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Research Letter

The Intrafamilial Variability of the 22q11.2 Microduplication Encompasses a Spectrum from Minor Cognitive Deficits to Severe Congenital Anomalies

Céline de La Rochebrochard,[1,2]* Géraldine Joly-Hélas,[1,2] Alice Goldenberg,[2] Isabelle Durand,[3] Annie Laquerrière,[4] Valentine Ickowicz,[5] Pascale Saugier-Veber,[2] Danièle Eurin,[5] Hélène Moirot,[1,2] Alain Diguet,[5] Fabrice de Kergal,[4] Coralie Tiercin,[1] Bertrand Mace,[1] Loïc Marpeau,[5] and Thierry Frebourg[2]

[1]Laboratory of Cytogenetics, Rouen University Hospital, Rouen, France
[2]Department of Genetics, Rouen University Hospital, Rouen, France
[3]Department of Pediatrics, Rouen University Hospital, Rouen, France
[4]Department of Pathology, Rouen University Hospital, Rouen, France
[5]Department of Obstetrics and Gynecology, Rouen University Hospital, Rouen, France

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To the Editor:

The DiGeorge/Velo-cardio-facial syndrome (DGS/VCFS) is a common microdeletion syndrome characterized by a typical facial appearance, conotruncal cardiac defects, velopharyngeal insufficiency, and learning disabilities. Most patients with DGS/VCFS harbor a 3-Mb 22q11.2 deletion, resulting from abnormal pairing and homologous recombination mediated by low-copy repeats (LCRs) [Edelmann et al., 1999a]. A corresponding 3-Mb 22q11.2 duplication was subsequently identified in patients with clinical presentations similar to the classical 22q11.2 deletion [Edelmann et al., 1999b]. Recent reports have highlighted the highly variable phenotype of this duplication, which includes dysmorphic facial features distinct from the DGS/VCFS syndrome, non-specific congenital heart defects, learning disabilities, hearing loss, and postnatal growth deficiency. The severity of the duplication phenotype ranges from severe congenital malformations to isolated mild learning disabilities [Ensenauer et al., 2003; Hassed et al., 2004a,b; Portnoï et al., 2005; Yobb et al., 2005]. In most published reports, the 22q11.2 duplication was detected in patients ascertained on the basis of DGS/VCFS-like features, which may bias the phenotype. We report here an antenatal case of 22q11.2 duplication and a lethal congenital heart defect.

A 23-year-old, gravida 3, para 1 woman was referred at 21 weeks gestation, because of the sonographic detection of a fetal cardiac defect. The parents were non-consanguineous, healthy, and phenotypically normal and had no family history of congenital heart defects. Level II ultrasound examination showed a singleton female fetus and confirmed the presence of a complex non-conotruncal heart defect including single atrium, small left ventricle, large right ventricle, double outlet right ventricle with transposed great arteries, subpulmonary ventricular septal defect, persistent left superior vena cava, and probable anomalous pulmonary venous return. In addition, abdominal *situs inversus totalis* with normal cardiac *situs* was also detected. RHG-banded chromosome analysis performed on cord blood lymphocytes revealed no abnormality. We performed FISH using the DiGeorge critical

*Correspondence to: Céline de La Rochebrochard, Laboratory of Cytogenetics, Rouen University Hospital, 1 rue de Germont, 76031 Rouen Cedex, France. E-mail: Celine.de-La-Rochebrochard@chu-rouen.fr
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region probe (Vysis, Downers Groove, IL), encompassing *TUPLE1/HIRA*, *D22S553*, *D22S609*, and *D22S942*. Unexpectedly, we observed three signals equal in size and intensity in 70 of 103 interphase cells. In 3 of 14 metaphase spreads, a double or more intense signal was detected on one chromosome 22. Therefore, the karyotype of the fetus was 46,XX.ish dup(22)(q11.2q11.2)(TUPLE1++) (Fig. 1A). To estimate the size of this duplication, we used a Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF) assay specific for the DiGeorge syndrome [Jacquet et al., 2002]. QMPSF is based on the simultaneous amplification of multiple short exonic fragments under quantitative conditions, and this specific assay interrogates 23 genes, including 15 genes located within the 3-Mb 22q11.2 DGS1 region (*PRODH*, *DGCR2*, *GSCL*, *TUPLE1*, *NLVCF*, *UFD1L*, *PNUTL1*, *TBX1*, *GNB1L*, *COMT*, *ARVCF*, *RANBP1*, *ZNF74*, *PIK4CA*, and *SNAP29*), six genes located at its boundaries (*TUBA8*, *USP18*, *UBE2L3*, *CECR1*, *VPREB1*, and *BCR*) and two genes located within the 10p14-DGS2 region (*GATA3*, *CUG-BP2*). Superposition of the QMPSF profiles generated from the fetal amniocyte DNA on that from a control DNA showed a 1.5-fold increase of the peak heights of the DGS1 amplicons, demonstrating the presence of the classic 3-Mb duplication (Fig. 1B,C).

