Clinical research

Submicroscopic chromosomal imbalances detected by array-CGH are a frequent cause of congenital heart defects in selected patients

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Aims Congenital heart defects (CHDs) are frequently caused by chromosomal imbalances, especially when associated with additional malformations, dysmorphism, or developmental delay. Only in a subset of such patients, a chromosomal aberration can be identified with current cytogenetic tests. Array Comparative Genomic Hybridization (Array-CGH) now enables the detection of submicroscopic chromosomal imbalances at high resolution. In this report, we evaluate for the first time the use of array-CGH as a diagnostic tool in a selected group of patients with a CHD.

Methods and results Sixty patients with a CHD of unknown cause but with features suggestive of a chromosomal aberration were selected. Array-CGH was performed using an in-house made 1 Mb micro-array.

Chromosomal imbalances not previously described as polymorphisms were detected in 18/60 patients (30%). Ten of these (17%) are considered to be causal. In three deletions, genes known to cause CHDs were implicated (NKX2.5, NOTCH1, NSD1, EHMT). One patient carried a duplication of chromosome 22q11.2, previously associated with CHD. In the other six patients, both the de novo occurrence as well as the size of the imbalance indicated causality. In addition, seven inherited aberrations unreported thus far were detected. Their causal relationship with CHDs remains to be established. Finally, a mosaic monosomy 7 was not considered as causal but did enable to make a diagnosis of Fanconi anaemia.

Conclusion This study shows that array-CGH is able to provide an etiological diagnosis in a large proportion of patients with a CHD, selected for a 'chromosomal phenotype'. Besides their usefulness in genetic counselling, identified chromosomal aberrations may aid in the medical follow-up of these individuals.

KEYWORDS
Congenital heart defects; Genetic tests; Chromosomes; Array-CGH

Introduction
Congenital heart defects (CHDs) are a major cause of mortality and morbidity, especially in individuals where the heart defect is associated with additional organ malformations. A precise etiological diagnosis can have important implications for treatment and follow-up, but also in counselling on recurrence risks. Chromosomal aberrations are a frequent cause of CHDs, especially when they are associated with growth or developmental delay, malformations affecting a second organ or dysmorphic features. However, with current cytogenetic techniques, a chromosomal aberration is identified only in a subset of such patients. This is in part explained by the limited resolution of standard karyotyping, which is unable to detect imbalances smaller than 5–10 megabases (Mb). Array Comparative Genomic Hybridization (array-CGH) is a novel technique for high-resolution, genome-wide screening for submicroscopic chromosomal imbalances. Recent studies demonstrated the ability of array-CGH to detect causal submicroscopic chromosomal imbalances in the range of a few megabases in 10–15% of patients with mental retardation and multiple congenital anomalies (reviewed in Menten et al.). Therefore, an increasing number of genetic laboratories are introducing this technique as a diagnostic tool. This study is the first to describe the use of array-CGH as a screening tool for the identification of cryptic chromosomal imbalances in selected patients with a CHD.

Methods
Patient selection and characterization
The patients were recruited from the congenital cardiology and genetic clinics of the University Hospital Leuven.
All patients had a CHD and additional features suggesting a chromosomal anomaly, i.e. (i) a second major malformation, and/or (ii) mental handicap or following special education and/or (iii) three or more minor physical anomalies.

In none of them, an etiological diagnosis could be reached after (i) expert dysmorphological examination, (ii) routine karyotyping using G-banding analysis at least at ISCN +550 bands, and (iii) the appropriate, additional investigations to exclude well-defined genetic disorders.

