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Independent De Novo 22q11.2 Deletions in First Cousins With DiGeorge/Velocardiofacial Syndrome

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Abstract

Deletions of chromosome 22q11.2 are found in the vast majority of patients with DiGeorge/velocardiofacial syndrome (DGS/VCFS). This most frequent microdeletion syndrome is estimated to occur in 1 in 4,000 live births. The majority of deletions are de novo, with 10% or less inherited from an affected parent. Here, we report two separate families with recurrence of a 22q11.2 deletion in first cousins. In each family, unaffected siblings (brother and sister) had an affected child. Fluorescence in situ hybridization (FISH) studies of the parents of each affected child were normal and hence, relatives were not considered at an increased risk for recurrence in another pregnancy. We used highly polymorphic microsatellite repeat markers from within 22q11.2 to determine the parental origin of each cousin's deletion and to assess whether parental germline mosaicism for the 22q11.2 deletion might be a factor in these cases. This analysis confirmed that in each case, the deletion occurred on a chromosome 22 derived from unrelated parents, consistent with independent de novo deletion events. Thus, we concluded that germline mosaicism as the underlying mechanism for affected cousins in these families was unlikely. Our findings underscore the high frequency with which the 22q11.2 deletion occurs in the general population and demonstrate the important role that PCR-based parental origin determination can have in recurrence risk counselling. Furthermore, relatives of affected individuals may benefit from genetic counselling and consider prenatal testing for the 22q11.2 deletion in future pregnancies, despite a low recurrence risk.

Keywords

chromosome 22q11.2 deletion; VCFS; DiGeorge syndrome; germline mosaicism

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INTRODUCTION

Collectively, DiGeorge, velocardiofacial, and conotruncal anomaly face syndromes (DGS/VCFS/CAFS) have been referred to as the 22q11.2 deletion syndrome since detectable deletions of chromosome 22q11.2 are found in approximately 90% of these patients [rev. in Emanuel et al., 2001]. The clinical features are variable and include conotruncal cardiac defects, craniofacial anomalies, learning difficulties, and palatal anomalies. Abnormal development of the thymus and parathyroid glands are seen with resulting immune defects and dysregulation of calcium metabolism.

The development of fluorescence in situ hybridization (FISH) assays for diagnostic purposes has improved detection of the deletion [Driscoll et al., 1992]. Over the past few years, clinical and molecular cytogenetic studies have demonstrated that de novo deletions of chromosome 22 occur with a high frequency, making them the most frequently occurring microdeletion syndromes found in humans. In fact, recent estimates indicate that the 22q11.2 deletion occurs in approximately 1 in 4,000 live births [Burn and Goodship, 1996]. The overwhelming majority (>85%) of patients are deleted for the same ~3 Mb region, although smaller variant deletions exist [Saitta et al., 1999; Emanuel et al., 2001]. The presence of “recombination permissive” duplicated DNA sequences (22-LCRs) in 22q11 are thought to play a major role in the frequent occurrence of the deletions [Edelmann et al., 1999; Shaikh et al., 2000]. Duplicated sequences arranged as direct repeats allow for mispairing and unequal crossing over between homologs (interchromosomal) or allow intrachromosomal recombination to take place within a single or between sister chromatids [Emanuel and Shaikh, 2001].

Less than 10% of deletions are familial, inherited from an affected parent with a 50% recurrence risk [McDonald-McGinn et al., 2001]. In de novo cases, we assume that the recurrence risk in subsequent pregnancies and for relatives is low except for the possibility of germline mosaicism [Hatchwell et al., 1998; Sandrin-Garcia et al., 2002]. Here, we report the use of parental origin determination utilizing highly polymorphic markers within two extended families with recurrent cases of the 22q11.2 deletion syndrome for the purpose of genetic counselling.