Due to the lethal heart defects, the parents elected to terminate the pregnancy. A 500 g female fetus (appropriate for gestational age) was delivered at 22 weeks gestation. Macroscopic examination showed superior placement of eyebrows and downslanting palpebral fissures, long philtrum, micrognathia, and dysplastic ears (Fig. 2A). Autopsy confirmed the cardiovascular abnormalities detected by sonography and the presence of total anomalous pulmonary venous return (TAPVR) in the right superior vena cava. It also confirmed abdominal *situs inversus totalis* with normal cardiac *situs* and revealed thoracic *heterotaxia* with right predominance and bilateral tri-lobed lungs. The urogenital tract was normal. Skeletal radiological examination was normal for the gestation.

FISH analysis, performed on peripheral blood lymphocytes of the parents, revealed that the 22q11.2 duplication was inherited from the father. Indeed, the majority of paternal interphase cells (69%, 92 of 134) exhibited three equal *TUPLE1* signals. Only 23% (9 of 39) of the metaphase cells exhibited a double or more intense signal on one chromosome 22. The duplication could not be seen by RBG and GBG chromosome analysis at the 550 band-level (Fig. 1D). Detailed physical, cardiac, and renal sonographic examinations of the father did not

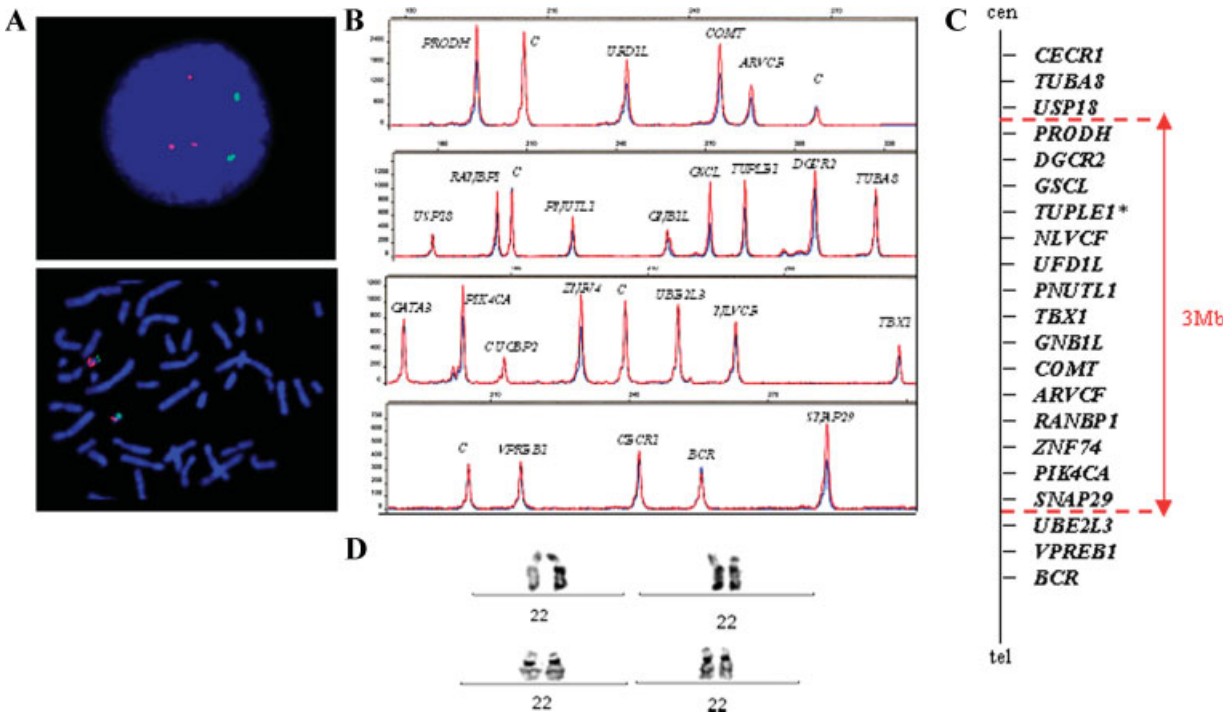

FIG. 1. Detection of the 22q11.2 duplication. **A**: FISH studies on fetal lymphocytes using a DiGeorge syndrome critical region probe at 22q11.2 (LSI *TUPLE1*, red signal) and a 22q13.3 telomere probe (LSI *ARSA*, green signal). The upper panel corresponds to an interphase cell whereas the lower panel corresponds to a metaphase cell. **B**: Characterization of the