Cytogenetic and Genome Research

Cytogenet Genome Res 2011;135:251–259
DOI: 10.1159/000331272

Published online: September 13, 2011

Challenges of Interpreting Copy Number Variation in Syndromic and Non-Syndromic Congenital Heart Defects

J. Breckpot[a] B. Thienpont[b] Y. Arens[c] L.C. Tranchevent[d] J.R. Vermeesch[a]
Y. Moreau[d] M. Gewillig[e] K. Devriendt[a]

[a]Center for Human Genetics, University Hospital Leuven, Leuven, Belgium; [b]Laboratory of Molecular Signalling and Laboratory of Developmental Genetics and Imprinting, Babraham Research Campus, Cambridge, UK; [c]Clinical Genetics, Cardiovascular Research Institute Maastricht, Academic Hospital Maastricht and Maastricht University, Maastricht, The Netherlands; [d]Bioinformatics Group, Department of Electrical Engineering, ESAT-SCD, Katholieke Universiteit Leuven, and [e]Department of Pediatric Cardiology, University Hospital Leuven, Leuven, Belgium

Key Words
Algorithm · Array CGH · Congenital heart defects (CHD) · Copy number variation (CNV) · Non-syndromic CHD

Abstract
Array comparative genomic hybridization (aCGH) has led to an increased detection of causal chromosomal imbalances in individuals with congenital heart defects (CHD). The introduction of aCGH as a diagnostic tool in a clinical cardiogenetic setting entails numerous challenges. Based on our own experience as well as those of others described in the literature, we outline the state of the art and attempt to answer a number of outstanding questions such as the detection frequency of causal imbalances in different patient populations, the added value of higher-resolution arrays, and the existence of predictive factors in syndromic cases. We introduce a step-by-step approach for clinical interpretation of copy number variants (CNV) detected in CHD, which is primarily based on gene content and overlap with known chromosomal syndromes, rather than on CNV inheritance and size. Based on this algorithm, we have reclassified the detected aberrations in aCGH studies for their causality for syndromic and non-syndromic CHD. From this literature overview, supplemented with own investigations in a cohort of 46 sporadic patients with severe non-syndromic CHD, it seems clear that the frequency of causal CNVs in non-syndromic CHD populations is lower than that in syndromic CNV populations (3.6 vs. 19%). Moreover, causal CNVs in non-syndromic CHD mostly involve imbalances with a moderate effect size and reduced penetrance, whereas the majority of causal imbalances in syndromic CHD consistently affects human development and significantly reduces reproductive fitness.
Copyright © 2011 S. Karger AG, Basel

Congenital heart disease (CHD) is a major cause of infant morbidity and mortality. The clinical outcome is largely dependent on the severity of the defect, the presence of extracardiac anomalies, and surgical complications. Congenital heart defects arise from an abnormal heart development, induced either by environmental influences [Jenkins et al., 2007], by an altered gene dosage or function [Bruneau, 2008], by stochastic factors [Kurnit et al., 1987], or by combinations thereof. CHD patients with a second major anomaly, developmental delay, or dysmorphism are considered syndromic and represent an

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2011 S. Karger AG, Basel
1424–8581/11/1354–0251$38.00/0

Accessible online at:
www.karger.com/cgr

Koenraad Devriendt
Center for Human Genetics, University Hospitals Leuven
Herestraat 49, bus 602
BE–3000 Leuven (Belgium)
Tel. +32 16 34 59 03, E-Mail Koenraad.Devriendt@uzleuven.be

estimated 22% of the total cohort [Oyen et al., 2009]. Human genetic studies have implicated mutations of numerous genes and chromosomal regions in isolated or syndromic CHD, which we have compiled in CHDWiki, a community repository based on Wiki-technology (http://homes.esat.kuleuven.be/~bioiuser/chdwiki/index.php/Main_Page) [Barriot et al., 2010]. Thus far, nucleotide mutations in over 20 genes have been implicated in sporadic or familial non-syndromic CHD and over twice as many in syndromic CHD. Array comparative genomic hybridization (aCGH) has lead to an increased detection of causal chromosomal imbalances in individuals with CHD. While the contribution of chromosomal imbalances to the genesis of syndromic CHD is now well established [Krepischi-Santos et al., 2006; Thienpont et al., 2007a; Richards et al., 2008; Breckpot et al., 2010], their implication in non-syndromic CHD is far less understood.

This review discusses some of the challenges that arise upon the introduction of aCGH as a diagnostic tool in a clinical cardiogenetic setting. Based on our own experience as well as those of others described in the literature, we outline the state of the art and attempt to answer a number of outstanding questions, such as the detection frequency of causal imbalances in different patient populations, the added value of higher-resolution arrays, the existence of predictive factors in syndromic cases and, importantly, decision trees used for the interpretation of detected imbalances.

Challenges of Copy Number Variant Interpretation

Interpretation of the clinical significance of copy number variants (CNVs) is challenging, as infrequent disease-causing copy number changes should be distinguished from the abundant CNVs without obvious major clinical significance. Initially, and even still today, many algorithms used to interpret molecular karyotyping results rely heavily on the de novo nature of chromosomal imbalances as a primary selection criterion for causality [Greenway et al., 2009; Koolen et al., 2009]. This criterion was erroneously extrapolated from experience with metaphase karyotyping, where it was noted that the large, cytogenetically visible (5–10 Mb) deletions or duplications under investigation were virtually absent from the normal population and arose de novo in nearly all patients in which they were detected.