CLINICAL REPORTS

Family 1

Proband III-1—The proband was diagnosed with tetralogy of Fallot at birth. Subsequently, the child was found to have a small left diaphragmatic hernia and absent thymus leading to the clinical diagnosis of DGS. Other features included nasal regurgitation of feeds due to velopharyngeal incompetence that was treated with a posterior pharyngeal flap procedure, frequent infections, and developmental delay requiring educational interventions. The child had several facial features suggestive of the 22q11.2 deletion, including hooded eyelids, overfolded helices, and bulbous nasal tip. She was diagnosed with a 22q11 deletion at age 7 years when FISH using the N25 probe became available [Driscoll et al., 1992; Vysis, Downers Grove, IL]. FISH analysis of the parents were normal.

Patient III-4—The first cousin of Patient III-1 had developmental delay, short stature, and the typical facial characteristics of the 22q11.2 deletion syndrome. His cousin’s FISH results prompted a similar study in him and at age 2, he was also found to have a 22q11 deletion. Parental FISH studies were normal in this case as well.

Family 2

Proband II-1—The proband presented at birth with truncus arteriosus. A normal thymus was noted and T cell studies were within normal limits. Mild hypocalcemia was noted. FISH

analysis demonstrated a 22q11 deletion. The baby died within the first week of life during cardiac surgery. The parents were tested and found to be normal.

Patient II-2—This infant, the first cousin of proband II-1, was diagnosed with a large ventricular septal defect (VSD), atrial septal defect (ASD), and coarctation of the aorta at birth. Feeding difficulties and gastro-esophageal reflux were severe, requiring a G-tube, although she now tolerates oral feeding. Height and weight were below the 5th centile for age. Immune function has been compromised with multiple respiratory and ear infections requiring frequent antibiotic coverage and calcium supplementation. Developmental delay in all areas was noted, with gross motor skills being the best. The child is in a special education program and receives occupational, physical, and speech therapy. She was diagnosed with the 22q11 deletion syndrome by FISH at 4 years of age [Driscoll et al., 1997a].

MATERIALS AND METHODS

Genotyping

To determine the parental origin of the deleted chromosome 22 in each affected child, six highly polymorphic, short tandem repeat polymorphism (STRP) markers from within the typically deleted region (3 Mb) on chromosome 22q11.2 were used for genotyping [Driscoll et al., 1997b]. Genomic DNA was extracted from peripheral blood or buccal swabs of affected children and their parents and PCR-amplified. For Family 1, PCR was performed using 50–100 ng of genomic DNA and PCR reagents (Perkin-Elmer, Concordville, CA): 200 μM dNTPs, standard 10× buffer, 2 U Taq polymerase, and 1 μM fluorescently-labeled primers (IDT, Coralville, IA). Reactions were performed in a Perkin-Elmer 9700 thermal cycler with conditions appropriate for the primers [Driscoll et al., 1997b]. PCR products were run on an ABI Prism 377 DNA sequencer. GeneScan software from ABI (Pleasanton, CA) was used to summarize the images and Genotyper to determine the genotypes from band fragment sizes. For Family 2, the same primers had been radiolabeled, the PCR products run on 6% polyacrylamide gels, and visualized by autoradiography as described previously [Driscoll et al., 1997a]. Data from informative markers was used for haplotype analysis to determine the parental origin of the deleted chromosome 22.

RESULTS

Each proband and set of parents was referred to our laboratory for further studies following the birth of a second affected child in the extended family. In each family, both sets of parents were clinically normal and were tested for a 22q11.2 deletion by FISH and were not deleted (data not shown).

Family 1

A pedigree of Family 1 with haplotypes represented by sizes of the markers in basepairs is shown in Figure 1. Informative alleles are given for each tested family member. The only informative alleles that the proband (III-1) inherits are paternal in origin (165, 466, 192) and no informative maternal alleles are inherited, indicating that the deletion of 22q11.2 is on the maternal chromosome. Genotype analysis of the affected cousin (III-4) is consistent with a deleted maternal chromosome as well; informative paternally-derived 190 and 156 alleles were transmitted. Since the origin of each deletion appears to be maternal and the two mothers are not related, the deletions are most likely independent events occurring by chance. Each cousin's deletion was sized using multiple FISH probes from 22q11.2 (data not shown) and found to correlate with the most commonly seen ~3 Mb deletion [Emanuel et al., 2001].

