RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Congenital Heart Defects in a Novel Recurrent 22q11.2 Deletion Harboring the Genes *CRKL* and *MAPK1*

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Received 10 May 2011; Accepted 7 December 2011

The proximal region of the long arm of chromosome 22 is rich in low copy repeats (LCR). Non-allelic homologous recombination (NAHR) between these substrates explains the high prevalence of recurrent rearrangements within this region. We have performed array comparative genomic hybridization in a normally developing girl with growth delay, microcephaly, and truncus arteriosus, and have identified a novel recurrent 22q11 deletion that spans LCR22-4 and partially affects the common 22q11.2 deletion syndrome and the distal 22q11 deletion syndrome. This deletion is atypical as it did not occur by NAHR between any of the major LCRs found on 22q11.2. However, the breakpoint containing regions coincide with highly homologous regions. An identical imbalance was reported previously in a patient with striking phenotypic similarity. Computational gene prioritization methods and biological evidence denote the genes *CRKL* and *MAPK1* as the highest ranking candidates for causing congenital heart disease within the deleted region.

Key words: *CRKL*; *MAPK1*; *ERK2*; 22q11 deletion; LCR22; congenital heart defects; prioritization

How to Cite this Article:
Breckpot J, Thienpont B, Bauters M, Tranchevent L-C, Gewillig M, Allegaert K, Vermeesch JR, Moreau Y, Devriendt K. 2012. Congenital heart defects in a novel recurrent 22q11.2 deletion harboring the genes *CRKL* and *MAPK1*.
Am J Med Genet Part A 158A:574–580.

INTRODUCTION

The proximal region of the long arm of chromosome 22 is rich in low copy repeats (LCR). Non-allelic homologous recombination (NAHR) between these recombination substrates explains the existence of recurrent rearrangements within the 22q11.2 region, and the high prevalence of de novo events. Proximal 22q contains eight LCRs known as LCR22s [Edelmann et al., 1999; McDermid and Morrow 2002]. The most common recombination event occurs between LCR22-2 and LCR22-4, and gives rise to a 3 Mb deletion, which is associated with DiGeorge syndrome (DGS) (OMIM: 188400) or velo-cardio-facial syndrome (VCFS)(OMIM: 192430) [Halford et al., 1993; Shaikh et al., 2000]. This common deletion syndrome is characterized by conotruncal heart defects, velopharyngeal insufficiency, learning difficulties, immune dysfunction, congenital hypocalcemia, urogenital defects, and a distinct facial gestalt, and is clinically indistinguishable from the embedded 1.5 Mb deletion, which arises from recombination between LCR22-2 and LCR22-3 [Carlson et al., 1997]. Similar

Additional supporting information may be found in the online version of this article.

Grant sponsor: FWO-Flanders; Grant sponsor: IWT; Grant number: SBO-60848; Grant sponsor: GOA/2012/015; Grant sponsor: SymBioSys Center of Excellence; Grant number: KUL PFV/10/016 SymBioSys.
Conflict of interest: None.

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Published online 8 February 2012 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.35217

features can be present in the reciprocal duplication event between LCR22-2 and LCR22-4, although this 22q11.21 duplication syndrome represents a rather variable phenotype, ranging from multiple congenital defects to mild learning difficulties or even a normal presentation [Ensenauer et al., 2003]. Of interest is a 1 Mb deletion between LCR22-3 and LCR22-4, corresponding to the distal half of the typical 3 Mb deleted region, which was detected in a dysmorphic boy with tetralogy of Fallot and in his asymptomatic father [Garcia-Minaur et al., 2002]. The same deletion was found in a boy with developmental delay and an anxiety disorder, but no heart defects [Rauch et al., 2005] (Fig. 1).

A cluster of recurrent 1.4 and 2.1 Mb rearrangements was found distal to LCR22-4 (also known as LCR22-D), adjacent to the common 3 Mb deletion region [Rauch et al., 1999; Saitta et al., 1999; Rauch et al., 2005; Mikhail et al., 2007; Ben-Shachar et al., 2008; Newbern et al., 2008; Rodningen et al., 2008; Busse et al., 2011; Verhoeven et al., 2011]. These distal 22q11.2 deletion syndromes are flanked proximally by LCR22-4 and distally either by LCR22-5 or LCR22-6 [Shaikh et al., 2007]. The associated phenotype is variable and relatively mild, with growth delay of prenatal onset, mild developmental delay, and a wide spectrum of congenital heart defects (CHD). From the data compiled in Table I, microcephaly emerges as a frequent feature of distal 22q11 deletions as well (Table I). All other atypical deletions of proximal 22q described thus far, represent unique findings.