Novel copy number profiling techniques such as aCGH revealed that this no longer holds true at higher resolution: several examples from literature have demonstrated that de novo CNVs found in patients are not necessarily causal [Itsara et al., 2010; Vermeesch et al., 2011], and imbalances >100 kb have shown to arise de novo in >1% of normal individuals [Sebat et al., 2007]. Nevertheless, de novo CNVs are found more frequently in some disease cohorts (such as syndromic CHDs) than in normal controls. Lack of inheritance is thus a valid yet insufficient argument in favor of causality.

At the flip-side of this coin, also inherited CNVs can cause a patient's phenotype [Mefford et al., 2008], typically in disorders with reduced penetrance and variable expressivity [Manolio et al., 2009]. This is similar to point mutations in many cardiac genes, such as *NKX2.5*, *GATA4*, and *NOTCH1* [Wessels and Willems, 2010]. Because of this reduced penetrance, clinically significant CNVs will also be found occasionally in the normal population [Mefford et al., 2008]. Nevertheless, common CNVs (copy number polymorphisms or CNPs) are unlikely to remain embedded in the normal population if they are under a significant selective pressure of debilitating or lethal diseases such as CHDs. In contrast to genome-wide association studies, aCGH in the clinical setting discussed here aims to identify rare or unique variants with a major phenotypic impact rather than frequent variants with minor effect sizes. Comparisons of a patient's CNVs with those frequently found in control populations are thus of interest to exclude CNPs from the list of potentially causal CNVs. Publicly available datasets, such as the Database of Genomic Variants, can be consulted for this purpose but should be interpreted with caution, as only a fraction of the catalogued CNVs have been validated [Lee et al., 2007]. We would argue that only CNPs that are reported to occur frequently (>1%) in the normal population in several independent studies should be discarded. Moreover, one should evaluate if the reported platform used to interrogate copy number states in the normal population is compatible with the platform used to investigate the patient. Ideally, an internal database of 'benign' CNVs is constructed from array experiments in healthy control individuals or from the non-transmitted alleles of the parents of patients if experiments are designed as trio analyses.

Besides reduced penetrance and variable expression, other issues such as the unmasking of recessive alleles [Breckpot et al., 2008], parental mosaicism, imprinting, and skewed X-inactivation [Thienpont et al., 2007b] should be considered when assessing any potentially pathogenic CNV. It is therefore clear that all rare CNVs are to be evaluated with respect to their role in the pathogenesis of a congenital defect such as CHD. Moreover, the