Family 2

In this study, the markers were radioactively labeled and visualized by autoradiography. Different sized alleles were each assigned a number. Two markers (D22S944 and D22S264) were informative in this family (Fig. 2). Haplotype analysis demonstrates that individual II-1 failed to inherit maternal alleles, while her first cousin II-2 failed to inherit paternal alleles. In this family, the origin of one deletion is maternal (II-1) and the other paternal (II-2). Here again, each chromosome with a 22q11 deletion came from the unrelated parent. Therefore, germline mosaicism is an unlikely explanation for the recurrence of the deletion in this family.

DISCUSSION

This study describes two separate families where first cousins are affected with the 22q11 deletion syndrome. The parents of these children, as well as their siblings, requested genetic counselling to determine if they were at increased risk of having offspring with the deletion. To address this issue and provide a more accurate assessment of their risk, we needed to establish if the deletions in the first cousins in each of these families were linked or occurred as independent events. If we could establish that the deletion occurred on a chromosome 22 shared by the parents who were siblings in each family, then we would consider germline mosaicism as a possible explanation for the recurrence of the deletion. The parents of each affected child were non-consanguineous and phenotypically normal. FISH analysis of peripheral blood lymphocytes was normal; none of them appeared to have a deletion. Haplotype analysis using STRPs from within the commonly deleted region of 22q11.2 further demonstrated that in both families, the deleted chromosomes were not from related parents of the affected cousins. In these cases, the results are most consistent with independent, de novo deletions in each of the affected children. However, we cannot totally exclude the possibility of germline mosaicism in any of these parents. Hence, antenatal monitoring of any future pregnancies would be offered for these parents and their relatives for reassurance, as in any case of a de novo deletion.

Genetic counselling is recommended for parents of an affected child with the 22q11 deletion. These counselling sessions should review the natural history of this disorder, including the spectrum of medical problems and cognitive difficulties that these infants may encounter. It is also helpful to arrange for a multidisciplinary team to evaluate the infant or child and arrange for appropriate care to meet the ongoing needs of the family and child [McDonald-McGinn et al., 1997]. In addition, it is important to discuss the recurrence risk for subsequent pregnancies. To accurately assess the parent's risk of having another child with the 22q11 deletion, it is valuable to determine if the deletion was transmitted from an affected parent or occurred as a sporadic, de novo event. We recommend that parents of an affected child should have deletion testing [McDonald-McGinn et al., 2001], preferably by FISH as there have been reports of low-level somatic mosaicism detected in peripheral blood lymphocytes from clinically unaffected parents [Kasprzak et al., 1998]. This degree of mosaicism might not be detectable by a purely PCR-based assay [Sandrin-Garcia et al., 2002]. If the deletion appears to have occurred de novo, then the recurrence risk in a future pregnancy is presumably low. However, apparently isolated germline mosaicism for intragenic deletions has been reported for several genetic disorders [Rimoin et al., 2002], including the 22q11.2 deletion syndrome [Hatchwell et al., 1998], raising the possibility of recurrence even when parental FISH studies are normal. The low, but as yet undefined risk of germline mosaicism in this disorder, often leads couples to consider prenatal testing in future pregnancies.

Although the likelihood that a second degree relative in these families will have a child with a 22q11 deletion is equal to the risk in the general population, they may benefit from genetic counselling to review their risk and antenatal testing options. They may wish to consider having

a level II ultrasound examination and/or deletion testing, particularly if they are having an amniocentesis or chorionic villus sampling (CVS) for advanced maternal age.

These cases illustrate the utility of PCR-based haplotype analysis as an adjunct to routine FISH-based diagnostic studies to provide more accurate recurrence risk counselling to similar families. Additionally, they underscore the high frequency of the deletion (1:4,000 live births) in the general population.