We report on a novel recurrent 22q11 deletion, which spans the distal part of the common 22q11.2 deletion syndrome and the proximal part of the distal 22q11 deletion syndrome. The deletion in our patient is flanked by highly homologous regions, and was described once previously [Ogilvie et al., 2009]. Both patients presented with CHD, growth delay and microcephaly, but without developmental delay.

CLINICAL DESCRIPTION

This 14-month-old girl was the first child of a healthy white woman. Maternal history was unremarkable with respect to congenital anomalies or developmental delay. No paternal data were available.

A type II common arterial trunk overriding a large non-restrictive outlet ventricular septal defect (VSD) was diagnosed prenatally at 34 weeks of gestation. No other congenital malformations were visualized on prenatal ultrasound. A cesarean was performed at 36 weeks of gestation due to acute fetal distress. Her birth weight was 1,925 g, length was 43 cm, and head circumference 28 cm (all below the 3rd centile). At birth she presented with a right-sided pre-auricular tag and a low nasal bridge. Cardiac ultrasound confirmed the presence of a type II common arterial trunk overriding a 7 mm perimembranous VSD. There was a tricuspid truncal valve with mild regurgitation and asingle coronary system originating from the right truncal cusp was diagnosed by cardiac catheterization aged 7 months. Ultrasound of the brain and abdomen was normal. Bilateral banding of both pulmonary arteries was performed at day 7, followed by truncus repair with a pulmonary homograft between the right ventricle and the pulmonary arteries, and VSD closure at the age of 9 months.

The patient presented to the genetic clinic at 5 months of age with growth delay and mildly dysmorphic facial features, including a unilateral pre-auricular tag, upslanting palpebral fissures, epicanthic folds, a low nasal bridge, and a thin vermilion of the upper lip. All biometric parameters were below the 3rd centile. Her weight was 4.82 kg (−3 SD), length 56.1 cm (−3.6 SD), and head circumference 36 cm (−4 SD). At the age of 14 months alternating occlusion with an eye patch was initiated because of convergent

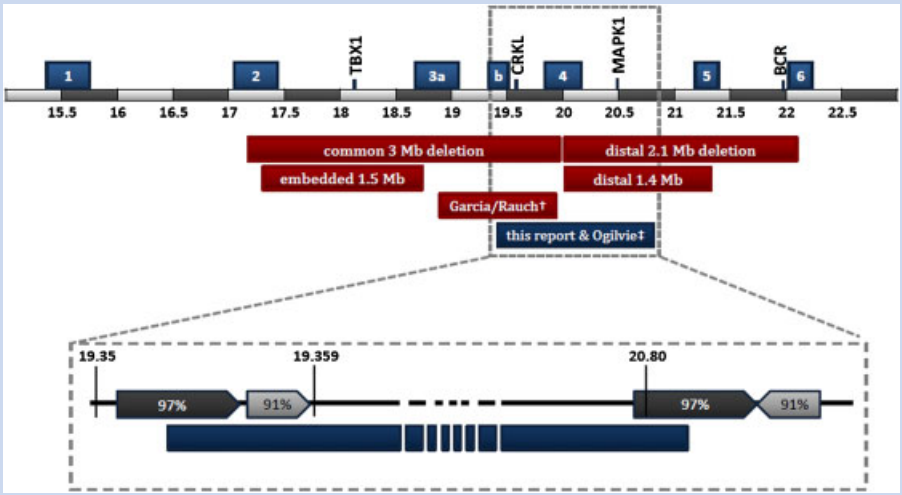

FIG. 1. Proximal 22q11.2 with LCR22-1 to LCR22-6 (blue boxes) and the positions of relevant genes on the axis. Recurrent 22q11.2 deletion syndromes are depicted as red boxes, the novel recurrent 22q11.2 deletion as a blue box. There are two blocks of high homology between the proximal and distal breakpoint containing regions: a 3.7 kb block of 97% homology and a 1.9 kb block of 91% homology (black and gray boxes, respectively). The latter are inverted and are unlikely to have mediated the recombination event. †Reference: [Garcia-Minaur et al., 2002; Rauch et al., 2005]. ‡Reference: [Ogilvie et al., 2009].