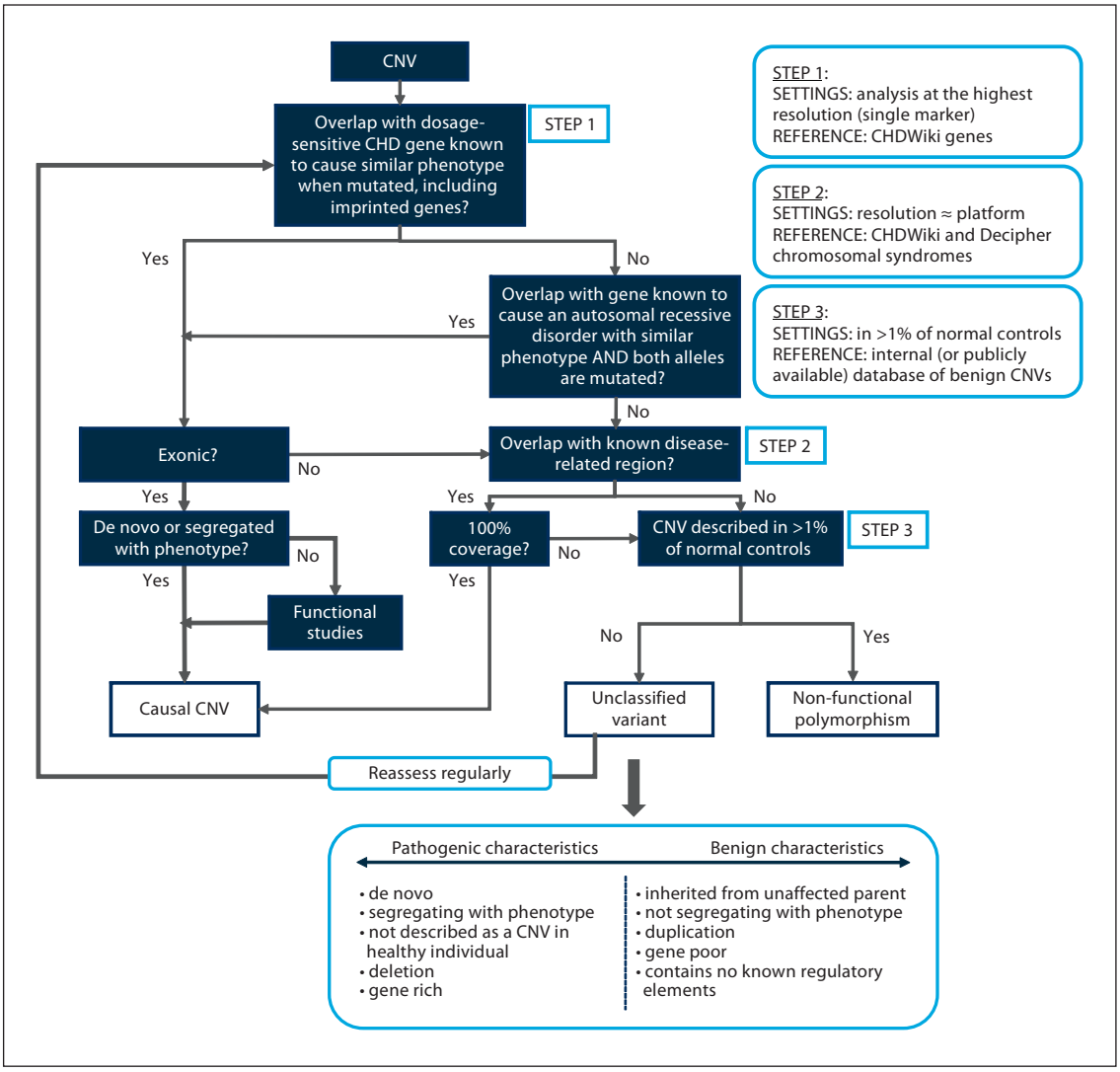


Fig. 1. Algorithm for interpretation of CNVs in patients with syndromic or non-syndromic CHD. Unclassified variants can be further ranked according to their probability of being causal, based on additional criteria such as a de novo origin, segregation in the family with the phenotype, size of the imbalance and gene count, and gene function of implicated genes.

decision tree to evaluate an imbalance – as any mutation – will differ depending on the clinical situation: sporadic or familial occurrence, syndromic or not.

We aimed to resolve these issues by introducing a step-by-step approach for CNV interpretation in CHDs, primarily based on gene content and overlap with known chromosomal syndromes (fig. 1).

STEP 1

Variants not recurrently found in normal individuals can be considered causal when they comprise dosage-sensitive genes known to cause CHD upon loss-of-function mutations or genes causing recessive forms of CHD when both alleles are mutated. They thus partially or fully explain the patient's phenotype. A list of such genes is compiled in CHDWiki. Higher-resolution array platforms will yield a larger amount of rare or unique CNVs per patient. Given the laborious nature of the ensuing confirmation experiments, array resolution will also impact the clinical interpretation algorithm and diagnostic work-up. When the intrinsic noise of a platform does not allow the reliable interpretation of single-probe altera-

tions, pooling of multiple array probes can be used to lower the false-positive ratio. However, array element pooling results in small potentially relevant CNVs escaping detection. One could thus envisage a hybrid strategy whereby in a first step the full set of aCGH data is analyzed using standard aberration calling thresholds, yielding a predefined number of false positives, depending on the diagnostic quality criteria required and available confirmatory analyses. Subsequently, a subset of the data overlapping with known cardiac genes is analyzed to identify CNVs with more definite clinical significance [Boone et al., 2010]. Thresholds for detection of such CNVs can be set lower, producing a higher ratio of false positives to true positives. Indeed, because the clinical significance of such CNVs is established, false negatives are diagnostically more costly.

STEP 2

Since several chromosomal imbalances exist for which the causal gene has not yet been identified, it is necessary to identify known chromosomal syndromes. We use the Decipher (https://decipher.sanger.ac.uk/application/syndrome) and CHDWiki (http://homes.esat.kuleuven.be/~bioiuser/chdwiki/index.php/CHD:Map) microdeletion and duplication syndromes as a reference set. As mentioned, all rare CNVs should be taken into account, irrespective of the nature of their inheritance. We propose that rare CNVs not affecting genes or regions known to be involved in CHDs should be regarded as unclassified and reassessed regularly as studies continue to elucidate the genetic basis of CHDs (step 3). As previously suggested for mutations detected at the base-pair level, unclassified variants can be further ranked according to their probability of being pathogenic, based on additional criteria such as a de novo origin, segregation in the family with the phenotype, size of the imbalance and gene count, and gene function of implicated genes [Breckpot et al., 2010].

Finally, algorithms can exceed the diagnostic level by implementing further steps to identify novel clinically relevant CNVs, potentially harboring novel candidate genes for CHDs. For this, in silico prioritization methods or animal models can be used. As an illustration, whole-mount in situ hybridization and subsequent morpholino knockdown in *Xenopus tropicalis* has been applied systematically on genes retrieved from rare CNVs in 262 heterotaxy patients and has uncovered 5 novel genes implicated in left-right body patterning [Fakhro et al., 2010].

Below, we discuss the application of aCGH on 2 patient populations: syndromic and isolated CHD. We describe the state-of-the-art and provide an overview of the studies performed thus far, supplemented with brief additional investigations where necessary to clarify matters further.

