Published in final edited form as:

Am J Med Genet A. 2007 August 1; 143A(15): 1778–1781. doi:10.1002/ajmg.a.31834.

Deletion 22q11.2: Report of a Complex Meiotic Mechanism of Origin

Sintia Iole Nogueira¹, April M. Hacker³, Fernanda T.S. Bellucco¹, Leslie Domenici Kulikowski¹, Denise Maria Christofolini¹, Mirlene C. Cernach², Maria Isabel Melaragno^{1,*}, and Beverly S. Emanuel³

¹ Disciplina de Genética, Departamento de Morfologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

² Centro de Genética Médica, Departamento de Morfologia, Universidade Federal de São Paulo, São Paulo, SP, Brazil

³ Department of Pediatrics, Division of Human Genetics, The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania

Abstract

We report on the case of a patient with a typical de novo 3 Mb 22q11.2 deletion. Haplotype reconstruction of the family, using polymorphic markers flanking the deleted region, demonstrated a complex mechanism of origin of the deletion, involving one intrachromosomal and two interchromosomal events.

Keywords

deletion 22q11.2; mechanism of deletion; haplotype reconstruction

INTRODUCTION

The 22q11.2 deletion syndrome is the most common deletion syndrome, and its prevalence is of 1/4,000 live births [Tezenas Du Montcel et al., 1996; Goldmuntz, 2005]. Most of these deletions (90%) occur de novo, and only 10% are inherited [Emanuel et al., 2001]. The high frequency of deletions in this region can be explained by the presence of chromosome-specific low-copy repeats (LCRs) that represent regions of chromosome-specific repeated DNA sequences [Shaikh et al., 2000]. There are some LCRs in chromosome 22 flanking (LCR A and LCR D) or within the DiGeorge chromosomal region (LCR A', B and LCR C) [Burn and Goodship, 1996; Edelmann et al., 1999; Shaikh et al., 2000]. These LCRs make the 22q11.2 region prone to chromosome rearrangements, probably due to misalignment during meiosis and unequal recombinational exchanges.

The aim of this work was to investigate the mechanism of formation of the 22q11.2 deletion. We report on a patient with 22q11.2 deletion syndrome, who has a 3 Mb de novo deletion originated by a complex meiotic exchange, with an intrachromosomal deletion flanked by two crossovers.

*Correspondence to: Maria Isabel Melaragno, Disciplina de Genética, Universidade Federal de São Paulo, Rua Botucatu, 740-CEP 04023-900, São Paulo, SP, Brazil. melaragno.morf@epm.br.

CLINICAL REPORT

A 6-year-9-month-old female patient, the first child of a nonconsanguineous couple with an unremarkable history, was referred for genetic evaluation. When she was born, her mother and father were 28 and 31 years old, respectively. The pregnancy was uneventful, and the delivery was at term, by Cesarean. Birth weight was 2,770 g (3rd centile), birth length 46 cm (3rd centile), and the head circumference was 32 cm (2nd centile). Soon after birth, the patient was admitted to the neonatal intensive care unit with cyanosis and respiratory distress. Echocardiography led to the diagnosis of atrioventricular canal defect. Craniofacial findings included: asymmetric face on crying, asymmetric ears, hypoplastic *alae nasi*, ocular hypertelorism (interpupillary distance 4.99 cm, >97th centile), malar hypoplasia, retrognathia, downturned oral commissures, and a bulbous nasal tip. Further findings included tapered digits and mild hypotonia.

Surgery performed at 1 year of age to correct the cardiac defect revealed an intermediate atrioventricular canal defect, characterized by two-hole valves, a ventricular septal defect of heart inflow, and an *ostium-primum*-type atrioseptal defect with cleft mitral valve, besides an *ostium-secundum*-type atrioseptal defect. The patient presented neither nasal speech nor velopharyngeal insufficiency.

Psychomotor development was mildly delayed: she walked at 15 months and spoke in sentences at 2 years. However, articulation and phonation problems were present. Speech therapy was initiated at age 3. Serum calcium levels were normal throughout her clinical history. At 6 years 9 months, she weighed 19,800 g (10th centile), was 115.5 cm tall (25th centile), and showed a mild global developmental delay.