TABLE I. Phenotypic Features of Patients With Atypical Recurrent 22q11 Deletion Syndromes

	This report	Ogilvie et al. [2009]	Garcia-Minaur et al. [2002]	Rauch et al. [2005]	Distal del 22q11: 12 cases[c]	Distal del 22q11: 5 cases[d]
LCR22	Overriding LCR4[b]	Overriding LCR4	LCR3-4[a]	LCR3-4[b]	LCR4-5	LCR4-6
Growth delay	+	+	−	NA	11/12	1/3
microcephaly	+	+	+	NA	6/6	0/2
CHD	Truncus, VSD	VSD, ASD	ToF	No CHD	7/11 truncus (4); VSD (3); RAA (1); BAV (1)	3/5 truncus (2); VSD (1)
Dysmorphism	Upslanting palpebral fissures, epicanthic folds, low nasal bridge, thin upper lip	No	Broad forehead, small mouth, posteriorly rotated ears with deficient upper helices	Small mouth, mild retrognathia, mild ptosis	Variable: Small alae nasi, dysplastic ears, smooth philtrum, micrognathia, arched eyebrows. . .	Variable: Small alae nasi, smooth philtrum, high arched palate, dysplastic ears. . .
Development	Normal	Normal	Normal?	Mild DD, anxiety	Mild to moderate DD	Mild to moderate DD
Other features	Pre-auricular tag	Imperforate anus	No	Recurrent infections	Cleft palate/bifid uvula behavioral problems	Recurrent infections cleft palate/bifid uvula

ASD: Atrial septal defect; BAV: Bicuspid aortic valve; DD: Developmental delay; LCR: Low copy repeat; RAA: Right aortic arch; ToF: Tetralogy of Fallot; VSD: Ventricular septal defect.

[a]Inherited from asymptomatic father.
[b]Father not available for testing.
[c]References: [Saitta et al., 1999; Ben-Shachar et al., 2008; Newbern et al., 2008; Rodningen et al., 2008; Busse et al., 2011; Verhoeven et al., 2011].
[d]References: [Rauch et al., 1999; Rauch et al., 2005; Mikhail et al., 2007; Ben-Shachar et al., 2008].

strabismus. Fundoscopy was normal. Thus far, she has normal motor and social development.

METHODS OF DETECTION

A standard metaphase karyotype by G-banding was normal for both the patient and her mother. Fluorescence in situ hybridization (FISH) with the probe LSI 22q11, performed as described [Thienpont et al., 2007], was normal. However, an atypical 22q11.2 deletion was detected in the patient by means of comparative genome hybridization (CGH) using a 105k oligo array platform (OGT CytoSure Syndrome Plus array, OGT Oxford, UK), performed according the manufacturer's instructions. The deleted region spans maximally 1.50 Mb (19,306,969–20,814,320) and does not involve a known copy number polymorphism. The proximal and distal breakpoint containing regions are situated between 19,306,969–19,383,355 and 20,774,217–20,814,320, respectively. This deletion was not found in the mother by means of FISH with probe KB-1440D3 on peripheral blood derived lymphocytes, nor on oral mucosa (Fig. 2). The father was not available for further testing.

There are two blocks of high homology between the proximal and distal breakpoint containing regions: A 3.7 kb block of 97% homology between 19,351,886–19,355,637 and 20,799,044–20,802,748, and a 1.9 kb block of 91% homology between 19,356,940–19,358,894 and 20,802,755–20,804,968 (Fig. 1). As the latter are inverted, they are less likely to have mediated the deletion. All genome coordinates were according to NCBI human genome build 36 (hg18, March 2006).

To identify candidate genes for CHD within the deleted region, we used Endeavour, a web application for computational gene prioritization [Aerts et al., 2006; Tranchevent et al., 2008]. Bioinformatics prioritization is an established tool for selecting candidate genes for a role in specific human disorders, such as CHD [Qiao et al., 2010; Thienpont et al., 2010]. For gene prioritization, Endeavour command line version 2.44 (August 2010) was used. Data sources are derived either from Ensembl 44 based on the NCBI build 36 or the corresponding databases (as of August 2008). As a training set for CHD, we extracted all 29 non-syndromic cardiac genes from CHDWiki, a collaborative knowledge base on cardiogenetics (http://homes.esat.kuleuven.be/~bioiuser/chdwiki/index.php/Main_Page) on December 2010 [Barriot et al., 2010].

