behavioral problems	4
25	t(4;12)(p12;q13.2~13.3)	244k	3	Hypertelorism, large ears, broad tip of the nose, short philtrum, thin upper lip, recurrent infections, no DD	2
26	t(4;16)(q33;q12.2)	244k	8	Microcephaly, moderate/severe DD, hypotonia	3
27	t(4;17)(q23;q21)	244k	4	Short stature (<P3), cerebral atrophy, strabism, scoliosis, severe DD, pes plani, autism, hypotonia	4
28	t(5;7)(p15.1;p22)	32k	5	upturned nose, mild DD, speech delay, autism, obsessive eating disorder	2
29	t(5;10)(q33;q25)	250k	4	High birth weight (P98), blepharophimosis, epicanthus, strabism, long face, prominent nose, aplasia of nails, hip dysplasia, obesity, sensorineural deafness, severe DD, hypotonia	5
30	t(5;17)(p15.3;q25.3)	32k	6	Macrocephaly, dolichocephaly, mild hypertelorism, epicanthus, short philtrum, micrognathia, overriding 2 <sup>nd</sup> and 4 <sup>th</sup> toes, mild conductive hearing loss, severe DD, speech delay, mild hypotonia	6
31	inv(5)(q14q33)	32k	2	Protruding tongue, down-slanting palpebral fissures, strabism, posteriorly rotated ears, hirsutism, moderate DD, speech delay, autistic spectrum disorder	3
32	t(6;9)(q21;p24)	250k	3	Severe DD, no speech, convulsions, mild hypotonia	2
33	t(6;11)(p12.3;p14.2)	32k	10	Macrocephaly, strabism, high palate, hypertrichosis lumbosacralis, cryptorchidism, camptylodactyly dig V, pes plani valgi and metatarsus adductus, moderate DD, speech delay, mild sensorineural deafness, mild hypotonia,	5
34	t(6;11)(q16.2;p15.1)	250k	5	Epicanthus, severe DD, no speech, mild hypotonia,	3
35	inv(6)(p11.1q21)	32k	6	Eye disorder, mild to moderate DD	2

36	t(7;15)(p14;p11.2)	244k	7	Microcephaly, small nose, dysplastic ears, low set left ear, clinodactyly, cryptorchidism, severe DD, West syndrome	5
37 <sup>31, pt 3</sup>	inv(7)(p22q21.3)	244k	5	Ectrodactyly of both hands and feet, atrovienous malformation of right hand, autism, no DD	1
38	t(8;14)(q21.2;q12)	32k	5	Microcephaly, partial agenesis corpus callosum, deep-set eyes, strabism, high palate, open mouth appearance, scoliosis, pectus excavatum, short distal phalanges, severe DD, absent speech, convulsions, obstipation	7
39	t(10;16)(q24.1;p11.2)	244k	8	Epicanthus, hypogonadism, obesity, mild DD, autism	2
40	t(12;14)(q13.1;q32.3)	244k	6	Trigonocephaly (familial), mild dysplastic ears, no DD	2
41	t(12;14)(q24.1;q11.2)	244k	5	Long narrow face, sparse hair, broad nasal bridge, umbilical hernia, scoliosis and asymmetrical thorax, mild DD, hypotonia with hypertonia of extremities	3
42 <sup>22, pt 8</sup>	t(12;15)(q24.1;q21.1)	244k	9	Marfan phenotype, broad nasal bridge, short philtrum, long and small fingers and toes, coeliac disease, PMR, hypotonia, no intellectual disability	3
43	t(13;17)(q32;q21)	32k	4	Deceased 1 day after uneventful pregnancy and birth, enlarged liver, steatosis	n.a.
44	t(17;22)(q23;q12.2)	32k	4	Long narrow face, down slanting palpebral fissures, retrognathia, severe scoliosis, pectus excavatum, arachnodactyly, short 4th metatarsals, hallux valgus, mild/moderate DD, cutis marmorata	5
45 <sup>29</sup>	t(18;20)(q21.1;q11.2)	32k	12	Broad, square face, high narrow palate, bilateral single palmar crease, pes planus, mild DD	3
46	t(19;21)(q13.3;q22.3)	244k	5	Microcephaly, epicanthus, high nasal bridge, overfolded helices, valvular pulmonary stenosis, pectus excavatum, mild webbing of the fingers, delayed speech development	6
<b>Complex Chromosome Rearrangements</b>					
47	ins(5;17)(pter;p13.33p13.1)	32k	9	Short stature (<P3), microcephaly, broad nasal bridge, thin upper lip, ASD, mild DD, persistent diarrhoea	6
48	ins(1;11)(p22;q23.1q24.3) inv(1)(p13q23)	32k	2	Short stature, mild trigonocephaly, epicanthus, upslant palpebral fissures, short nose, hearing loss, carp mouth, short 4 <sup>th</sup> metatarsal bone left, pes planus, moderate DD, convulsions, obesity	5
49	der(2)ins(8;2)(q2?;p15p21), der(8)ins(8;2)inv(p?;q?)	32k	4	Short stature (<P3), microcephaly, epicanthus, dysplastic ears, carp mouth, severe gastro-oesophageal reflux, kyphoscoliosis, contractures, rocker bottom feet, severe DD, absent speech, mild hypotonia	8
50	der(2),der(10),der(18)	250k	4	Microcephaly, severe PMR, hypotonia	3
51	der(6)t(6;9)(p21.3;q22) ins(6;13)(p21.3;q?21q31), der(9)t(6;9),der(13)ins(6;13)	32k	5	Birth weight <P3, short stature (<P3), microcephaly, enlarged 4 <sup>th</sup> ventricle, ptosis, strabism, broad high nasal bridge, low dysplastic ears, cleft palate, micrognathia, ASD, clinodactyly dig V, severe speech delay, hypotonia, compulsive behaviour, obstipation	9