Array CGH as a Diagnostic Tool in Syndromic CHD

After clinical evaluation, standard karyotyping, and targeted mutation analysis or fluorescence in situ hybridization for clinical recognizable syndromes, a genetic diagnosis is reached in about 55% of individuals with syndromic CHD [Meberg et al., 2007; Oyen et al., 2009]. In the remainder, aCGH has led to an increased detection of causal chromosomal imbalances [Krepischi-Santos et al., 2006; Thienpont et al., 2007a; Richards et al., 2008; Breckpot et al., 2010]. The yield of aCGH studies in this population lies between 16 and 28%, excluding clinical recognizable 22q11 deletions (table 1). In a small subset of 7 patients with syndromic heart defects, Krepischi-Santos et al. [2006] detected 3 causal variants by means of a 1-Mb resolution array: a de novo 1p36 deletion, a clinical recognizable de novo 22q11 deletion, and a large 8q21 deletion of unknown inheritance. Using the same platform in our series of 150 patients [Thienpont et al., 2007a; Breckpot et al., 2010], causal CNVs were detected in 26 subjects (17%). Of the 22 CNVs originally described as unclassified variants, 9 were considered to be phenotypically indifferent polymorphisms during follow-up. Two higher-resolution studies using oligonucleotide arrays yielded comparable results: 5 causal CNVs, including 3 unbalanced translocations and 1 clinical recognizable 22q11 deletion, were detected in 20 syndromic heart patients [Richards et al., 2008], and 4 in 19 patients with tetralogy of Fallot and developmental delay [Rauch et al., 2010]. Based on our algorithm for CNV interpretation (fig. 1), we have reclassified the detected aberrations in each study for their causality for CHDs. The results are displayed in table 1. In addition, numerous unique or rare CNVs in syndromic heart patient are compiled in collaborative databases such as DECIPHER (https://decipher.sanger.ac.uk/), ECARUCA http://umcecaruca01.extern.umcn.nl:8080/ecaruca/ecaruca.jsp), and CHDWiki. Several known as well as novel regions recurrently linked to CHDs in patients emerge from the compiled genomic data. These provide an entry point for the identification of novel genes linked to human CHDs [Vissers et al., 2004; Kleefstra et al., 2006; Thienpont et al., 2010]. Moreover, the identification of genes implicated in the

Table 1. The yield of aCGH studies in syndromic CHD patients

Study	Platform	Patients	Causal CNV[a] (%)	Inheritance		22q11 del[b]	Yield (%)
				de novo	unknown		
Krepischi-Santos et al. [2006]	1 Mb BAC/PAC	7[c]	3/7 (42)	2	1	1/3	2/6 (33)
Thienpont et al. [2007a]	1 Mb BAC/PAC	60	10/60 (16.6)	8	1	0/10	10/60 (16.6)
Richards et al. [2008]	385k Nimblegen	20	5/20 (25)	3	2	1/5	4/19 (21)
Rauch et al. [2010]	100k Affymetrix	19	4/19 (21)	3	1	0/4	4/19 (21)
Breckpot et al. [2010]	1 Mb BAC/PAC	90	16/90 (17.7)	12	2	0/16	16/90 (17.7)
Breckpot et al. [2010]	244k Agilent	29[d]	2/29 (6.8)	2	0	0/2	2/29 (6.8)
Total		196	40/196 (20)	30	7	2/40	38/194 (19.5)

[a] Causal and unclassified CNVs were defined based on the CNV interpretation algorithm depicted in figure 1 (as of May 2011).
[b] Clinical recognizable 22q11 deletions were excluded (taken account of the age at presentation) as FISH is the gold standard for diagnosis.
[c] Subgroup of syndromic CHD within a total of 95 patients with developmental delay.
[d] Subgroup of 29 patients normal on BAC/PAC aCGH with 1-Mb resolution.

aberrant region can be of great prognostic value, necessitating or enabling a personalized clinical follow-up [Thienpont et al., 2007a].

An additional question is to what extent increased resolution offered by technological advances further increases the diagnostic pick-up rate [Richards et al., 2008; Breckpot et al., 2010; Wincent et al., 2011]. In our study, 29 syndromic heart patients, normal on 1-Mb aCGH, were reanalyzed by a 244k oligo-microarray with a resolution of 30–40 kb. We detected 75 variants not listed as clinically neutral polymorphisms, of which 2 were considered to be causal [Breckpot et al., 2010] (table 1). The low frequency of additional causal imbalances detected by higher-resolution aCGH contrasted sharply with the higher number of rare inherited variants that were detected and with the concomitant laborious evaluations. Similarly, Wincent et al. [2011] showed that, despite the higher effective resolution, the diagnostic yield of a 244k oligo-microarray was approximately equal to that of a 38k BAC array in a cohort of 160 subjects with developmental delay and multiple congenital anomalies. Moreover, no causal imbalances <300 kb were detected. This suggests that causal chromosomal imbalances in individuals with syndromic CHDs are typically large and thus detectable by lower-resolution aCGH (fig. 2). It also implies that, in individuals with a normal aCGH result, irrespective of the resolution, one should reconsider the possibility of a monogenic condition, as indicated by the fact that during clinical follow-up of our patient cohort, a monogenic disorder was diagnosed in 7% of individuals with a normal 1-Mb aCGH result [Breckpot et al., 2010].