First, a leave-one-out cross-validation was performed on the set of these 29 non-syndromic cardiac genes using all available human models. The Area Under the ROC Curve (AUC) was calculated and used as an estimate of the performance for each model. Only the best performing models with an AUC above 65% were kept for the prioritization of genes within the deleted region (see online Supporting Information eTable I). The overall AUC was 94%. In addition, a heart specific mouse microarray dataset was added [Thienpont et al., 2010].

We tested as a positive control whether Endeavour would identify *TBX1* as a candidate for CHD within proximal 22q. When using the non-syndromic CHDWiki gene set as training, *TBX1* ranked first. *TBX1* was not identified as a CHD candidate gene when using random training genes unrelated to CHD (data not shown). Then, we used this approach to prioritize all 34 genes within this novel recurrent 22q11.21 deletion (19,351,886–20,802,748). The

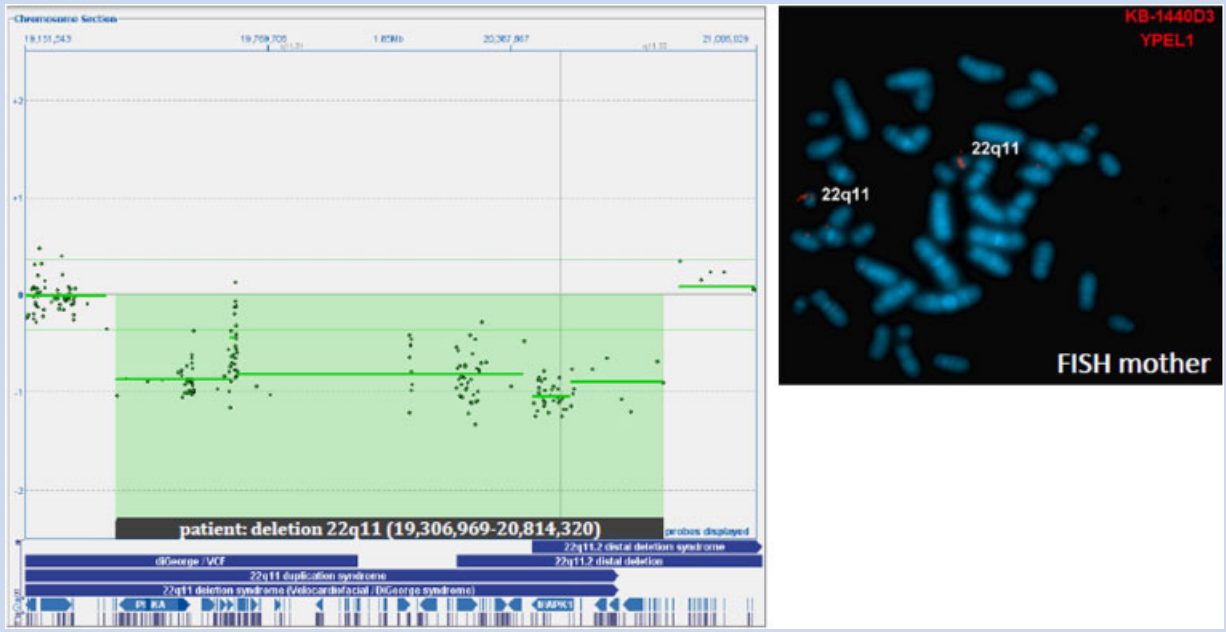

FIG. 2. The chromosomal profile of the patient on 22q11.2 (OGT CytoSure Syndrome Plus array) shows a 1.5 Mb deletion (19,306,969–20,814,320). This deletion was not found in the mother by means of FISH using probe KB-1440D3 (20,291,184–20,445,034). Genome coordinates were according to NCBI human genome build 36 (hg18, March 2006).

highest ranking genes were *MAPK1* and *CRKL* (Table II). The corresponding *P*-values (0.002 and 0.004, respectively) were highly significant, and no significant prioritization ($P < 0.01$) was obtained for any other gene in the deleted region. Subsequently, to assess the potential influence of *TBX1* on the prioritization results, we added the *TBX1* gene to the training set (see online Supporting Information eTable I). However, adding *TBX1* did not alter the prioritization results (Table II). Taken together, these data indicate that these genes are likely candidates for CHD.

