The human genome; you gain some, you lose some

Kriek, M.

Chapter III

Case report based findings

Chapter III-1

A complex rearrangement on chromosome 22 affecting both homologues; haplo-insufficiency of the Cat eye syndrome region may have no clinical relevance

Marjolein Kriek[1], Karoly Szuhai[2], Sarina Kant[1], Stefan White[1], Hans Dauwerse[1], Heike Fiegler[3], Nigel P. Carter[3], Jeroen Knijnenburg[2], Johan den Dunnen[1], Hans Tanke[2], Martijn Breuning[1], Carla Rosenberg[2,4]

[1]Center for Human and Clinical Genetics, Leiden University Medical Center, The Netherlands
[2]Dept. Molecular Cell Biology, Leiden University Medical Center, The Netherlands
[3]Wellcome Trust Sanger Institute, Wellcome trust Genome Campus, Hinxton, Cambridge, UK.
[4]Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil.

Hum Genet. 2006 Aug;120(1):77-84.

Abstract

The presence of highly homologous sequences, known as low copy repeats, predisposes for unequal recombination within the 22q11 region. This can lead to genomic imbalances associated with several known genetic disorders. We report here a developmentally delayed patient carrying different rearrangements on both chromosome 22 homologues, including a previously unreported rearrangement within the 22q11 region. One homologue carries a deletion of the proximal part of chromosome band 22q11. To our knowledge, a 'pure' deletion of this region has not been described previously. Four copies of this 22q11 region, however, are associated with Cat eye syndrome (CES). While the phenotypic impact of this deletion is unclear, familial investigation revealed five normal relatives carrying this deletion, suggesting that haplo-insufficiency of the CES region has little clinical relevance. The other chromosome 22 homologue carries a duplication of the Velocardiofacial/DiGeorge syndrome (VCFS/DGS) region. In addition, a previously undescribed deletion of 22q12.1, located in a relatively gene-poor region, was identified. As the clinical features of patients suffering from a duplication of the VCFS/DGS region have proven to be extremely variable, it is impossible to postulate as to the contribution of the 22q12.1 deletion to the phenotype of the patient. Additional patients with a deletion within this region are needed to establish the consequences of this copy number alteration. This study highlights the value of using different genomic approaches to unravel chromosomal alterations in order to study their phenotypic impact.

Introduction

The 22q11 region contains highly homologous regions known as low copy repeat (LCR) sequences. Despite the difference in size and organisation of these repeats, the overall sequence identity is 97–98% (Shaikh *et al*. 2000). It has been demonstrated that the presence of these LCRs can initiate misaligned (non-) allelic homologous recombination of the region flanked by these duplicons, resulting in a deletion and an obligate reciprocal duplication (McDermid and Morrow 2002; Bailey *et al*. 2002). As a result, 22q11 is associated with different genomic disorders (Table 1). The 22q11 related disorders display a wide variety of clinical features, with no obvious correlation between the size of the genomic imbalance and the severity of the clinical characteristics.

The most common genetic disorder in this region is the Velocardiofacial syndrome/DiGeorge syndrome (VCFS/DGS) [MIM # 192430; MIM #188400], affecting 1:4,000–6,000 individuals. This syndrome is caused by haplo-insufficiency of the 22q11.2 region. Over 90% of patients suffering from VCFS/DGS have a 3 Mb deletion between LCR22-2 and LCR22-4 (Fig. 1), that share a particularly high degree of homology. The rest of the patients have a smaller rearrangement (1.5 Mb) with breakpoints located in LCR22-2 and LCR22-3a. Fluorescent in situ hybridisation (FISH) analysis is 100% accurate in detecting VCFS. However, the DGS phenotype can also be caused by other genetic (e.g. 10p13 deletion) or non-genetic causes (Robin and Sprintzen 2005). Most of the affected organs (thymus, (para)thyroid gland, outflow area of the heart) in VCFS/ DGS (Table 1) are derived from the third, fourth and sixth branchial arch in early development. Recently, it became apparent that VCFS/DGS are due to developmental deficiency of the endodermal pharyngeal pouches and the pharyngeal mesoderm, rather than (migration) defects of the neural crest cells (Graham 2003). As the *TBX1* gene is strongly expressed in the branchial arches, it is assumed that mutations in this gene are responsible for the majority of the features of VCFS/DGS (Jerome and Papaioannou 2001; Lindsay *et al*. 2001; Mercher *et al*. 2001).