boundaries of the 22q11 duplication using QMPSF in fetal DNA. This QMPSF investigates 23 genes simultaneously within 22q11 [Jacquet et al., 2002] and the 10p14 chromosomal regions. In each panel, the electropherogram of the patient (in red) was superimposed on that of a normal control (in blue) by adjusting the peak heights obtained for the control amplicon (C) to the same level. The Y-axis displays fluorescence and the X-axis indicates the size in bp. Peak height ratios between 1.4 and 1.6 indicate three copies. All QMPSF analyses were performed at least twice. Primers are available upon request. **C**: Schematic representation of the position of the 22q11 amplicons along chromosome 22. **D**: RBG- (upper panel) and GBG (lower panel)-banded partial karyotype performed in the father's lymphocytes. Note that the derivative chromosome 22 does not exhibit evidence of duplication of the 22q11.2 region.

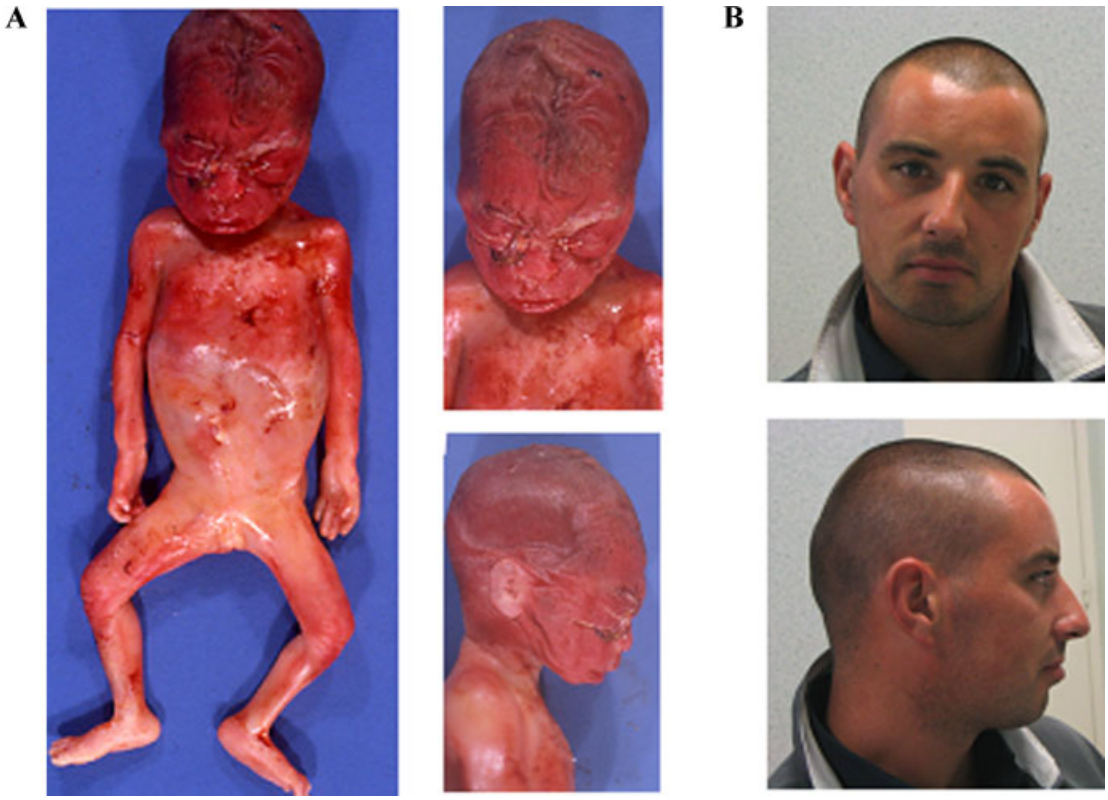

FIG. 2. **A**: Phenotype of the fetus: note moderate craniofacial dysmorphism with long philtrum, micrognatism, and dysplastic ears. **B**: Phenotype of the father: note normal facial appearance. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

reveal any abnormalities (Fig. 2B). The father had undergone surgery at the age of 11 months for a hiatal hernia. He had normal growth (height: 1.80 m, weight: 81 Kg, OFC: 57 cm). His global IQ score, tested with the Wechlser Adult Intelligence Scale (WAIS III) was 72 (verbal IQ 71, performance IQ 76) with preserved verbal comprehension (79) and perceptual organization (83). Memory performances evaluated with the Grober and Buschke test were within the normal range [Grober et al., 1988]. He had no history of behavior disorders.