DISCUSSION

The current deletion spans LCR22-4 and partially affects the regions corresponding to the common 22q11.2 deletion syndrome and the distal 22q11 deletion syndrome. This 22q11 deletion is atypical as it did not occur by NAHR between any of the major LCR found on chromosome 22q11.2. However, the proximal and distal breakpoint containing regions were found to coincide with two blocks of high homology. We assume that the 3.7 kb homologous regions served as recombination substrates for this novel recurrent 22q11.2 deletion, overriding the boundaries of the common and the distal 22q11.2 deletion syndrome. An identical deletion was described recently as a de novo event in a normally developing girl with growth delay, imperforate anus, and multiple septal defects [Ogilvie et al., 2009]. Interestingly, both patients presented with a CHD, growth delay and microcephaly, but no developmental delay (Table I).

CHD are a frequent finding associated with proximal 22q imbalances. Prevalence of CHD ranges from 30 to 65% in adolescents and adults [Bassett et al., 2005; Lima et al., 2011] to 80% in neonates [Momma 2010]. The latter may be inflated by ascertainment bias, since a CHD is the prime reason for 22q11 imbalance screening at neonatal age. The *TBX1* gene, mapped between LCR22-2 and LCR22-3, 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 typical 22q11.21 deletion syndrome [Yagi et al., 2003] as well as in the reciprocal duplication syndrome [Ensenauer et al., 2003]. However, haplo-insufficiency of other genes within proximal 22q, like *UFD1L* [Yamagishi et al., 2003] and *CRKL* [Guris et al., 2001; Guris et al., 2006], or genetic modifiers outside the region, may modify *TBX1* expression or may independently affect the genesis of CHD. Newbern et al. [2008] showed that deletions located beyond LCR22-4 did not produce a cis-acting position effect on *TBX1* expression, and vice versa. These findings suggest that the similar cardiac phenotypes in the proximal and distal deletion syndromes result from the deletion of distinct regions containing a unique set of genes.

Both patients with the atypical 22q11.21 deletion described here, presented with CHD. Automated gene prioritization was applied to identify candidate genes for CHD within this region. The highest ranking genes were *CRKL* and *MAPK1*, independent of the inclusion of *TBX1* into the training set. Previously, in silico prioritization experiments using various sets of DGS-related training genes, identified *YPEL1* as the highest ranking candidate for pharyngeal arch development within the distal 22q11 deleted region. Ypel1 knockdown zebrafish embryos exhibited an underdeveloped jaw and defects in pharyngeal arch cartilage formation, but no cardiac

TABLE II. Combined ENDEAVOUR Prioritization Results

Rank	Ensembl ID	Gene	P-val	△rank TBX1
1	ENSG00000100030	MAPK1	0.002	0
2	ENSG00000099942	CRKL	0.004	0
3	ENSG00000133511	PIK4CA	0.114	−3
4	ENSG00000100038	TOP3B	0.116	−1
5	ENSG00000169635	HIC2	0.133	−2
6	ENSG00000184436	THAP7	0.148	3
7	ENSG00000185651	UBE2L3	0.171	−1
8	ENSG00000099937	SERPIND1	0.190	−1
9	ENSG00000100023	PPIL2	0.223	5
10	ENSG00000099949	LZTR1	0.263	0
11	ENSG00000099940	SNAP29	0.270	−1
12	ENSG00000100034	PPM1F	0.300	1
13	ENSG00000183506	PI4KAP2	0.345	0
14	ENSG00000183773	AIFM3	0.365	0
15	ENSG00000206152	AC002470.1	0.412	0
16	ENSG00000128228	SDF2L1	0.458	0
17	ENSG00000099957	P2RXL1	0.458	0
18	ENSG00000206140	TMEM191C	0.538	−1
19	ENSG00000161179	YDJC	0.540	−1
20	ENSG00000161180	CCDC116	0.595	2
21	ENSG00000161149	AC002472.8	0.650	0
22	ENSG00000099960	SLC7A4	0.703	−1
23	ENSG00000187905	AC002472.13	0.704	1
24	ENSG00000206145	P2RX6P	0.736	0
25	ENSG00000197549	PRAMEL	0.751	0
26	ENSG00000211637	IGLV4-69	0.780	0
27	ENSG00000211638	IGLV8-61	0.780	0
28	ENSG00000100027	YPEL1	0.788	0
29	ENSG00000133475	GGT2	0.805	0
30	ENSG00000196934	RIMBP3B	0.924	−1
31	ENSG00000183246	RIMBP3C	0.945	−2
32	ENSG00000169668	BCRP2	0.953	2
33	ENSG00000169662	BCRP6	0.968	1
34	ENSG00000128389		0.991	0