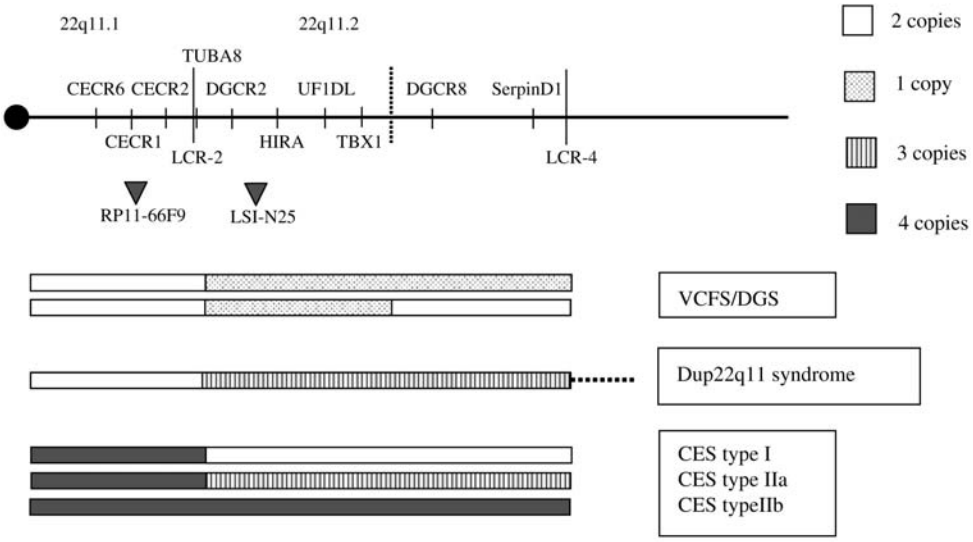
In 1999, the first report of the reciprocal duplication of the VCFS/DGS region was published. The phenotypic variability associated with the duplication of the VCFS/ DGS region emerged as a healthy mother and grandmother had the same duplication as the affected individual (Edelmann *et al*. 1999). Ensenauer *et al*. (2003) summarised the clinical characteristics of 13 patients with 22q11 duplications of variable sizes (3, 4, 6 Mb). More recently, the clinical features of another seven

Table 1. Overview of different 22q11 related syndromes.

Name of syndrome	Rearrangement	Clinical features
Velocardiofacial syndrome/DiGeorge syndrome	Deletion of the 22q11.2 region	DD, facial dysmorphisms (micrognathia, short philtrum and ear anomalies), cleft palate, cardiac outflow tract defects, Tetralogy of Fallot, nasal speech, hypocalcemia, thymic hypoplasia and behavioural problems (especially schizophrenia).
22q11.2 duplication syndrome	Duplication of the 22q11.2 region	Extremely variable. Clinical features of these patients could show similarities with those described in VCFS/DGS [DD (± motor delay), poor growth, dysmorphic features, velopharyngeal malformation ± cleft palate, urogenital malformations, hearing loss) However, dysmorphic features and behavioural problems not related to the VCFS/DGS spectrum have also been described (see text).
Cat eye syndrome	Quadruplication of the 22q11.1 region	Ocular coloboma, downslanting palpebral fissures, preauricular tags and/or pits, anal atresia with fistula, frequent occurrence of congenital heart and renal malformations and normal to near-normal mental development.

DD developmental delay

Figure 1 Overview of three 22q11 related syndromes in relation to the location of the different MAPH and BAC probes (RP11-66F9, N25) used in this study.



The size of the majority of the deletions within the VCFS/DGS regions is 3 Mb. The remaining deletions of this region encompass 1.5 Mb. The distal breakpoint of the duplications of the VCFS/DGS region is not always localised within LCR-4 (indicated by a *dotted line*) (Ensenauer *et al.* 2003). Different types of CES are depicted. This figure is based on Fig. 2 of McDermid and Morrow *et al.* (2002).

22q11 duplication syndrome patients were described, showing a very wide range of clinical variability. Furthermore, the first triplication of 22q11.2 was described (Yobb *et al*. 2005).

The Cat eye syndrome (CES [MIM #115470]) has three different subtypes: CES type I, CES type IIa and CES type IIb (Fig. 1). The endpoint of CES type I colocalises with LCR-2 and consists of two extra copies of the CES region only. Patients with CES type IIa have four copies of the CES region combined with three copies of the VCFS/DGS region. CES type IIb consists of four copies of both the CES region and the VCFS/ DGS region. The endpoint of both CES type IIa and IIB is mapped to LCR-4 (McDermid and Morrow 2002).

So far, a deletion of the Cat eye critical region has never been reported.

In this report, we describe the clinical features of a patient with complex chromosome 22 rearrangements, including a previously undescribed familial deletion of CES region in one homologue and, a duplication of VCFS/DGS region of the other homologue, in addition to a deletion of 22q12.1. These imbalances were characterised using different techniques: multiplex amplifiable probe hybridisation (MAPH), multiplex ligation-dependent amplification (MLPA), fluorescence in situ hybridisation (FISH), array-based comparative genomic hybridisation (array-CGH).