**Cytogenetic analysis**

Array-CGH was performed using an in-house made micro-array containing the clones from the 1 Mb BAC/PAC clone set generously donated by the Sanger Institute (Hinxton, UK), as described.5 Interphase and metaphase FISH and real-time quantitative (RTQ-)PCR were performed as described.5 An additional RTQ-PCR analysis was performed on patient 3 to confirm deletion of the NKX2.5 gene. To precisely delineate the detected imbalances, additional FISH studies were performed for patient 10, additional RTQ-PCR analyses for patient 16 (RTQ-PCR primers: see Table 1), and additional array-CGH analyses for patients 7, 8, and 18 using arrays containing a full tiling path clone set for either chromosome 14, 19, or X.6

The study was approved by the Ethics Committee at the University Hospital Leuven. Appropriate informed consent was obtained from all participating patients or their legal representatives. This study complies with the Declaration of Helsinki.

**Results**

Array-CGH revealed copy number variations in most of the 60 patients, ranging in size from 0.15 to 14 Mb. The vast majority of these are listed as phenotypically indifferent polymorphisms (http://projects.tcag.ca/variation/)7,8 and were not further investigated. Eighteen patients (30%) carried imbalances that are undescribed in phenotypically normal individuals, with the exception of the duplication of chromosome 22q11.2 observed in patient 9 (Tables 2 and 3). All imbalances were confirmed using an independent technique: FISH or RTQ-PCR.

The de novo nature of these imbalances was investigated through parental analysis using FISH or RTQ-PCR, except for patients 4, 14, and 15. In eight patients, the imbalance was shown to have occurred de novo. In six patients, it was inherited (Tables 2 and 3); for these six patients, the parents were phenotypically normal, with the exception of the father of patient 9 who carried the duplication in chromosome 22q11.2 and had learning difficulties.

Patient 4 carried a terminal deletion of chromosome 5q, extending to the Sotos syndrome region.9,10 His phenotype, with macrocephaly, large anterior fontanel, widely spaced nipples, and low set ears corresponds to the phenotype of terminal 5q deletions, which include the Sotos syndrome gene NSD1.10 His parents were reportedly normal, but parental samples were unavailable. Given the severity and high penetrance of Sotos syndrome and of the phenotype associated with terminal 5q deletions, chances that this deletion was inherited are low.

In patient 18, a mosaic monosomy 7, initially detected by array-CGH, was confirmed by interphase FISH to be present in 8% of white blood cells, and not found in fibroblasts and buccal smear cells. The de novo nature of this imbalance was therefore not an issue.7 This newborn presented with coarctation of the aorta, absent thumbs, hydrenephrosis, and severe prenatal growth retardation, features suggestive of Fanconi anaemia.11 Monosomy 7 is a frequent finding in Fanconi anaemia associated with myelodysplasia,11 and this was subsequently confirmed in this infant. This chromosomal aberration is not considered causal for the constitutional features, but did contribute to establishing a diagnosis.

In patient 13, the 11qter duplication was inherited from an unaffected parent. Inheritance of the same 11qter

| Table 1 Primers used for RTQ-PCR and result of RTQ-PCR analysis |
|------------------|------------------|--------------------|------------------|------------------|
| Clone name       | Location of the amplicon chromosome, start/ end [bp] | Forward primer | Reverse primer | Patient no. | Result of RTQ-PCR copy number in patient |
| CTC-281H14       | 5, 172 592 027–172 592 094 | AACACCTGATCCTCCCTGGATT | GCCGGTGGGACAGAAAA | 3 | 1 |
| CTC-3032J10      | 19, 017 666 809–017 666 778 | TCAGTGGCGGTCGGCAAA | TCCCTTGCAAGCTGGATGTG | 8 | 3 |
| RP11-203G14      | 19, 015 025 603–015 025 669 | CAGTTGTGCTCTGTGTTACTC | CTCCTCCATTTCCTCCCTTGG | 8 | 2 |
| RP11-872H1       | 19, 018 641 817–018 641 878 | CCTCTCAATTTGCCTCTTGG | GTTACGGTTCGCCCTCACAT | 8 | 4 |
| RP1-85M6         | 11, 033 044 046–033 044 118 | TGCAGGCGCCAGAAAAT | GTTAGCAAATTCCTACATCATCTT | 12 | 3 |
| RP11-469N6       | 11, 134 003 932–134 003 996 | GTGACCCCAGGCAGCACA | CTGTTGCAAGGTTGCTGATGA | 13, 14, 15 | 3 |
| CTC-459H11       | 16, 015 063 108–015 063 174 | GACGGATTTCGAGTAGTTC | GGGACTGGGTGTGATGACAA | 16 | 2 |
| RP11-109D4       | 16, 018 701 665–018 701 734 | GGGCGACGCAGTG | TTCCTTCTGGCTGATTAGTCCTT | 16 | 2 |
| RP1-292H14       | X, 029 059 049–029 059 123 | CACATCATGCGACAGCTGAA | TGGACTGTGGTCATGTCAGACA | 17 | 2 |
Table 2  Detected causal chromosomal aberrations, phenotype, and causality evaluation of the proband