Cytogenetic and FISH Studies

Cytogenetic and fluorescence in situ hybridization (FISH) analyses were performed on metaphase spreads from peripheral blood lymphocyte cultures. FISH using the TUPLE1 probe (Cytocell®, Cambridge, UK) showed a deletion of the 22q11.2 region. This was a *de novo* deletion, since the patient's parents had normal cytogenetic results. In order to determine the size of the deletion, FISH using cosmid probes flanking the LCRs of the DiGeorge chromosomal region was performed, as described by Saitta et al. [2004]. The probes used were c103a2 (*PH11*), c68a1 (*N41*, D22S788), c2c9 (*HCF2*), and c45c9 (*LN80*), located between LCR A and LCR A', LCR A' and LCR B, LCR C, and LCR D, and after LCR D, respectively. The deleted chromosome 22 showed positive hybridization only with the c45c9 probe, revealing a 3-Mb deletion from LCR A to LCR D.

Haplotype Reconstruction

DNA was extracted from transformed lymphoblasts or whole blood of the patient, her parents and all four grandparents. In order to perform haplotype reconstruction, six markers flanking the standard 3-Mb deletion were chosen. Three of the markers map proximally (centromeric) to LCR A (F8VWFP2, D22S420, D22S427), and three map distally (telomeric) to LCR D (D22S303, D22S257, *TOP1P2*) [Saitta et al., 2004]. Additionally, the D22S302 marker, located at 22q13.1, was used as a control to assess recombination in this region (Fig. 1).

Fluoresceinated PCR primers for these DNA markers were designed, using TET and FAM fluors conjugated to the 5' ends of the forward primers. PCR in a total volume of 25 µl was performed, using 50–100 ng of genomic DNA, 10X AccuPrime PCR Buffer I/II (containing Mg⁺⁺, dNTPs), AccuPrime Taq DNA polymerase, 10 pmol of primers and ultra pure water (all reagents from Invitrogen®, Carlsbad, CA). The PCR conditions were in accordance with previously published primer conditions [Saitta et al., 2004]. PCR products were analyzed on

an ABI Prism 3730 DNA analyzer, in the Nucleic Acid/Protein core facility at The Children's Hospital of Philadelphia. Gene Mapper 3.0 (Applied Biosystems®, Foster City, CA) was used to analyze the size of each product.

The haplotype reconstruction results (Fig. 2) showed a complex meiotic rearrangement as the origin of the deletion. Three meiotic events in paternal meiosis were found to be involved: two crossovers (one between F8VWFP2 and D22S420 and the other between D22S303 and D22S257) and one intrachromosomal exchange. A crossover, associated with a normal chromosome 22, also occurred in the maternal meiosis.

DISCUSSION

Many chromosomal rearrangements are not random events, but the consequence of a complex genome architecture that may create genomic instability [Shaw and Lupski, 2004]. Breakpoints were identified in some chromosome regions, such as 7q11.2 (Williams–Beuren syndrome) [Bayes et al., 2003], 15q11.2 (Prader-Willi/Angelman) [Amos-Landgraf et al., 1999], 17p11.2 (Smith–Magenis syndrome) [Chen et al., 1997], and 22q11.2 (DiGeorge/Velocardiofacial syndrome) [Edelmann et al., 1999]. These regions are flanked by LCRs, chromosome-specific repeated DNA sequences that make the chromosomes susceptible to rearrangements because of the similarity between their sequences [Shaw and Lupski, 2004]. Although the LCRs in the 22q11.2 region are different in content and organization, the modules that are common between them share 97–98% sequence identity with one another [Shaikh et al., 2000; Saitta et al., 2004]. So, these elements may lead to misalignment during meiosis and unequal interchromosomal recombinations between directly oriented LCRs in homologous chromosomes, or to intrachromosomal recombination within a single or between sister chromatids [Baumer et al., 1998; Emanuel and Shaikh, 2001].

The rearrangements might be mediated by non-allelic homologous recombination (NAHR) between highly homologous LCR substrates. Unequal crossover can occur between two segments flanked by LCRs, resulting in deletion and/or duplication or genomic inversion. Exchanges between LCRs located in different chromosomes, within a chromosome or within a chromatid result in interchromosomal, intrachromosomal, or intrachromatid rearrangements, respectively [Inoue and Lupski, 2002].