Clinical report

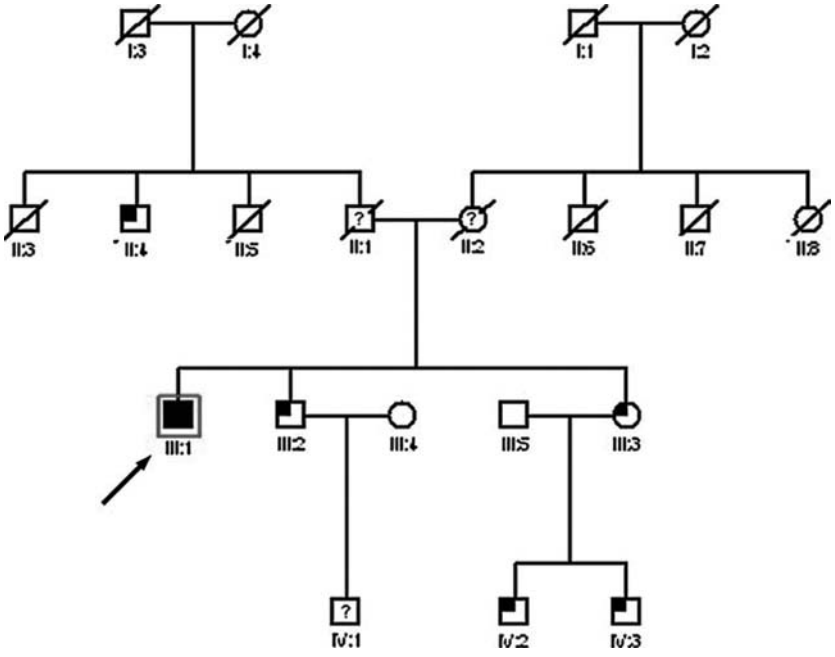
The male patient was born by forceps delivery after an uneventful pregnancy. At birth, a cleft palate was diagnosed and he was reported to have a double set of teeth. The cleft palate was corrected by surgical treatment at the age of two and five. He attended special education because of hearing loss and moderate mental retardation. From his early adolescence onwards, he has been living in a support home. His further medical history included cataract and myopia.

At the time of evaluation in the clinical genetics department, this patient was 52 years old (Fig. 2). Physical examination showed hypertonia with wooden movements. His speech was slow and difficult to comprehend and he tended to avoid eye contact. He had a normal height of 172 cm (–1.5 SD), microcephaly (head circumference 51.2 cm: –3.8 SD), round face with hypotonic expression, proptosis of the eyes, prominent simple ears, earpits on both sides and short fifth fingers. His heart tones were normal and no murmur was diagnosed. His medical record shows no history of cardiac problems.

Figure 2 Picture of the proband.

Note the microcephaly, myotonic facial expression, the proptosis of the eyes and the prominent simple ears. [See appendix: colour figures.]

Figure 3. The pedigree of proband III-1.



A square symbol and an arrow mark the proband. The symbol (▪) represents all five unaffected family members with a deletion of the CES region.

Familial history

The pedigree of the family is shown in Fig. 3. Familial history included two siblings with children and grandchildren, all of them healthy. The index patient's father died at the age of 81 years of unknown causes. His mother died due to a cardiovascular accident at the age of 79 years. The overall familial history showed no other individuals with developmental delay, nor any other genetic disorders.

Additional investigation

Additional investigation showed a normal male karyotype and a normal number of CGG repeats of the *FMR1* gene. FISH analysis was performed for the detection of a deletion of chromosome band 22q11.2 (using TUPLE1 probe) and for the detection of a deletion of 4p16.3 (Wolf–Hirschhorn syndrome) (using LSI-WHS probe). No rearrangements were detected. DNA testing for myotonic dystrophy type 1 showed normal CTG repeats on both alleles.

Materials and methods

Patients

This study was approved by the Institutional Review Board of the Leiden University Medical Center, conforming to Dutch law. All subjects or their representatives gave informed consent for DNA studies.

MAPH and MLPA

Multiplex amplifiable probe hybridisation was performed as described by White *et al.* (2002). The probe set used contained 19 probes from genes on chromosome 22 with approximately 1 Mb spacing, and ten additional genes in the 22q11 region.

A modified protocol of MLPA (Schouten *et al.* 2002) was performed as described by White *et al.* (2004).

Array-comparative genomic hybridisation (array-CGH)

The array-CGH procedures were performed as described (Knijnenburg *et al.* 2004). Briefly, slides containing triplicates of ~3,500 BAC DNA probes spaced at ~1 Mb density over the full genome were produced in the Leiden Technology Center (LGTC). The BAC set used to produce these arrays was received from the Welcome Trust Sanger Institute (UK), and information regarding the full set is available in the genome browser, Ensembl (http://www.ensembl.org/).