<table>
<thead>
<tr>
<th>No.</th>
<th>Short karyotype</th>
<th>ISCN karyotype</th>
<th>Flanking normal clone(s)</th>
<th>Cardiac defect</th>
<th>Associated anomalies</th>
<th>de novo</th>
<th>Min–max size (Mb)</th>
<th>Min–max number of affected genes</th>
<th>Reports of similar patients</th>
<th>Known haplo-insufficient or dosage-sensitive genes</th>
<th>Causality criteria fulfilled</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>dup(4)(q34)</td>
<td>46,XY.ish(der4)(4pter→4q28::4q33→4q28::4q32.2→4q22.2::4q34.1→4q35.1→4qter)</td>
<td>f</td>
<td>AS</td>
<td>Cleft palate, dysmorphism, MR</td>
<td>Y</td>
<td>0.35, 12.90</td>
<td>1, 41</td>
<td>29</td>
<td>FBN2 (Beals syndrome)²⁷</td>
<td>3/4</td>
</tr>
<tr>
<td>2</td>
<td>del(5)(q23q23)</td>
<td>46,XX arr cgh 5q23q23 (CTC-461G12→RP1-241C15)x1</td>
<td>ASD</td>
<td></td>
<td></td>
<td>Y</td>
<td>13.97–14.40</td>
<td>68–78</td>
<td>30</td>
<td></td>
<td>4/4</td>
</tr>
<tr>
<td>3</td>
<td>del(5)(q35.1q35.1)</td>
<td>46,XX arr cgh 5q35.1 (RP1-20022→CTB-549F1)x1</td>
<td>ToF</td>
<td>Microcephaly, bronchomalacia, dysmorphism (+)</td>
<td></td>
<td>Y</td>
<td>1.77–4.00</td>
<td>28–42</td>
<td>31</td>
<td>NKX2.5 (CHD)³³</td>
<td>4/4</td>
</tr>
<tr>
<td>4</td>
<td>del(5)(q35.3qter)</td>
<td>46,XY arr cgh 5q35.3qter (CTB-87L24→CTB-240G13)x1</td>
<td>CTC-355H1</td>
<td>ToF</td>
<td>MRD, dysmorphism, (+)</td>
<td>ND</td>
<td>5.00–5.72</td>
<td>118–130</td>
<td>10</td>
<td>NSD1 (Sotos syndrome, CHD)²⁸</td>
<td>3/4</td>
</tr>
<tr>
<td>5</td>
<td>del(9)(q34.3) dup(20)(q13.33)</td>
<td>46,XX arr cgh 9q34.3qter (RP11-135P4 RP1-719C8)x1</td>
<td>RP11-153P4</td>
<td>Coarctation aorta (aortic valve normal)</td>
<td></td>
<td>Y</td>
<td>3.20–4.86</td>
<td>5.66–5.78</td>
<td>32</td>
<td>NOTCH1 (CHD)³⁴</td>
<td>4/4</td>
</tr>
<tr>
<td>6</td>
<td>del(13)(q21.31) del(13)(q31) dup(13)(q22q33)</td>
<td>46,XX.ish(der13)(13pter→13q21.31:13q31.1→13q31.1:13q33.3→13q31.3:13q33.1→13qter)</td>
<td>e</td>
<td>ToF</td>
<td>Microcephaly, dysmorphism, MR</td>
<td></td>
<td>0.15–2.06, 9.08–11.47, 14.03–16.22</td>
<td>1–2, 32–36, 101–105</td>
<td>33</td>
<td></td>
<td>3/4</td>
</tr>
<tr>
<td>7</td>
<td>del(14)(q22.1q23.1)</td>
<td>46,XX.arr cgh 14q22.1q23.1 (RP11-262A8→RP11-246G6)x1</td>
<td>RP11-121P6</td>
<td>TGA</td>
<td>Microtia, anophthalmia, polydactyly, (+)</td>
<td></td>
<td>6.70</td>
<td>61</td>
<td>34</td>
<td>OTX2 (anophthalmia)³⁶</td>
<td>4/4</td>
</tr>
<tr>
<td>9</td>
<td>dup(22)(q11.2q11.2)</td>
<td>46,XX arr cgh 22q11.2q11.2 (RP11-151G10 RBK-144D3)x3</td>
<td>RP11-652D6</td>
<td>Truncus arteriosus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>dup(22)(q12.2q12.2)</td>
<td>46,XX arr cgh 22q12.2q12.2 (RP11-664C16→RP11-794O4)x1</td>
<td>RP11-1036C6</td>
<td>TGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