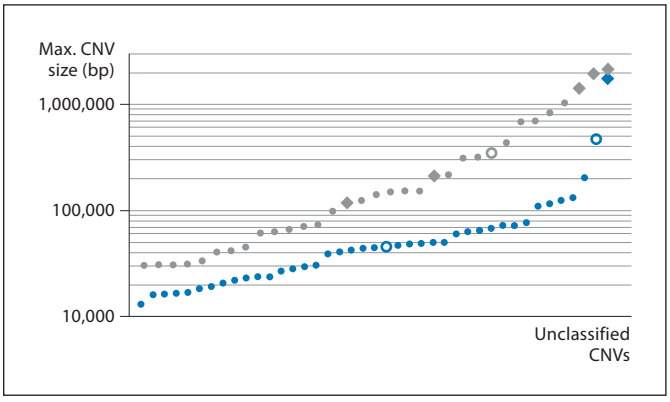


Fig. 2. Maximal sizes of unclassified CNVs detected by aCGH on Agilent 244k arrays. CNVs are sorted on the x-axis by their maximal size, which is shown on the y-axis (bp, $\log_{10}$ scale). In the graph, 41 deletions are indicated in blue, 34 duplications in grey. Empty dots indicate de novo aberrations and diamonds aberrations already detected by aCGH on 1-Mb arrays. This graph shows that most aberrations larger than 1 Mb were already detected on 1-Mb arrays, and that unreported duplications are in general larger than deletions. This difference can be partly explained by the lower detection threshold for deletions than for duplications. Duplications larger than 100 kb are much more frequent than deletions (19/34 vs. 7/41). Adapted from Breckpot et al. [2010].

Richards et al. [2008] showed that within a small cohort of 20 syndromic CHD patients some subpopulations are at higher risk for causal imbalance detection. Taking account of the limited sample size, the association of a neurologic abnormality, defined as either developmental

Table 2. The yield of aCGH studies in non-syndromic CHD patients

Study	Platform	Cases	Reported CNV (%)	Causal CNV[a] (%)	Inheritance		Syndromic[b]	Yield (%)
					de novo	unknown		
Erdogan et al. [2008]	tiling path BAC array	105	18/105 (17)	6/105 (4.7)	1	3	3/6	3/102 (2.9)
Richards et al. [2008]	385k Nimblegen	20	3/20 (0)	0/20 (0)	0	0	0	0/20 (0)
Greenway et al. [2009]	Affymetrix SNP 6.0	114	11/114 (9.6)	6/114 (5.3)	6	0	1/6	5/113 (4.4)[c]
This study	Affymetrix SNP 6.0	46	2/46 (4.3)	2/46 (4.3)	2	0	0/2	2/46 (4.3)
Total		285	33/285 (11.6)	14/285 (4.9)	9	3	4/14	10/281 (3.6)

[a] Causal and unclassified CNVs were defined based on the CNV interpretation algorithm depicted in figure 1 (as of May 2011).
[b] Causal CNVs retrospectively associated with syndromic features were excluded.
[c] Including 2 common 22q11 deletions without additional congenital defects.

delay or a structural brain anomaly, resulted in a greater probability of a causal imbalance when compared with other types of extracardiac defects or with isolated CHD. Within a cohort of 150 syndromic CHD cases assessed by 1-Mb aCGH, systematic comparison of the clinical features of 26 patients with a causal imbalance to the remaining 124 patients revealed the number of dysmorphic features as the only feature with a significant predictive value for detecting a causal CNV by 1-Mb aCGH (estimated odds ratio 1.322 with a 95% CI 1.107–1.579) [Breckpot et al., 2010]. The presence of dysmorphism should thus prompt the clinician to further genetic testing by aCGH. This formally confirms the clinical experience: clinical geneticists commonly use the term 'chromosomal phenotype' which includes the presence of multiple minor anomalies [de Vries et al., 2001; Hennekam, 2011]. Further prospective studies are required to confirm these findings.

Table 3. Patients' cardiac phenotypes

CHD type	n = 46
UVH (left/right ventricle)	29 (20/9)
AVSD	11
Critical LVOTO	6

AVSD = Atrioventricular septal defect; CHD = congenital heart defect; LVOTO = left ventricle outflow tract obstruction; UVH = univentricular heart. Full description of the patients' heart defects is available in supplementary table 1.

Array CGH in Non-Syndromic Sporadic CHD

The implication of chromosomal imbalances in non-syndromic CHD is far more controversial. The first study to address this issue was performed by Erdogan and colleagues [2008], which found de novo CNVs in 3 out of 105 patients with various CHDs; 2 of these were of unknown significance, while the third was a 17p11.2 deletion encompassing *RAI1*. They also reported on an additional 8 'rare' inherited CNVs, defined as being 'absent in 700 other aCGH analyses'; 6 of these were of unknown significance, while the remaining 2 were known to be associated with CHDs (a duplication of 22q11 and a deletion of 1q21). An additional 7 rare CNVs were of unknown inheritance; 5 of these were of unknown significance, while the remaining 2 were respectively a common 22q11 deletion and a distal 22q11 duplication. In the aforementioned study by Richards and colleagues [2008], no obvious disease-causing CNVs were detected in 20 patients with non-syndromic CHD. Finally, Greenway and colleagues [2009] reported that 11 out of 114 (10%) sporadic patients with non-syndromic tetralogy of Fallot carried de novo CNVs. These included 2 deletions of 22q11, a duplication of 1q21, and 3 imbalances affecting the genes *NOTCH1*, *JAG1,* and *RAF1*, respectively. An overview of these studies is depicted in table 2.