The parents returned to our center in the context of a new pregnancy. Considering the phenotypic variability of the 22q11.2 duplication, the counseling was based both on fetal FISH analysis and sonographic examination. At 16 weeks gestation, prenatal diagnosis was performed on amniocytes. FISH analysis showed in 55% (56 of 102) of interphase cells three *TUPLE1* signals. A double or more intense signal on one chromosome 22 was detected in only 2 of 9 metaphase cells (data not shown). The 22q11.2 QMPSF assay confirmed the presence of the 3-Mb 22q11.2 duplication in the second fetus. The parents were informed of the phenotypic variability of the 22q11.2 duplication and decided to continue the pregnancy. Repeated level II ultrasound examinations at 21, 27, and 32 weeks of gestation, focused in particular on the heart, revealed no abnormality. At birth, clinical examination, weight, size, and OFC were normal.

Among the 48 patients reported with a 22q11.2 duplication [Edelmann et al., 1999b; Papenhausen et al., 2002; Ensenauer et al., 2003; Beiraghi et al., 2004; Hassed et al., 2004a; Hassed et al., 2004b; Lamb et al., 2004; Somerville et al., 2004; Portnoï et al., 2005; Sparkes et al., 2005; Yobb et al., 2005], only 11 patients (23%), (including the fetus reported here) exhibited heart defects (Table I), and in 5 of 11 cases these defects were conotruncal. The fact that most of the patients had been initially ascertained on the basis of DG/VCFS may have led to an overestimation of the frequency of the conotruncal defects associated with the 22q11.2 duplication. The case that we report confirms that the spectrum of congenital heart defects in the 22q11.2 duplication syndrome also includes non-conotruncal defects. The observation of TAPVR in a fetus with a 22q11.2 duplication is of particular interest. Indeed, TAPVR has been reported in about 20% of patients with Cat Eye syndrome (CES). CES is a rare disorder associated with a trisomy-tetrasomy extending from the 22q centromere to part of 22q11.2. Importantly, the CES critical region (CES-CR) is centromeric to the DGS/VCFS critical region [McDermid and Morrow, 2002]. The

TABLE I. Clinical and Molecular Findings in 50 Patients With 22q11.2 Duplication, Corresponding to 31 Families and Reported in 12 Studies

References (number of patients)	Postnatal dysmorphic facial features	Congenital heart defect	Cleft palate	Velo-pharyngeal insufficiency without cleft palate **	Postnatal growth retardation	ADHD**	Cognitive deficits**	Estimated size of the duplication
Edelmann et al. [1999b] (3)	3/3	0/3	0/3	1/3	0/3	0/3	1/3	3 Mb
Papenhausen et al. [2002] (1)	1/1	1/1 (Aortic coarctation)	0/1	0/1	1/1	0/1	1/1	NT
Ensenauer et al. [2003] (13)	12/12	2/12 (1 TOF, 1 IAA with HLH)	5/13	2/8	7/12	4/9	11/11	3 Mb (7 fam) 4 Mb (1 fam) 6 Mb (2 fam)
Hassed et al. [2004a] (4)	4/4	0/4	1/4	0/3	1/4	1/4	2/4	NT
Beiraghi et al. [2004] (4)	2/3	0/3	0/3	3/3	3/3	3/3	2/3	NT
Hassed et al. [2004b] (3)	3/3	1/3 (Aortic insufficiency and Mitral valve prolapse)	0/3	2/3	0/3	0/3	0/3	NT
Lamb et al. [2004] (1)	1/1	1/1 (TOF)	0/1	0/1	0/1	0/1	1/1	NT
Somerville et al. [2004] (4)	0/4	1/4	0/4	NM	0/4	2/4	2/4	NT
Sparkes et al. [2005] (3)	0/3	2/3 (1 TOF, 1 HLH)	0/3	0/3	0/3	0/1	1/1	NT
Yobb et al. [2005]* (7)	5/7	2/7 (1 TOF, 1 HLH)	1/5 (Arched palate)	1/5	3/7	3/7	4/7	3 Mb (7 fam) (1 patient had a 3 Mb triplication)
Portnoï et al. [2005] (4)	4/4	0/4	1/4	1/3	1/4	1/3	2/3	NT
This study (3)	1/2	1/3 (complex congenital heart defect)	0/3	0/1	0/1	0/1	0/1	3 Mb
Total 50 patients, 31 families	36/47 (77%)	11/48 (23%) (5 conotruncal, 5 non-conotruncal, 1 unspecified)	8/47 (17%)	10/34 (29%)	16/46 (35%)	14/40 (35%)	27/41 (66%)	3 Mb (16/19; 84%) 4 Mb (1/19; 5%) 6 Mb (2/19; 11%)