 Patients 3 and 5 were also included in the study by Menten et al.³⁵

*CLP, cleft lip and palate; MR, mental retardation; MRD, multicystic renal dysplasia; VPI, velopharyngeal insufficiency; (+), deceased neonatally.

²AS, aortic valve stenosis; ASD, atrial septum defect; AVSD, atrioventricular septal defect; ToF, tetralogy of Fallot; TGA, transposition of the great arteries; VSD, ventricular septum defect.

³Y, yes; P, paternally inherited; ND, not determined.

⁴Number of Ensembl gene IDs assigned to this region (Ensembl version 38).

⁵Genes known to cause a phenotype when respectively deleted or duplicated.

⁶Cytogenetic characterization was described in Thiengo et al.³⁶

⁷The deletion of 13q21.31q21.31 was flanked by RP11-359P14 and RP11-67L17, the deletion of 13q31.1q31.3 was flanked by RP11-533P8 and RP11-632L2, the duplication of 13q31.3q33.3 was flanked by RP11-95C14 and RP11-513N16.
<table>
<thead>
<tr>
<th>No.</th>
<th>Short karyotype</th>
<th>ISCN karyotype</th>
<th>Flanking normal clone(s)</th>
<th>Cardiac defect(^a)</th>
<th>Associated anomalies(^b)</th>
<th>(\text{de novo})(^c)</th>
<th>Min–max size (Mb)</th>
<th>Min–max number of affected genes(^d)</th>
<th>Reports of similar patients</th>
<th>Known haplo-insufficient or dosage-sensitive genes(^e)</th>
<th>Causality criteria fulfilled</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>del(9)(p22.3p22.2)</td>
<td>46,XY arr cgh 9p22.2 (RP11-132E11)x1</td>
<td>R11-109M15 RP11-123J20</td>
<td>VSD</td>
<td>Cleft palate</td>
<td>P</td>
<td>1.51</td>
<td>5-5</td>
<td>0/4</td>
<td>1.51</td>
<td>5-5</td>
</tr>
<tr>
<td>12</td>
<td>dup(11)(p13p13)</td>
<td>46,XY arr cgh 11p13p13 (RP1-85M6)x3</td>
<td>RP11-4809 RP1-316D7</td>
<td>IAA type A</td>
<td>Dysmorphism, MR</td>
<td>P</td>
<td>0.14-0.62</td>
<td>5-5</td>
<td>0/4</td>
<td>0.14-0.62</td>
<td>5-5</td>
</tr>
<tr>
<td>13</td>
<td>dup(11)(q25qter)</td>
<td>46,XX arr cgh 11q25qter (RP11-469N6)x3</td>
<td>RP11-149G17</td>
<td>PDA</td>
<td>Unilateral hemimelia leg, hip dysplasia,</td>
<td>P</td>
<td>0.15-0.75</td>
<td>2-5</td>
<td>0/4</td>
<td>0.15-0.75</td>
<td>2-5</td>
</tr>
<tr>
<td>14</td>
<td>dup(11)(q25qter)</td>
<td>46,XY arr cgh 11q25qter (RP11-469N6)x3</td>
<td>RP11-149G17</td>
<td>AVSD</td>