LCR-mediated NAHR does not explain all cases of genomic rearrangement. Several genomic disorders are associated with rearrangements whose breakpoints do not cluster within LCRs [Shaw and Lupski, 2004]. Another mechanism utilizes two nonhomologous sequences as substrates for recombination. The two sequences are joined via nonhomologous end joining (NHEJ) with deletion of the intervening fragment [Shaw and Lupski, 2004].

Previous reports have shown that interchromosomal exchanges are the predominant cause of micro-deletions [Thomas et al., 2006]. For the 22q11.2 deletion, interchromosomal exchanges were found in 8 out of the 10 cases reported by Baumer et al. [1998], in all three cases studied by Trost et al. [2000], but in none of the two cases studied by Edelmann et al. [1999]. In a larger sample, Saitta et al. [2004] studied 20 informative three-generation families of patients and found that 95% (19/20) of the deletions occurred through an interchromosomal event, while an intrachromosomal event was present in only one family.

From the haplotype reconstruction study using data from three generations of the patient's family, we found that three chromosomal events occurred in the 22q11.2 region: one intrachromosomal and two interchromosomal rearrangements, an uncommonly complex mechanism involving both intra- and interchromosomal events in the same meiosis (paternal). The intrachromosomal rearrangement was responsible for the deletion, since the deleted region is flanked by segments of the same homolog.

The mechanism that could explain this rearrangement is a loop formation by an intrachromatid misalignment of directed repeats in one chromosome 22 (Fig. 3). The other chromosome 22 homolog had to perform a loop too, for sequence alignment. An exchange of fragments inside the loop, followed by an excision, could produce the 3-Mb deletion. This event could probably provide the substrate for the homologous recombination (interchromosomal exchange). Considering that crossover is not a frequent event, especially in the proximal 22q region [Saitta et al., 2004; Sun et al., 2004], we suggest that the loop and the intrachromosomal recombination could predispose to the interchromosomal recombination events between the two chromosomes 22.

The present report provides for a better understanding of the mechanisms of deletion formation, not only for chromosome 22, but also for other chromosomes with similar architecture. Additional studies of such rearrangements are likely to provide insights into the mechanisms, which control chromosomal stability and recombination.

Acknowledgments

Grant sponsor: CAPES; Grant sponsor: CNPq; Grant sponsor: NIH; Grant number: DC02027; Grant number: CA39926; Grant sponsor: Charles E. H. Upham endowed chair.

This work was supported by CAPES and CNPq (Brazil) and grants DC02027, CA39926 from the NIH (BSE) and funds from the Charles E. H. Upham endowed chair (BSE).

References

- Amos-Landgraf JM, Ji Y, Gottlieb W, Depinet T, Wandstrat AE, Cassidy SB, Driscoll DJ, Rogan PK, Schwartz S, Nicholls RD. Chromosome breakage in the Prader-Willi and Angel-man syndromes involves recombination between large, transcribed repeats at proximal and distal breakpoints. *Am J Hum Genet* 1999;65:370–386. [PubMed: 10417280]
- Baumer A, Dutly F, Balmer D, Riegel M, Tükel T, Krajewska-Walasek M, Schinzel AA. High level of unequal meiotic crossovers at the origin of the 22q11.2 and 7q11.23 deletions. *Hum Mol Genet* 1998;7:887–894. [PubMed: 9536094]
- Bayes M, Magano LF, Rivera N, Flores R, Perez Jurado LA. Mutational mechanisms of Williams-Beuren syndrome deletions. *Am J Hum Genet* 2003;73:131–151. [PubMed: 12796854]
- Burn J, Goodship J. Developmental genetics of the heart. *Curr Opin Genet Dev* 1996;6:322–325. [PubMed: 8791511]
- Chen KS, Manian P, Koeuth T, Potocki L, Zhao Q, Chinault AC, Lee CC, Lupski JR. Homologous recombination of a flanking repeat gene cluster is a mechanism for a common contiguous gene deletion syndrome. *Nat Genet* 1997;17:154–163. [PubMed: 9326934]
- Edelmann L, Pandita RK, Morrow BE. Low-copy repeats mediate the common 3-Mb deletion in patients with velocardio-facial syndrome. *Am J Hum Genet* 1999;64:1076–1086. [PubMed: 10090893]
- Emanuel BS, Shaikh TH. Segmental duplications: An ‘expanding’ role in genomic instability and disease. *Nat Rev Genet* 2001;2:791–800. [PubMed: 11584295]
- Emanuel BS, McDonald-McGinn D, Saitta SC, Zackai EH. The 22q11.2 deletion syndrome. *Adv Pediatr* 2001;48:39–73. [PubMed: 11480765]
- Goldmuntz E. DiGeorge syndrome: New insights. *Clin Perinatol* 2005;32:963–978. ix–x. [PubMed: 16325672]
- Inoue K, Lupski JR. Molecular mechanisms for genomic disorders. *Annu Rev Genomics Hum Genet* 2002;3:199–242. [PubMed: 12142364]
- Saitta SC, Harris SE, Gaeth AP, Driscoll DA, McDonald-McGinn DM, Maisenbacher MK, Yersak JM, Chakraborty PK, Hacker AM, Zackai EH, Ashley T, Emanuel BS. Aberrant interchromosomal exchanges are the predominant cause of the 22q11.2 deletion. *Hum Mol Genet* 2004;13:417–428. [PubMed: 14681306]
- Shaikh TH, Kurahashi H, Saitta SC, O’Hare AM, Hu P, Roe BA, Driscoll DA, McDonald-McGinn DM, Zackai EH, Budarf ML, Emanuel BS. Chromosome 22-specific low copy repeats and the 22q11.2