Tiling path array

The chromosome 22 tile path array and its hybridisation and analysis were performed as described by Woodfine *et al.* (2004).

Fluorescence in situ hybridisation (FISH)

The FISH experiments were performed by standard procedures (Dauwerse *et al.* 1992).

The CES region specific BAC RP11-66F9 were visualised using Alexa594 (green). For the identification of chromosome 22, the telomere specific BACs LSI-ARSA and RP11-3018K1 (22q13) (Flint and Knight 2003) was used and visualised using FITC (green). The VCFS/ DGS region was tested by N25 Probe (Vysis). This probe consists of N25 in red (SpectrumOrange).

Results

MAPH and MLPA

Multiplex amplifiable probe hybridisation analysis of the index patients DNA revealed a deletion of probes within the CES region, (*CECR1* (GeneID: 51816), *CECR2* (GeneID: 27443), *CECR6* (GeneID: 27439) sequence) and a duplication of the probes containing sequences within VCFS/DGS region (*DGCR2* (GeneID: 9993), *DGCR8* (GeneID: 54487), *TBX1* (GeneID: 6899), *UF1DL* (GeneID: 7353), *HIRA* (GeneID: 7290), *SERPIND1* (GeneID: 3053). The *TUBA8* gene (GeneID: 51807), localised between CECR and DGCR, showed a normal copy number (Fig. 4).

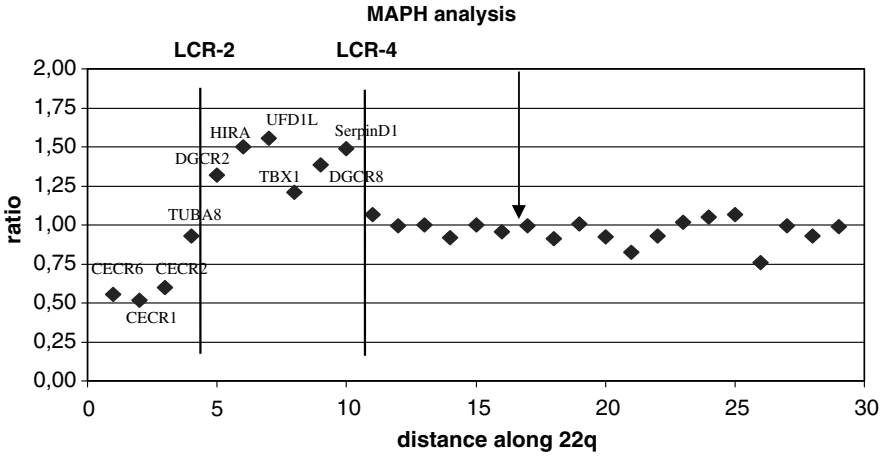
It was not possible to test the patient's parents; however, we were able to test several other healthy family members. The results are summarised in Fig. 3 and show that both siblings, two of their children and a brother of the patients' father carried the same deletion in the Cat eye region as the index patient. The duplication of 22q11.2 was absent in all family members tested.

After verification of these findings with MLPA using sequences of *CECR2* gene and *DGCR2* gene, the characteristics of the genetic rearrangements of index patient were refined by different techniques.

Array-CGH

Array-CGH using a 3500 BAC array was initially carried out to define the length of each of the two rearrangements. However, this analysis revealed a third chromo-

Figure 4. MAPH analysis of chromosome 22.