<td>Eye coloboma, lung sequestration, dysmorphism, (+)</td>
<td>ND</td>
<td>0.15-0.75</td>
<td>2-5</td>
<td>0/4</td>
<td>0.15-0.75</td>
<td>2-5</td>
</tr>
<tr>
<td>15</td>
<td>dup(11)(q25qter)</td>
<td>46,XY arr cgh 11q25qter (RP11-469N6)x3</td>
<td>RP11-149G17</td>
<td>Coarctation aorta</td>
<td>Microcephaly, hypospadias, MR Coloboma, renal agenesis, choanal atresia,</td>
<td>ND</td>
<td>0.15-0.75</td>
<td>2-5</td>
<td>0/4</td>
<td>0.15-0.75</td>
<td>2-5</td>
</tr>
<tr>
<td>16</td>
<td>dup(16)(p13.11p12.3)</td>
<td>46,XX arr cgh 16p13.11p12.3 (RP11-489O1—RP11-288I3)x3</td>
<td>RP11-82D18 RP11-489A11</td>
<td>ASD</td>
<td></td>
<td>M</td>
<td>1.81-3.64</td>
<td>16-17</td>
<td>0/4</td>
<td>1.81-3.64</td>
<td>16-17</td>
</tr>
<tr>
<td>17</td>
<td>dup(X)(p21.3p21.3)</td>
<td>46,XY arr cgh Xp21.3 (RP11-37E19—RP6-27C10)x2</td>
<td>RP11-169J21 RP4-662N3</td>
<td>Heterotaxy</td>
<td>Microcephaly, epilepsy, MR Absent thumbs, hydrenephrosis, severe prenatal growth retardation</td>
<td>M(^f)</td>
<td>0.75</td>
<td>1-1</td>
<td>0/4</td>
<td>0.75</td>
<td>1-1</td>
</tr>
<tr>
<td>18</td>
<td>del(7)(pterqter) mosaic 8%</td>
<td>46,XX arr cgh del(7)(pterqter).ish 46,XX[92]/45,XX.-7[8]</td>
<td>RP11-169J21 RP4-662N3</td>
<td>Coarctation aorta</td>
<td>Microcephaly, epilepsy, MR Absent thumbs, hydrenephrosis, severe prenatal growth retardation</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Patients 17 and 18 were also included in the study by Menten et al.\(^5\) The aberration in patient 18 was considered not causal (see results section).

\(^a\)IAA, interrupted aortic arch; PDA, persistent ductus arteriosus; VSD, ventricular septum defect.

\(^b\)CHD7-2 no mutation in the CHD7 gene detected.

\(^c\)M, maternally inherited; P, paternally inherited; ND, not determined.

\(^d\)Number of Ensembl gene IDs assigned to this region (Ensembl version 38).

\(^e\)Genes known to cause a phenotype when respectively deleted or duplicated.

\(^f\)X-inactivation in the mother of patient 17 was not skewed.

\(^g\)This region shows partial overlap with a known polymorphic region. However, this imbalance was confirmed using RTQ-PCR primers targeted to a region not shown to be polymorphic thus far.
duplication detected in patients 14 and 15 was not studied (Table 3).