ADHD, aggressive behavior and/or attention deficit hyperactivity disorder; Fam, family(ies); HLH, hypoplastic left heart; IAA, interrupted aortic arch; NT, not tested; NM, not mentioned; TOF, tetralogy of fallot.
*Patients non-previously reported.
**Patients over 2 years.

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CES manifests ocular colobomas, preauricular pits and tags, anorectal abnormalities, urogenital malformations, congenital heart defects, and variable mental retardation [Berends et al., 2001; Rosias et al., 2001]. The TAPVR cardiac defect has also been reported in a child exhibiting some CES features (preauricular pits and unilateral absence of kidney) who had an interstitial 22q11–q12 duplication encompassing the DGS/VCFS region [Knoll et al., 1995]. The *CECR1* gene is a candidate gene for TAPVR as it is expressed in the embryonic cardiac outflow tract and atrium [Riazi et al., 2000] and because the gene is located within the CES-CR. The absence of *CECR1* duplication in the present fetus harboring TAPVR does not support this hypothesis. It is interesting to note that the fetus described here exhibited lateralization defects with abdominal *situs inversus totalis* and thoracic *heterotaxia*. It will be important to determine whether or not this feature, which has never been documented so far in 22q11.2 microduplication syndrome, is a recurrent finding in this syndrome. The possibility that some or all of the defects in this fetus have a cause distinct from the 22q11.2 duplication cannot be excluded.

Other remarkable clinical features within this family are the absence of mental retardation and facial dysmorphic features in the father harboring the 22q11.2 duplication. Previous reports have demonstrated that some patients with the 22q11.2 duplication have no evident abnormal phenotype except minor hand abnormalities or craniofacial dysmorphic features [Edelmann et al., 1999a; Hassed et al., 2004a; Yobb et al., 2005]. It has been previously suggested that the 22q11.2 duplication was associated with specific facial dysmorphic features, such as hypertelorism, superior placement of eyebrows, downslanting palpebral fissures with or without ptosis, mild micro-/retrognathia, and a long, narrow face [Ensenauer et al., 2003]. The frequency of nonspecific dysmorphic features in the 22q11.2 microduplication syndrome in the published studies is 77% (Table I). Our report confirms that facial dysmorphic features are inconstant and variable in this syndrome. Cognitive defects have been previously reported in 66% of patients with 22q11.2 duplication. The observation that the phenotypic expression of the 22q11.2 duplication can be restricted to border line IQ with preserved verbal comprehension, perceptual organization, and memory performances is consistent with this literature and confirms that the severity of the cognitive deficits in the 22q11.2 microduplication syndrome is highly variable.

Although genomic deletions and duplications are theoretically expected to occur in equal proportions, relatively few duplications have been detected among human genomic disorders [Lupski, 1998; Stankiewicz and Lupski, 2002]. This discrepancy may be due, in part, to the remarkable phenotypic variability of the duplications [Potocki et al., 2000], which may complicate the clinical recognition of the corresponding syndromes. It also may be partially explained by methodological bias, since microduplications are more difficult to detect than are microdeletions. Our report demonstrates the utility of FISH studies on interphase nuclei for the detection of genomic duplications. Indeed, the 22q11.2 microduplication was evident in the majority of interphase cells, whereas it was detected in only 22% of metaphase cells. One possible explanation is that the lower level of condensation of the chromatin in the interphase nucleus allows a better discrimination of two close fluorescent signals. It also demonstrates that QMPSF is a useful tool for the detection of genomic duplications and that this simple molecular method facilitates the characterization of the boundaries of genomic rearrangements.

In conclusion, the family that we describe here illustrates the wide phenotypic variability of 22q11.2 genomic duplications. This phenotypic variability suggests that this duplication syndrome remains underdiagnosed. Identification of the genetic factors underlying the phenotypic variability of deleterious microduplications will be essential in the future to refine prognostic assessments of this disorder.

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