52	t(10;18;14)(p15.3;q12.2;q32.3)	244k	6	Sotos syndrome (NSD1 mutation), height and head circumference >P99, broad high forehead, hypertelorism, broad nasal bridge, overfolded helices, pectus excavatum, mild DD, autism	n.a.
53 <sup>34</sup>	der(2),der(3),der(7),der(11)	32k	7	Hypertelorism, everted large nose, full lips, pectus carinatum, short fingers, convulsions, severe DD, absent speech	5
54	t(2;6;12;3)(q24;q23;q12;p13)	250k	5	PMR, hypotonia	1

\* Based on conventional karyotype and FISH-analysis

# 32k= 32k BAC array; 105k= Agilent 105k oligonucleotide array; 244k= Agilent 244k oligonucleotide array; 250k= Affymetrix 250k SNP array

¶The total number of copy number variations (CNVs) detected, including well-known benign CNVs. See table 4 for potentially causative copy number alterations.

\$ ASD= atrial septal defect; DD= developmental delay; PMR= psychomotor retardation

& See table 2, n.a. = not applicable

**Table 4. Summary of patients with an imbalance at the breakpoint regions (A) or elsewhere in the genome (B) detected by genome-wide array analysis**

Pt	Karyotype	Imbalance		Size [Mb] Position [Mb]	De novo/ inherited	Clinical Relevance*	Array platform	Confirmation method
<b>A</b>	<b>At breakpoint region</b>							
6	t(1;6)(p22.1;q15)	del 1p21.3	(51 oligos)	1.07 95.81-96.88	<i>de novo</i>	No	244k Agilent	FISH
		del 6q14.1q15	(680 oligos)	9.21 81.72-90.93	<i>de novo</i>	Yes		
19	t(2;15)(p22.2;p11)	del 2p22.3p22.1	(84 BACs)	7.2 32.8-40.0	<i>de novo</i>	Yes	32k BAC	FISH
24	t(4;8)(p16.1;p23.1)	del 4p16.3p16.1	(501 oligos)	4.52 4.33-8.85	<i>de novo</i>	Yes	244k Agilent	FISH
34	t(6;11)(q16.2;p15.1)	del 6q16.1q16.2	(57 oligos)	0.66 98.44-99.10	<i>de novo</i>	Yes	250k SNP	FISH
42 <sup>22</sup>	t(12;15)(q24.1;q21.1)	del 15q21.1q21.2	(496 oligos)	4.25 46.12-50.37	<i>de novo</i>	Yes	244k Agilent	FISH
47	ins(5;17)(pter;p13.3p13.1)	del 17p13.3p13.2	(32 BACs)	3.3 2.8-6.2	<i>de novo</i>	Yes	32k BAC	FISH
48	ins(1;11)(p22;q23.1q24.3) inv(1)(p13q23)	del 1p22.1p13.3	(176 BACs)	15.3 94.2-109.5	<i>de novo</i>	Yes	32k BAC	None <sup>#</sup>