Discussion

Incidence and causality of cryptic chromosomal imbalances in patients with CHD and additional anomalies identified using array-CGH

Our results indicate that array-CGH can lead to an etiological diagnosis in a high proportion of selected patients with a CHD and additional features suggestive of a chromosomal aberration. However, at the present time, the interpretation of a detected imbalance is not always straightforward, given the presence in the human genome of a high level of copy number variations. Traditionally, the causality of a chromosomal aberration can be evaluated using several arguments (after exclusion of known polymorphisms) including (1) a gene in the deleted or duplicated region is known to cause CHDs or another dominant monogenic disorder through a dosage effect, (2) a patient with a similar chromosomal imbalance and a similar phenotype has previously been described, (3) the aberration occurred de novo, and (4) the size of the imbalance.

1. A gene, known to cause CHDs or another dominant monogenic disease through a dosage effect, is deleted or duplicated.

The precise delineation of a chromosomal aberration by array-CGH allows the identification of all affected genes. When haplo-insufficiency or over-expression of a gene located within the chromosomal imbalance is known to cause a distinct phenotype, the presence of this phenotype in the patient is a very strong argument in favour of a causal aberration. For this reason, RTQ-PCR was performed on patient 3 to confirm the deletion of NKK2.5, a gene known to be involved in CHDs. In two additional patients, genes known to cause CHDs were deleted: NSD1 (patient 4) and NOTCH1 and EHMT (patient 5).

Three patients carry a deletion that includes a gene causing a distinct phenotype different from CHDs when hemizygous, e.g. patient 7 had anophthalmia, and the deletion on chromosome 14q22.1q23.1 included the OTX2 gene, known to be involved in anophthalmia (Table 2). The same phenotype is present in other individuals carrying a chromosomal imbalance in the same chromosomal region.

This criterion partially overlaps with the previous one. For instance, a submicroscopic deletion or duplication of chromosome 22q11.2 in multiple patients with heart defects led to the identification of the deletion 22q11 (DiGeorge) syndrome or the duplication 22q11 syndrome. In total, nine patients in this study presented distinct phenotypic manifestations previously reported in other individuals with a chromosomal imbalance in the same chromosomal region (Table 2).

3. The aberration occurred de novo.

In traditional cytogenetics, de novo chromosomal imbalances detected by routine karyotyping are large, encompass in general hundreds of genes, and are generally accepted to explain the patient’s phenotype. At the present time, it is not certain whether this also applies to the smaller aberrations detected by array-CGH, and the frequency at which de novo aberrations occur in phenotypically normal individuals is not exactly known. Recently, it was found that copy number polymorphisms in genomic regions flanked by low copy repeats are typically inherited polymorphisms.

Data from deletions and duplications in the Duchenne muscular dystrophy gene, which has a size of 2.5 Mb, suggest that one in eight newborns carries a de novo imbalance of several kilobases. It is therefore likely that some de novo chromosomal imbalances will be phenotypically irrelevant. However, the majority of these imbalances are beyond the resolution of our BAC/PAC micro-array, and it is thus unlikely that the high frequency of de novo imbalances in the patients we studied is reflecting the naturally occurring rate of de novo phenotypically irrelevant imbalances. In conclusion, although the argument is still strong, the de novo nature of an imbalance can no longer be sufficient for considering an aberration as causal. We identified de novo imbalances in eight patients in this study (Table 2).