These studies thus yielded quite disparate numbers of de novo CNVs. The high frequency of pathogenic de novo CNVs in non-syndromic CHDs is rather surprising. This could be attributed to a historic ascertainment bias causing an overestimation of the frequency of syndromic features in patients carrying these CNVs. Alternatively, it could be due to inaccurate phenotyping of the studied patient cohorts. One could question whether de novo

Table 4. CNVs overlapping with known CHD-related genes

Proband	CHD	Gain/loss	Chromosome	Start	Size	Gene	Status	Reference	De novo?
P338, P339	UVH	gain	4q12	54,810,448	58 bp	*PDGFRA*	CNP	Bleyl et al. [2010]	no
P362, P894	UVH	loss	4q12	54,810,448	58 bp	*PDGFRA*	CNP	Bleyl et al. [2010]	no
P370	AVSD	loss	9q34.3	138,551,164	398 bp	*NOTCH1*	CNP	Garg et al. [2005]	unknown
P436	AVSD	gain	22q11.21	17,270,419	2.5 Mb	*TBX1*	causal	Yagi et al. [2003]	yes

AVSD = Atrioventricular septal defect; CNP = copy number polymorphism; UVH = univentricular heart.

Table 5. CNVs overlapping with known CHD-related genes or chromosomal syndromes

Proband	CHD	Gain/loss	Chromosome	Start	Size, Mb	Gene	Status	Reference	De novo?
P517	right UVH	gain	1q21.1	144,643,813	1.65	*GJA5*?	causal	Mefford et al. [2008]	yes
P436	AVSD	gain	22q11.21	17,270,419	2.52	*TBX1*	causal	Yagi et al. [2003]	yes

AVSD = Atrioventricular septal defect; UVH = univentricular heart.

CNV rates in the range of 10% are compatible with the mortality and inheritance rates documented in isolated CHD or tetralogy of Fallot populations, arguing against CNVs as being the sole cause for the high de novo rate or concomitant reduced reproductive fitness documented in these populations. Associated malformations or developmental delay (as in syndromic cases) may provide alternative explanations. Erdogan et al. [2008] indeed noted that the 17p11.2 deletion and the 22q11.2 deletion were retrospectively associated with syndromic features, and so was the large *RAF1* duplication reported by Greenway and colleagues [2009]. This suggests that at least some of these pathogenic CNVs were detected in – retrospectively – misclassified patients (table 2).

In order to attempt shedding some more light on these issues, we investigated the involvement of CNVs in a cohort of sporadic CHD cases. We took great care in the selection of non-syndromic cases, using stringent criteria and careful clinical examination of patients and both parents by an experienced clinician. We focused on complex or severe CHDs, hypothesizing that de novo CNVs or rare inherited variants with a substantial phenotypic impact occur primarily in severe and non-familial cases, as seen in non-syndromic neuropsychiatric disorders [Marshall et al., 2008; Stefansson et al., 2008]. High-resolution SNP array (Affymetrix® SNP 6.0) was performed in 50 subjects with non-syndromic CHD and in 99 controls without CHD (online suppl. table 1, www.karger.com/doi/10.1159/000331272). Four patients were excluded because of repeated experiment failure resulting from suboptimal DNA quality. Table 3 and online supplement table 1 depict the cardiac phenotypes of the remaining 46 patients (male/female: 32/14). We detected 3 CNVs covering or within a known CHD-related gene (table 4). A de novo duplication including the *TBX1* gene was detected in a girl with an atrioventricular septal defect. *TBX1* is required for normal development of the cardiac outflow tract in a gene dosage-dependent manner [Theveniau-Ruissy et al., 2008] and is considered responsible for the cardiac phenotype in the velo-cardio-facial syndrome [Yagi et al., 2003] as well as in the reciprocal duplication syndrome [Yobb et al., 2005; Portnoi, 2009]. In another patient an intronic deletion in *NOTCH1* was detected. Mutations in *NOTCH1* cause non-syndromic left ventricle outflow tract obstruction [Garg et al., 2005]. However, since this deletion was described as a common variant in normal controls [Jakobsson et al., 2008] and given its intronic position, this CNV is unlikely to have major functional consequences, even though a small effect in a multifactorial model cannot be excluded by this study. A 58-bp intronic variant of the *PDGFRA* gene was found either deleted or duplicated in 4 unrelated patients. Knockdown of *PDGFRA* in chicken and mouse results in a spectrum of inflow tract defects including totally abnormal pulmonary venous return (TAPVR). In humans, one *PDGFRA* variant with reduced penetrance was found in 2 unrelated

TAPVR patients [Bleyl et al., 2010]. Thus far, this region has not been described as a polymorphism, but we detected 19 deletions and 4 duplications of this area in our control cohort. Therefore, this variant is unlikely to contribute to the cardiac phenotype in these patients.

We detected 2 de novo duplications in this patient cohort. As described above, a 22q11.2 duplication was detected in a girl with an atrioventricular septal defect. Another duplication of chromosome 1q21.1 was detected in a patient with a univentricular heart (table 5). Duplications of the 1q21.1 region were found to be enriched in persons with various developmental disorders, including CHD [Christiansen et al., 2004; Mefford et al., 2008; Greenway et al., 2009]. Such duplications were also described in patients with univentricular [Mefford et al., 2008] or complex heart disease [Brunetti-Pierri et al., 2008], in association with developmental delay or autism. The present patient developed normally and showed no autistic features. The *GJA5* gene (also known as *Cx40*) is considered to be a candidate gene for CHD within this region, since absence or reduced expression of *GJA5* increases the probability of cardiac malformations in mice [Gu et al., 2003]. In our study, 2 out of 46 isolated CHD patients (4%) were thus found to carry de novo CNVs.