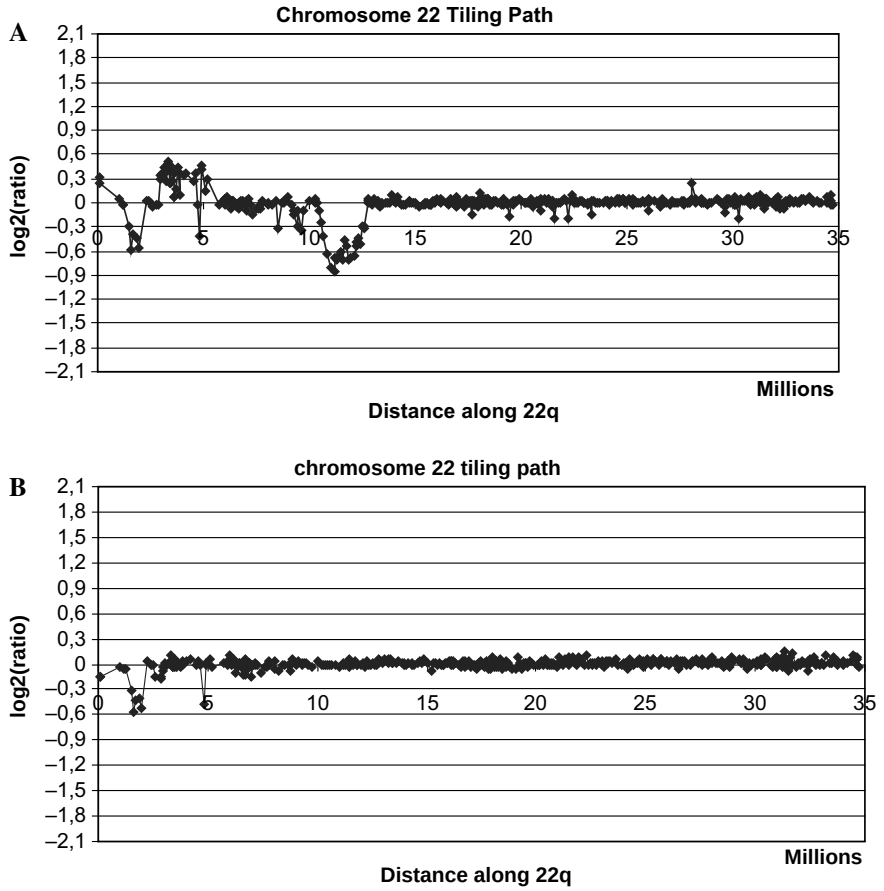
MAPH plot of chromosome 22 revealing a deletion of the CES region and a duplication of the VCFS/DGS region in the proband. A probe covering 22q12.1 was not included. The *arrow* indicates the locus of the 22q12.1 deletion.

some 22 alteration, namely a deletion of 22q12.1. The deleted area was localised about 25 Mb distal from the 22q11.2 region, between BACs CTA-57G9 and CTB-48E9. As this deletion was present in a relatively gene-poor region, the chromosome 22-MAPH-probe set did not contain a probe in this region. The duplication of 22q11.2 and the deletion of chromosome 22q12.1 were not present in the healthy brother of the index patient.

Chromosome-22-tiling-path array

To map the breakpoints of the alterations at a higher level of resolution, the patient's DNA and that of his brother were analysed on a chromosome-22-tiling-path array, as shown in Fig. 5. The sizes of the deletion and the duplication are 1.5 and 4.1 Mb, respectively. The transition of the deletion and the duplication within the 22q11 region maps to the LCR22-2. As the size of the duplication is larger than 3 Mb, the distal breakpoint of the alteration is not localised within LCR22-4, being localised more distally. The distal deletion on chromosome band 22q12.1 encompasses 2.3 Mb on chromosome band 22q12.1 and is not flanked by intrachromosomal LCRs. This region, however, is flanked by sequences that share high homology with sequences localised on other chromosomes.

Figure 5. Chromosome 22 tiling path array.



A Tiling path array analysis of the proband revealed the deletion and duplication of 22q11 subregions and a distal deletion of chromosome band 22q12.1. The sizes of the rearrangements are 1.5, 4.1 and 2.3 Mb, respectively. One BAC within the VCFS/DGS region shows an aberrant ratio. The cause of this aberration is currently unknown. B Tiling path array analysis of the healthy brother of the proband confirmed the presence of the proximal 22q11 deletion but the absence of the duplication of 22q11 and the deletion of 22q12.1.

Additional familial investigation using MLPA showed that none of the family members with a 22q11 deletion carried the distal deletion.

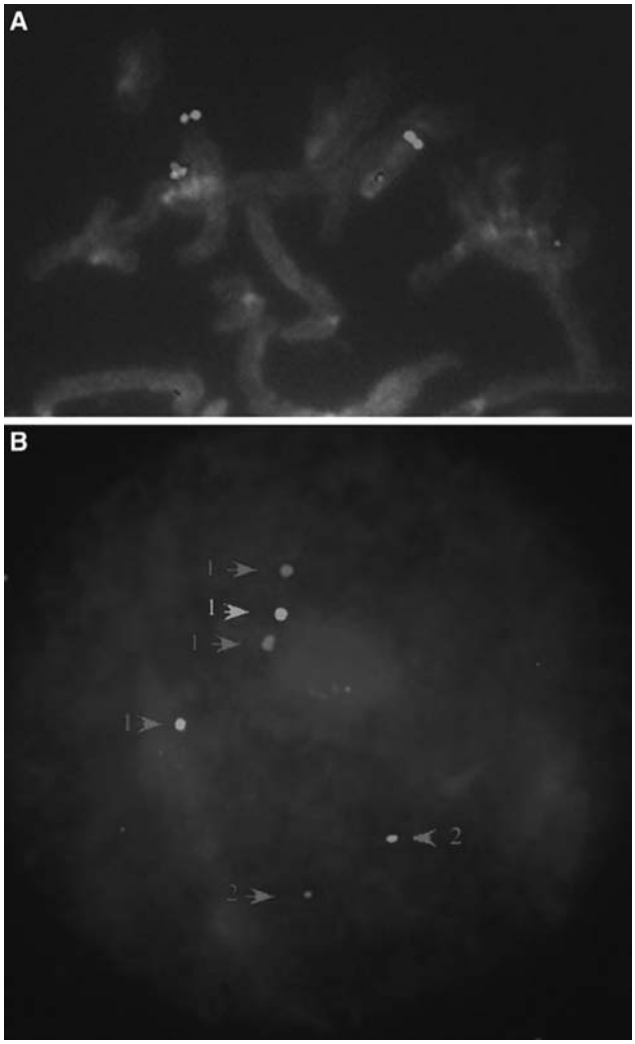
Fluorescence in situ hybridisation (FISH)