The duplication of chromosome 22q11.2 detected in patient 9 illustrates that the de novo nature of an imbalance is not a condictio sine qua non for its causality: in many instances this duplication is inherited from phenotypically ‘normal’ or mildly affected parents. As has been observed for nucleotide changes (e.g. mutations in THRAP2 or in NKK2.5), it is known that the heart defects associated with certain chromosomal imbalances will show reduced penetrance or variable expressivity (as seen in the del22q11.2 or DiGeorge syndrome, and the del7q11 or Williams syndrome). Studying larger groups of patients and controls is then necessary to establish causality, penetrance, and extent of the phenotypic spectrum of the imbalance. Whether the inherited imbalances described here (Table 3) represent new susceptibility loci or modifiers for CHDs, as described for instance for VEGF polymorphisms in patients with a conotruncal heart defect or with a deletion of 22q11.2, or if they represent non-functional rare variants, remains to be determined.

4. The size of the aberration.

As discussed before, the size of the chromosomal imbalance does matter. In one study, the size of de novo aberrations detected in patients (median 2.74 Mb) was significantly larger than inherited aberrations (median size 0.43 Mb). In the present study, imbalances not yet detected in normal individuals have a median size between 5.85 and 6.21 Mb (minimal and maximal size of the median aberration), compared to a median size between 0.15 and 1.35 Mb for imbalances detected in normal individuals.

This size difference most likely reflects the increasing chance of affecting a dosage-sensitive gene with an increasing aberration size. The median number of genes implicated in the de novo aberrations we detected in this study (Table 2) was between 65 and 70, compared to five genes in the five previously undescribed inherited aberrations (Table 3). The largest imbalance detected in a normal individual (the mother of patient 16) in the present study was maximally 3.7 Mb, affecting 17 genes at most. This individual is currently 42 years old, and never experienced cardiovascular manifestations. A recent medical examination did not reveal any manifestations of a heart defect. The largest imbalance inherited from a normal individual in a previous study was 3.8 Mb, affecting 10 genes. Based on these data, the number of affected genes can be included as a criterion in the evaluation of causality, with a proposed threshold of 20 affected genes. By no means do we claim...
that imbalances affecting more genes will never be detected in normal individuals and therefore, the criterion of 20 genes needs to be confirmed in future studies.

Taken together, in 10/60 patients we considered the identified aberration to be causal (17%; binomial distribution 95% CI: 8–29%) (Table 2). Seven other aberrations were not previously described as polymorphisms (Table 3) but were also carried by a healthy parent and did not score positive on any of the other three criteria. For this reason, we consider them as not being causal, but representing rare variants.

Clinical importance of molecular karyotyping

The high resolution of array-CGH offers the additional opportunity to delineate the aberrant chromosomal region with high accuracy. For certain aberrations, where characterized genes are implicated, this enables a better assessment of the patients’ prognosis and at times a person-alized clinical follow-up. Patient 10 carries a deletion of chromosome 22q12.2, including the FBN2 gene. Hemizygosity of the NF2 locus causes Neurofibromatosis type 2.26 Diagnosis of the microdeletion in this patient at age 15 years led to the detection of bilateral acoustic neurenomas before clinical manifestation. Patient 2 carries a deletion of chromosome 5q23, including the FBN2 gene. FBN2 haplo-insufficiency causes congenital contractual arachnodactyly (Beals syndrome).27 Since this syndrome is associated with an aortic root dilatation in about 15% of cases,28 this patient will need follow-up for this. Finally, patient 3 carries a deletion of chromosome 5q35.1, encompassing the NKX2.5 gene. NKX2.5 haplo-insufficiency causes CHDs and an increased risk for AV-block.13 These examples demonstrate the usefulness of a precise delineation of the aberrant chromosomal region for patient management.

In conclusion, the present study illustrates that array-CGH allows the detection of a high number of clinically significant chromosomal imbalances in individuals with syndromic CHDs. However, the interpretation of the imbalances detected must be done with caution, using several arguments, including parental genotypic and phenotypic analysis, and therefore requires a close interaction between the cardiologist, clinical geneticist, and cytogeneticist. If these limitations are taken into account, the technique will offer novel opportunities in the diagnostics of patients with a congenital heart defect and a syndromic phenotype.

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References


Submicroscopic chromosomal imbalances are a cause of CHDs