deletion syndrome: Genomic organization and deletion endpoint analysis. *Hum Mol Genet* 2000;9:489–501. [PubMed: 10699172]

Shaw CJ, Lupski JR. Implications of human genome architecture for rearrangement-based disorders: The genomic basis of disease. *Hum Mol Genet* 2004;13(Spec No 1):R57–64. [PubMed: 14764619]

Sun F, Oliver-Bonet M, Liehr T, Starke H, Ko E, Rademaker A, Navarro J, Benet J, Martin RH. Human male recombination maps for individual chromosomes. *Am J Hum Genet* 2004;74:521–531. [PubMed: 14973780]

Tezenas Du Montcel S, Mendizabai H, Ayme S, Levy A, Philip N. Prevalence of 22q11 microdeletion. *J Med Genet* 1996;33:719. [PubMed: 8863171]

Thomas NS, Durkie M, Potts G, Sandford R, Van Zyl B, Youings S, Dennis NR, Jacobs PA. Parental and chromosomal origins of microdeletion and duplication syndromes involving 7q11.23, 15q11-q13 and 22q11. *Eur J Hum Genet* 2006;14:831–837. [PubMed: 16617304]

Trost D, Wiebe W, Uhlhaas S, Schwindt P, Schwanitz G. Investigation of meiotic rearrangements in DGS/VCFS patients with a microdeletion 22q11.2. *J Med Genet* 2000;37:452–454. [PubMed: 10928854]

Fig. 1.

Physical map of loci tested. The DNA markers used for this study are depicted as vertical lines. LCR-A and LCR-D are depicted as black boxes. F8VWP2, D22S420, and D22S427 are proximal markers to LCR-A. D22S303, D22S257, and TOP1P2 are distal markers to LCR-D. D22S302 is a control marker.

Fig. 2.

Haplotype reconstruction of the patient's family, using seven DNA markers flanking the deleted region. DNA marker names are given on the left. Data show that the deleted chromosome is of paternal origin. Two interchromosomal events (crossovers) and one intrachromosomal exchange produced the deletion.

Fig. 3.

Schematic representation of a paternal meiotic recombination between homologous chromosomes 22. The figure shows an intrachromatid misalignment of directed repeats (intrachromatid loop) with an excision resulting in the deletion. Two interchromosomal exchanges occurred between homologous chromosomes, one proximal and one distal to the deleted region. This resulted in a deletion between LCRS A to D and an acentric fragment. The crosses represent the rearrangement sites. Numbers 1 to 7 represent DNA markers F8VWFP2, D22S420, D22S427 (proximal to the deletion region), D22S303, D22S257, TOP1P2 (distal to the deletion region), and D22S302 (control at 22q13), respectively. **A**, **B**, **C**, and **D** represent LCRs A, B, C and D, respectively. The drawing is not in scale.