49	der(2)ins(8;2)(q2?;p15p21), der(8)ins(8;2)inv(p?;q?)	del(8)(q21.11)	(7 BACs)	0.7 75.4-76.1		?	32k BAC	FISH
		del(8)(q22.1)	(11 BACs)	1.1 04.2-95.3		?		
		del(8)(q24.21)	(26 BACs)	2.4 126.7-131.1	<i>de novo</i>	Yes		
50	der(2)(10qter->10q2?5::2p?5: :10q2?4::2p2?5->2qter),der(10) (10pter->10q24::18q2?3->18qter), der(18)(2pter->2p2?5::18p11.?2 ->18q?::18p11.?3->18pter)	del 18q21.1	(29 oligos)	0.24 51.19-51.34	<i>de novo</i>	Yes	250K SNP	FISH
51 <sup>34</sup>	der(6)t(6;9)(p21.3;q22),ins(6;13) (p21.3;q?21q31),der(9)t(6;9), der(13)ins(6;13)	del 13q21.33-q21.2	(68 BACs)	6.2 69.5-75.7	<i>de novo</i>	Yes	32K BAC	FISH and MLPA
		del 13q22.3	(13 BACs)	1.1 76.8-77.9	<i>de novo</i>	Yes		
53	der(2)t(2;7)(p21;q22),der(3) (7pter->7p13::3p26.3->	See also table 4B del 3q13.11q13.13	(53 BACs)	2.8 106.8-109.6	<i>de novo</i>	Yes	32k BAC	MLPA

3q21.3::11p13->11pter),der(7)  
 (2pter->2p23.3: :7p13->7q22:  
 :3q21.3->3qter),der(11)(3pter  
 ->3p26.3::2p12.2->2p23.3:  
 :11p13->11qter)

del 11p15.1 (3 BACs) 0.1 21.3-21.4 unknown ?

<b>B</b>	<b>Elsewhere in the genome</b>							
10	t(1;17)(p36.1;q11)	dup 1p34	(65 oligos)	0.65	unknown	?	244k Agilent	MLPA
16	t(2;10)(q22;q22.3)	del 5p13.1	(23 oligos)	45.67-46.32 0.27	unknown	?	244k Agilent	Illumina
18 <sup>28</sup>	t(2;14)(q37.3;q13)	dup 1p34.1p33	(10 oligos)	38.54-38.81 0.65	<i>de novo</i>	Yes <sup>28</sup>	244k Agilent	FISH
29	t(5;10)(q33;q25)	del 2q33.3q34	783 oligos)	45.86-46.51 4.90	<i>de novo</i>	Yes	500K SNP	FISH
		del 9q21.13q21.2	(32 oligos)	207.86-212.76 0.20	<i>de novo</i>	?		
		del 12p11.22	(104 oligos)	78.27-78.47 0.26	paternal	No		
30	t(5;17)(p15.3;q25.3)	del 16p13.11	(10 BACs)	29.98-30.24 0.9	maternal	Yes <sup>25,26</sup>	32k BAC	MLPA & FISH
32	t(6;9)(q21;p24)	dup 1q23.3	(34 SNPs)	15.4-16.3 0.24	maternal	No <sup>23</sup>	250K SNP	MLPA
51	der(6)t(6;9)(p21.3;q22),ins(6;13) (p21.3;q?21q31),der(9)t(6;9), der(13)ins(6;13)	del 1q21.1	(39 BACs)	160.75-160.99 1.5	paternal	Yes <sup>27</sup>	32K BAC	FISH and MLPA
		See also table 4A		144.7-146.3				

\* See discussion

# Because of the size of the deletion, no confirmation was performed