Based on FISH studies on both metaphase and interphase nuclei using FISH probes RP11-3018K1 and LSI-ARSA (both corresponding to the subtelomeric region of chromosome 22q), N25 (corresponding to the VCFS/ DGS region) and RP11-66F9 (corresponding to the CES region), it was concluded that the deletion of the CES region and the duplication of the VCFS/DGS region are localised on different homologues of chromosome 22 (Fig. 6).

Discussion

The complex rearrangement described here is, to our knowledge, the first report of a 'pure' deletion (e.g. not as a part of an unbalanced translocation) located in the CES region. The question is whether this rearrangement is related to a specific phenotype. Haplo-insufficiency of the CES region was found among five healthy relatives of the index patient. In addition, the family study indicates that the patient's father was an obligate carrier of the deletion of the CES region, as one of his brothers was a carrier of this deletion. A recent publication (Banting *et al*. 2005) showed that the vast majority of mice heterozygous for *CECR2* gene mutations were normal and capable of reproduction, whereas mice homozygous for mutations in the *CECR2* gene (correspondingly located on chromosome band 22q11.1 in the human genome) had a high penetrance of exencephaly. They established that CECR2 plays a role in neurulation during embryogenesis. These results suggest that, although the *CECR2* gene is essential during early development, a 50% decrease of gene dosage might not be associated with an aberrant phenotype. Although this data involves only one gene within the CES region, it is in accordance with our findings that a deletion in this region has no (obvious) phenotype and might therefore be present in the healthy population. In fact, the lack of clinical phenotype would explain the absence of reports on this deletion. Another reason for the lack of reported deletions of the CES region is that there is no commercial FISH probe available for this region, so it cannot be found 'coincidentally' as the duplication of the VCFS/DGS region was detected (Edelmann *et al*. 1999). Furthermore, ascertainment bias might account for this deletion. People with a mild phenotype will not be tested using high resolution-or whole genome techniques.

Figure 6. FISH analysis of chromosome 22.



A A partial metaphase of the patient, hybridised with the telomere probe RP11-3018K1 (*green*; chromosome region 22q13), N25 (*red*; VCFS/DGS region) and RP11-66F9 (*green*; CES region). On the right chromosome, green signals of RP11-3018K1 (telomeric side of chromosome 22) and a red signal N25 corresponding to the VCFS/DGS region are present; however, the signal of RP11-66F9 is lacking, indicating a deletion of the CES region. On the left chromosome, in addition to the green signals of RP11-3018K1, a red signal corresponding to the VCFS/DGS regions and a green signal corresponding to the CES region are both present. These latest two signals are partly overlapping. On this chromosome, the signal of N25 is stronger than the signal on the right chromosome, suggesting a duplication of the VCFS/DGS region. These findings are confirmed by the result of the interphase nucleus depicted in part b of this figure. B The different chromosomes 22 are marked 1 and 2. The signal of LSI ARSA, corresponding to the telomeric side of chromosome 22, is indicated with a *blue arrow*. The *red arrow* indicates the N25 signal (corresponding to the VCFS/DGS region), which is duplicated in chromosome 22 nr.1 (two red signals). The *green arrow* indicates the signal of RP11-66F9 (corresponding to the CES region). This signal is missing on chromosome 22 nr.2, demonstrating the deletion of the CES region. [See appendix: colour figures.]