Conclusions

Based on the compiled data in table 1 and table 2, it can be observed that the frequency of causal CNVs in non-syndromic CHD populations is lower than that in syndromic CHD populations. Moreover, causal CNVs in non-syndromic CHD mostly involve chromosomal regions 22q11 and 1q21 (8/10). Such imbalances have an effect size that is likely to be lower than that of the other reported imbalances, since carriers do not always have manifestations [Mefford et al., 2008]. This reduced penetrance challenges genetic counseling. However, compared to complex disorders the probability of these clinical disorders is much higher, and therefore, the finding of such an imbalance in an individual with a heart defect is significant, even when inherited. In syndromic CHD genetic counseling is more straightforward, as the majority of causal imbalances consistently affects human development and significantly reduces reproductive fitness. In concordance with that, inheritance of causal CNVs in table 1 was described in only 3 out of 33 syndromic heart patients, for whom parental DNA was available: a duplication of Xq21 with 100% skewing in the unaffected mother, and, not surprisingly, duplications of 1q21.1 and 22q11.2 [Thienpont et al., 2007a; Breckpot et al., 2010].

Whether aCGH is warranted in a non-syndromic CHD population depends on local practical and financial constraints. Moreover, the clinical setting in which aCGH is to be used needs to be assessed. The age at which patients are tested as well as the quality of phenotyping, which is in part dependent on age, will influence the number of patients that receive an etiological diagnosis by aCGH. Although the frequency of detectable causal CNVs in a truly non-syndromic patient population may be low, the mixed population typically studied may present a far greater incidence of causal CNVs.

Acknowledgements

The authors wish to thank the patients and their family for their kind cooperation. Special thanks to Sigrun Jackmaert and Thierry Voet for sharing their experience with high resolution SNP arrays.

J.B. is an aspirant investigator and K.D. a senior clinical investigator of the FWO (Fonds voor Wetenschappelijk Onderzoek) – Flanders. This work was made possible in part by grants from the IWT (SBO-60848) and GOA/2012/015, and the SymBioSys Center of Excellence (KUL PFV/10/016 SymBioSys) to J.R.V. and K.D. CHDWiki is one of the work-packages of a European FP7 project CHeartED, which aims to identify genetic and environmental pathways involved in CHD (http://www.chearted.eu/).

References

Barriot R, Breckpot J, Thienpont B, Brohee S, Van Vooren S, et al: Collaboratively charting the gene-to-phenotype network of human congenital heart defects. Genome Med 2:16 (2010).

Bleyl SB, Saijoh Y, Bax NA, Gittenberger-de Groot AC, Wisse LJ, et al: Dysregulation of the *PDGFRA* gene causes inflow tract anomalies including TAPVR: integrating evidence from human genetics and model organisms. Hum Mol Genet 19:1286–1301 (2010).

Boone PM, Bacino CA, Shaw CA, Eng PA, Hixson PM, et al: Detection of clinically relevant exonic copy-number changes by array CGH. Hum Mutat 31:1326–1342 (2010).

Breckpot J, Takiyama Y, Thienpont B, Van Vooren S, Vermeesch JR, et al: A novel genomic disorder: a deletion of the *SACS* gene leading to spastic ataxia of Charlevoix-Saguenay. Eur J Hum Genet 16:1050–1054 (2008).