All 34 genes within the deleted region (chr22:19,351,886–20,802,748) were ranked toward their potential involvement in CHD development, using 29 non-syndromic genes (retrieved from CHDWiki) as a training set. The gene rankings of every dataset were combined in a general ranking. For each gene, the *P*-value represents the probability to observe such a ranking based on similarity with the training set, rather than by chance alone. The highest ranking genes were *CRKL* and *MAPK1*. To assess the potential influence of *TBX1* on the prioritization results, we added the *TBX1* gene to the training set. The right column displays the impact of *TBX1* addition on the gene ranking (e.g., *PIK4CA* drops 3 positions after *TBX1* inclusion).

defects [Aerts et al., 2006]. In our study, no significant prioritization was obtained for *YPEL*, or for any other gene within the deleted region.

The mitogen-activated protein kinase 1 (*MAPK1* or *ERK2*) is mapped distally from LCR22-4. The craniofacial and cardiac outflow tract defects observed in patients with distal 22q11 deletions were ascribed to deficiencies in neural crest MAPK1 (ERK2) signaling, as homozygous conditional elimination of MAPK1 in murine developing neural crest resulted in VSDs and conotruncal heart defects with variable penetrance [Newbern et al., 2008]. Although its role in human CHD is unexplored thus far, Erk1/2 autophosphorylation was recently shown to mediate cardiac hypertrophy [Lorenz et al., 2009]. Further studies are required to investigate whether haplo-insufficiency of *MAPK1* might infer an increased risk of cardiac hypertrophy and early heart failure in patients with 22q11.2 deletions reaching beyond LCR22-4.

The adaptor-protein-encoding gene *CRKL* is located within the distal half of the common 3 Mb deletion. *CRKL* is expressed ubiquitously, but is highly abundant in the pharyngeal region, neural tissues, and neural crest derivatives [Guris et al., 2001]. Homozygous Crkl knock-out mouse embryos exhibited multiple aspects of DGS, including malformations of the thymus, the parathyroid glands, and the heart [Guris et al., 2001]. Heterozygous $Crkl^{+/-}$ mice were generally normal, although mild craniofacial and thymic defects did sporadically occur. However, compound heterozygosity of both Crkl and Tbx1 induced a higher penetrance of thymic, parathyroid, and cardiovascular defects than that generated by heterozygosity of Crkl or Tbx1 alone [Guris et al., 2006]. Since sensitivity to gene dosage differs between mice and humans, haploinsufficiency of *CRKL* alone might contribute significantly to the defective cardiac phenotype present in the typical 3 Mb 22q11.21 deletion, as well as in atypical deletions described here and previously [Garcia-Minaur et al., 2002; Ogilvie et al., 2009].

Interestingly, CRKL and MAPK1/ERK2 interact within a common genetic pathway involved in craniofacial and outflow tract morphogenesis. Pharyngeal ERK1/2 activation in response to Fgf8 signaling was found to be Crkl dependent [Moon et al., 2006]. Moreover, Fgf8 is a direct downstream target of Tbx1, and Fgf8 hypomorphic mutant mice phenocopy many features of DGS [Vitelli et al., 2002; Hu et al., 2004]. These findings imply that the common craniofacial and cardiovascular abnormalities observed in individuals with the common 3 Mb deletion or with the distal 22q11 deletion arise from perturbation of the same pathway [Newbern et al., 2008].

This novel recurrent 22q11.2 deletion was associated with growth delay and CHD, but did not seem to impair psychomotor development, despite the presence of microcephaly in both patients. Therefore, we assume that no dosage-dependent genes with a major cognitive function were affected. Moreover, it is likely that this deletion is under-diagnosed, given the predominance of intellectual disability as a motive to perform genetic diagnostic tests.

In conclusion, we report on a novel recurrent 22q11 deletion overriding LCR22-4, detected in two normally developing girls with microcephaly, growth delay, and CHD. We hypothesized that the recombination event is mediated by a 3.7 kb highly homologous region. Both computational prioritization methods as biological evidence denote the genes *CRKL* and *MAPK1* as the highest ranking candidates for causing congenital heart disease within the deleted region.

ACKNOWLEDGMENTS

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. The authors would like to thank the patient and her mother for their cooperation. Many thanks to Natalie Sohier for performing the OGT array analysis.

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