Patients with duplication 22q11.2 syndrome show a wide variety of clinical features ranging from unaffected to severely affected individuals (Edelmann *et al*. 1999; Kriek *et al*. 2004; Yobb *et al*. 2005). Despite this, Ensenauer *et al*. (2003) described six clinical features that are frequent among 22q11.2 patients. Five of these (cognitive deficit, poor growth, dysmorphic features, cleft palate and hearing loss) were present in our index patient. The most characteristic dysmorphic features for the duplication 22q11.2 syndrome, however, such as superior placement of eyebrows, widely spaced eyes and downslanting of the eyes, were absent in our patient. Furthermore, our patient has several features (myotonic facial expression, proptosis of the eyes and a double set of teeth) that have not been described previously in other dup22q11.2 patients. Notably, the patients described by Ensenauer *et al*. (2003) show an ascertainment bias towards VCFS/DGS related features. All 653 patients included in this study were previously referred for 22q11 deletion screening using FISH on metaphase nuclei. In 2005, the clinical characteristics of another seven patients showing a duplication of the VCFS/DGS region were summarised (Yobb *et al*. 2005). This group of patient has a partial ascertainment bias for VCFS/DGS related features. Five were detected using FISH for 22q11 deletion screening, two were found by screening a cohort of 275 samples that was referred for fragile X screening. The clinical features of the latest two patients did not show similarity with VCFS/DGS spectrum. This last paper highlights the extreme variability of this alteration.

It is known that genetic factors localised outside the 22q11 region contribute to the variable clinical manifestations of 22q11 related alterations. It appeared that Fibroblast Growth Factor 8 (FGF8) mutant mice show close resemblance to the phenotype of del22q11.2 syndrome patients (Frank *et al*. 2002). Therefore, the *FGF8* gene, localised in the ectoderm and endoderm of the developing pharyngeal arches, might contribute to the 22q11 features. Stalmans *et al*. (2003) argued, based on mouse experiments, that the vascular endothelial growth factor gene (*VEGF* gene) modifies the expression of the VCFS/DGS syndrome, especially the cardiovascular birth defects. These, or other, as yet unidentified, modifiers localised outside the 22q11 region could also contribute to the phenotype of 22q11 duplication cases. Phenotypic variability due to the presence of a so far unknown modifier of a rearrangement might also play a role in to the phenotype of the patients with a deletion of the CES region.

In short, the clinical features described can, in theory, be caused by the unique combination of the three copy number changes on chromosome 22. However, as the deletion of the CES region probably has no clinical consequences, there is no previous MR-related literature regarding the deletion of 22q12.1 and the contribution of 22q11

rearrangements could be altered by other factors, it is not possible to determine the isolated influence of the different genetic imbalances.

To date, only a few cases with a duplication of the VCFS/DGS region have been described. It is probable that the majority of these duplications have not been detected so far due to a combination of phenotypic diversity (mentioned above) and the difficulty of diagnosis. A good example of the second argument is the clinical report described here; our patient was tested for a possible deletion in the VCFS/DGS region using FISH on the metaphase cells and the duplication present in the same region could not be seen, as two signals were overlapping. To overcome these problems, one has to focus on applying techniques in a routine diagnostic setting that are capable of detecting both duplications and deletions, within the same assay. In this way, it will be possible to increase the number of patients with a genetic diagnosis and, in parallel, learn more about possible causes of clinical features as we have demonstrated in this study of 22q11 rearrangement.

Recent initiatives such as those of the Sanger Institute (www.sanger.ac.uk/PostGenomics/decipher/) to create platforms for compiling molecular cytogenetic data from clinical genetic studies will hopefully provide a base for understanding the role of different DNA copy number alterations in genetic diseases. Collecting and understanding larger sets of data generated by different genomic approaches, as described here, will improve our ability to determine which copy number alterations contribute to abnormal phenotypes, and eventually result in a more consistent application of these techniques for genetic counseling.

References

Bailey JA, Gu Z, Clark RA, Reinert K, Samonte RV, Schwartz S, Adams MD, Myers EW, Li PW, Eichler EE (2002) Recent segmental duplications in the human genome. *Science* **297**:1003–1007

Banting GS, Barak O, Ames TM, Burnham AC, Kardel MD, Cooch NS, Davidson CE, Godbout R, McDermid HE, Shiekhattar R (2005) CECR2, a protein involved in neurulation, forms a novel chromatin remodeling complex with SNF2L. *Hum Mol Genet* **14**:513–524

Dauwerse JG, Jumelet EA, Wessels JW, Saris JJ, Hagemeijer A, Beverstock GC, Van Ommen GJB, Breuning MH (1992) Extensive cross-homology between the long and short arm of chromosome 16 may explain leukemic inversions and translocations. *Blood* **79**:1299–1304

Edelmann L, Pandita RK, Spiteri E, Funke B, Goldberg R, Palanisamy N, Chaganti RS, Magenis E, Shprintzen RJ, Morrow BE (1999) A common molecular basis for rearrangement disorders on chromosome 22q11. *Hum Mol Genet* **8**:1157–1167