Breckpot J, Thienpont B, Peeters H, de Ravel T, Singer A, et al: Array comparative genomic hybridization as a diagnostic tool for syndromic heart defects. J Pediatr 156:810–817 (2010).
Bruneau BG: The developmental genetics of congenital heart disease. Nature 451:943–948 (2008).
Brunetti-Pierri N, Berg JS, Scaglia F, Belmont J, Bacino CA, et al: Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities. Nat Genet 40:1466–1471 (2008).
Christiansen J, Dyck JD, Elyas BG, Lilley M, Bamforth JS, et al: Chromosome 1q21.1 contiguous gene deletion is associated with congenital heart disease. Circ Res 94:1429–1435 (2004).
de Vries BB, White SM, Knight SJ, Regan R, Homfray T, et al: Clinical studies on submicroscopic subtelomeric rearrangements: a checklist. J Med Genet 38:145–150 (2001).
Erdogan F, Larsen LA, Zhang L, Tümer Z, Tommerup N, et al: High frequency of submicroscopic genomic aberrations detected by tiling path array comparative genome hybridisation in patients with isolated congenital heart disease. J Med Genet 45:704–709 (2008)
Fakhro KA, Choi M, Ware SM, Belmont JW, Towbin JA, et al: Rare copy number variations in congenital heart disease patients identify unique genes in left-right patterning. Proc Natl Acad Sci USA 108:2915–2920 (2010).
Garg V, Muth AN, Ransom JF, Schluterman MK, Barnes R, et al: Mutations in *NOTCH1* cause aortic valve disease. Nature 437:270–274 (2005).
Greenway SC, Pereira AC, Lin JC, DePalma SR, Israel SJ, et al: De novo copy number variants identify new genes and loci in isolated sporadic tetralogy of Fallot. Nat Genet 41:931–935 (2009).
Gu H, Smith FC, Taffet SM, Delmar M: High incidence of cardiac malformations in connexin40-deficient mice. Circ Res 93:201–206 (2003).
Hennekam RC: A newborn with unusual morphology: some practical aspects. Semin Fetal Neonatal Med 16:109–113 (2011).
Itsara A, Wu H, Smith JD, Nickerson DA, Romieu I, et al: De novo rates and selection of large copy number variation. Genome Res 20:1469–1481 (2010).
Jakobsson M, Scholz SW, Scheet P, Gibbs JR, VanLiere JM, et al: Genotype, haplotype and copy-number variation in worldwide human populations. Nature 451:998–1003 (2008).
Jenkins KJ, Correa A, Feinstein JA, Botto L, Britt AE, et al: Noninherited risk factors and congenital cardiovascular defects: current knowledge: a scientific statement from the American Heart Association Council on Cardiovascular Disease in the Young: endorsed by the American Academy of Pediatrics. Circulation 115:2995–3014 (2007).
Kleefstra T, Brunner HG, Amiel J, Oudakker AR, Nillesen WM, et al: Loss-of-function mutations in euchromatin histone methyl transferase 1 *(EHMT1)* cause the 9q34 subtelomeric deletion syndrome. Am J Hum Genet 79:370–377 (2006).
Koolen DA, Pfundt R, de Leeuw N, Hehir-Kwa JY, Nillesen WM, et al: Genomic microarrays in mental retardation: a practical workflow for diagnostic applications. Hum Mutat 30:283–292 (2009).
Krepischi-Santos AC, Vianna-Morgante AM, Jehee FS, Passos-Bueno MR, Knijnenburg J, et al: Whole-genome array-CGH screening in undiagnosed syndromic patients: old syndromes revisited and new alterations. Cytogenet Genome Res 115:254–261 (2006).
Kurnit DM, Layton WM, Matthysse S: Genetics, chance, and morphogenesis. Am J Hum Genet 41:979–995 (1987).
Lee C, Iafrate AJ, Brothman AR: Copy number variations and clinical cytogenetic diagnosis of constitutional disorders. Nat Genet 39: S48–S54 (2007).
Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, et al: Finding the missing heritability of complex diseases. Nature 461:747–753 (2009).
Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, et al: Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 82:477–488 (2008).
Meberg A, Hals J, Thaulow E: Congenital heart defects – chromosomal anomalies, syndromes and extracardiac malformations. Acta Paediatr 96:1142–1145 (2007).
Mefford HC, Sharp AJ, Baker C, Itsara A, Jiang Z, et al: Recurrent rearrangements of chromosome 1q21.1 and variable pediatric phenotypes. N Engl J Med 359:1685–1699 (2008).
Oyen N, Poulsen G, Boyd HA, Wohlfahrt J, Jensen PK, Melbye M: Recurrence of congenital heart defects in families. Circulation 120: 295–301 (2009).
Portnoi MF: Microduplication 22q11.2: a new chromosomal syndrome. Eur J Med Genet 52:88–93 (2009).
Rauch R, Hofbeck M, Zweier C, Koch A, Zink S, et al: Comprehensive genotype-phenotype analysis in 230 patients with tetralogy of Fallot. J Med Genet 47:321–331 (2010).
Richards AA, Santos LJ, Nichols HA, Crider BP, Elder FF, et al: Cryptic chromosomal abnormalities identified in children with congenital heart disease. Pediatr Res 64:358–363 (2008).
Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, et al: Strong association of de novo copy number mutations with autism. Science 316:445–449 (2007).
Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, et al: Large recurrent microdeletions associated with schizophrenia. Nature 455:232–236 (2008).
Theveniau-Ruissy M, Dandonneau M, Mesbah K, Ghez O, Mattei MG, et al: The del22q11.2 candidate gene *TBX1* controls regional outflow tract identity and coronary artery patterning. Circ Res 103:142–148 (2008).
Thienpont B, Mertens L, de Ravel T, Eyskens B, Boshoff D, et al: Submicroscopic chromosomal imbalances detected by array-CGH are a frequent cause of congenital heart defects in selected patients. Eur Heart J 28: 2778–2784 (2007a).
Thienpont B, de Ravel T, Van Esch H, Van Schoubroeck D, Moerman P, et al: Partial duplications of the *ATRX* gene cause the ATR-X syndrome. Eur J Hum Genet 15:1094–1097 (2007b).
Thienpont B, Zhang L, Postma AV, Breckpot J, Tranchevent LC, et al: Haploinsufficiency of *TAB2* causes congenital heart defects in humans. Am J Hum Genet 86:839–849 (2010).
Vermeesch JR, Balikova I, Schrander-Stumpel C, Fryns JP, Devriendt K: The causality of de novo copy number variants is overestimated. Eur J Hum Genet [Epub ahead of print] (2011).
Vissers LE, van Ravenswaaij CM, Admiraal R, Hurst JA, de Vries BB, et al: Mutations in a new member of the chromodomain gene family cause CHARGE syndrome. Nat Genet 36:955–957 (2004).
Wessels MW, Willems PJ: Genetic factors in non-syndromic congenital heart malformations. Clin Genet 78:103–123 (2010).
Wincent J, Anderlid BM, Lagerberg M, Nordenskjold M, Schoumans J: High-resolution molecular karyotyping in patients with developmental delay and/or multiple congenital anomalies in a clinical setting. Clin Genet 79:147–157 (2011).
Yagi H, Furutani Y, Hamada H, Sasaki T, Asakawa S, et al: Role of *TBX1* in human del22q11.2 syndrome. Lancet 362:1366–1373 (2003).
Yobb TM, Somerville MJ, Willatt L, Firth HV, Harrison K, et al: Microduplication and triplication of 22q11.2: a highly variable syndrome. Am J Hum Genet 76:865–876 (2005).