Ensenauer RE, Adeyinka A, Flynn HC, Michels VV, Lindor NM, Dawson DB, Thorland EC, Lorentz CP, Goldstein JL, McDonald MT, Smith WE, Simon-Fayard E, Alexander AA, Kulharya AS, Ketterling RP, Clark RD, Jalal SM (2003) Microduplication 22q11.2, an emerging syndrome: clinical, cytogenetic, and molecular analysis of thirteen patients. *Am J Hum Genet* **73**:1027–1040

Flint J, Knight S (2003) The use of telomere probes to investigate submicroscopic rearrangements associated with mental retardation. *Curr Opin Genet Dev* **13**:310–316

Frank DU, Fotheringham LK, Brewer JA, Muglia LJ, Tristani-Firouzi M, Capecchi MR, Moon AM (2002) An Fgf8 mouse mutant phenocopies human 22q11 deletion syndrome. *Development* **129**:4591–4603

Graham A(2003) Development of the Pharyngeal Arches. *Am J Med Genet* **119A**:251–256

Jerome LA, Papaioannou VE (2001) DiGeorge syndrome phenotype in mice mutant for the T-Box gene, *TBX1*. *Nat Genet* **27**:286–291

Knijnenburg J, Szuhai K, Giltay J, Molenaar L, Sloos W, Poot M, Tanke HJ, Rosenberg C (2004) Insights from genomic micro-arrays into structural chromosome rearrangements. *Am J Med Genet* **132**(1):36–40

Kriek M, White SJ, Bouma MC, Dauwerse HG, Hansson KB, Nijhuis JV, Bakker B, van Ommen GJ, Den Dunnen JT, Breuning MH (2004) Genomic imbalances in mental retardation. *J Med Genet* **41**:249–255

Lindsay EA, Vitelli F, Su H, Morishima M, Huynh T, Pramparo T, Jurecic V, Ogunrinu G, Sutherland HF, Scambler PJ, Bradley A, Baldini A (2001) TBX1 haploinsufficiency in the DiGeorge syndrome region causes aortic arch defects in mice. *Nature* **410**:97–101

McDermid HE, Morrow BE (2002) Genomic disorders on 22q11. Am J Hum Genet 70:1077–1088

Merscher S, Funke B, Epstein JA, Heyer J, Puech A, Lu MM, Xavier RJ Demay MB, Russell RG, Factor S, Tokooya K, Jore BS, Lopez M, Pandita RK, Lia M, Carrion D, Xu H, Schorle H, Kobler JB, Scambler P, Wynshaw-Boris A, Skoultchi AI, Morrow BE, Kucherlapati R. (2001) TBX1 is responsible for cardiovascular defects in velo-cardio-facial/DiGeorge syndrome. *Cell* **104**:619–629

Robin NH, Shprintzen RJ (2005) Defining the clinical spectrum of deletion 22q11.2. *J Pediatr* **147**: 90–96

Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G (2002) Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res* **30**:e57

Shaikh TH, Kurahashi H, Saitta SC, O'Hare AM, Hu P, Roe BA, Driscoll DA, McDonald-McGinn DM, Zackai EH, Budarf ML, Emanuel BS (2000) Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. *Hum Mol Genet* **9**:489–501

Stalmans I, Lambrechts D, De Smet F, Jansen S, Wang J, Maity S, Kneer P *et al* (2003) VEGF: a modifier of the del22q11 (DiGeorge) syndrome? *Nat Med* **9**:173–182

White S, Kalf M, Liu Q, Villerius M, Engelsma D, Kriek M, Vollebregt E, Bakker B, van Ommen GJ, Breuning MH, Den Dunnen JT (2002) Comprehensive detection of genomic duplications and deletions in the DMD gene, by use of multiplex amplifiable probe hybridization. *Am J Hum Genet* **71**:365–374

White SJ, Vink GR, Kriek M, Wuyts W, Schouten J, Bakker B, Breuning MH, Dunnen JT (2004) Two-color multiplex ligation-dependent probe amplification: detecting genomic rearrangements in hereditary multiple exostoses. *Hum Mutat* **24**:86–92

Woodfine K, Fiegler H, Beare DM, Collins JE, McCann OT, Young BD, Debernardi S, Mott R, Dunham I, Carter NP (2004) Replication timing of the human genome. *Hum Mol Genet* **13**:191–202

Yobb TM, Somerville MJ, Willatt L, Firth HV, Harrison K, Mackenzie J, Gallo N, Morrow BE, Shaffer LG, Babcock M, Chernos J, Bernier F, Sprysak K, Christiansen J, Haase S, Elyas B, Lilley M, Bamforth S, McDermid HE (2005) Microduplication and triplication of 22q11.2: a highly variable syndrome. *Am J Hum Genet* **76**:865–876