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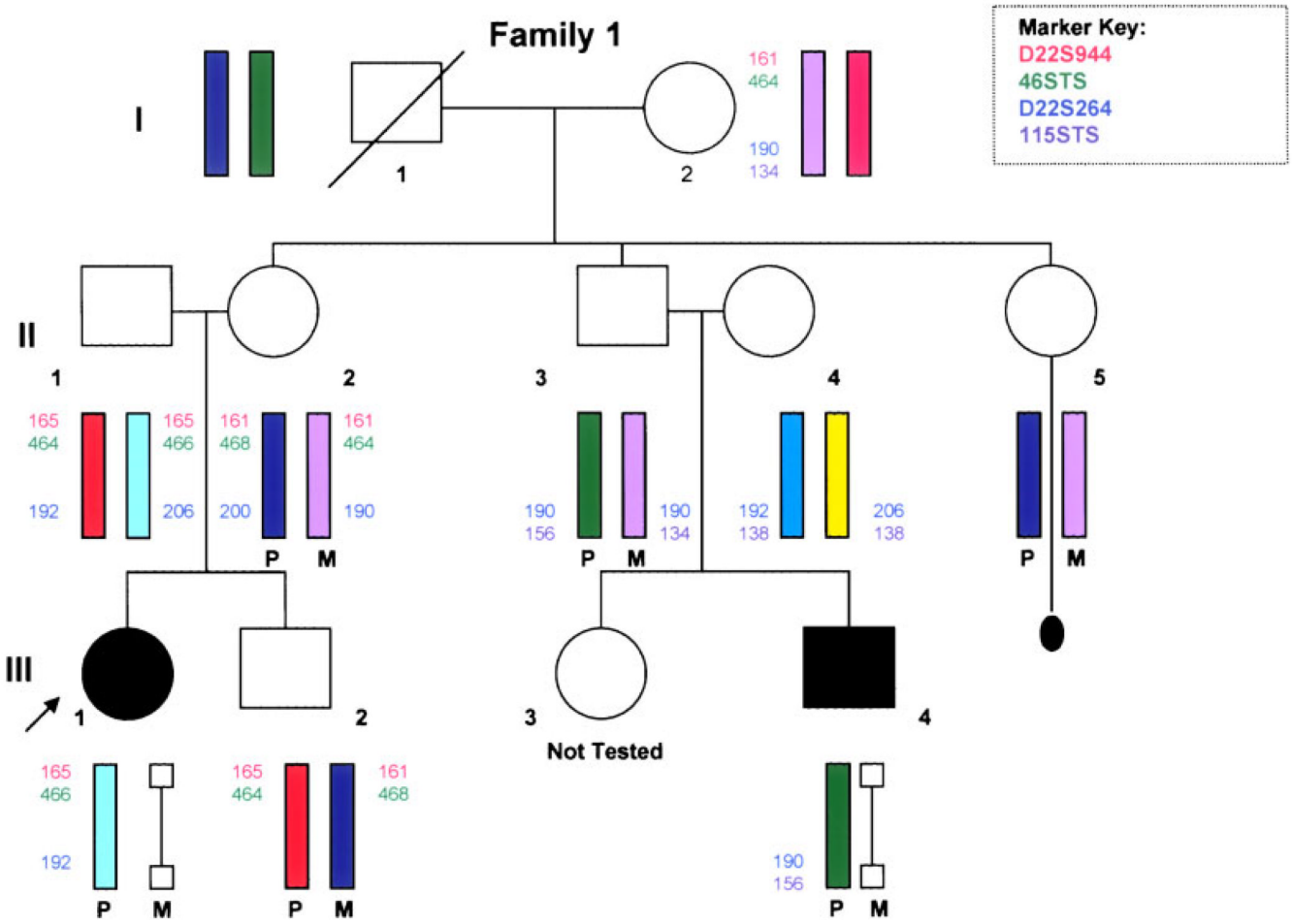

Fig. 1.

Haplotype analysis and pedigree of Family 1 with polymorphic allele sizes in base pairs. The markers used for the analysis are all located within the typical 3 Mb deleted region of 22q11.2 and are listed in the marker key. The proband is denoted by an arrow and deleted chromosomes are depicted by unfilled bars. The letter M (maternal) or P (paternal) beneath a chromosome indicates the parental origin of the alleles and chromosome. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

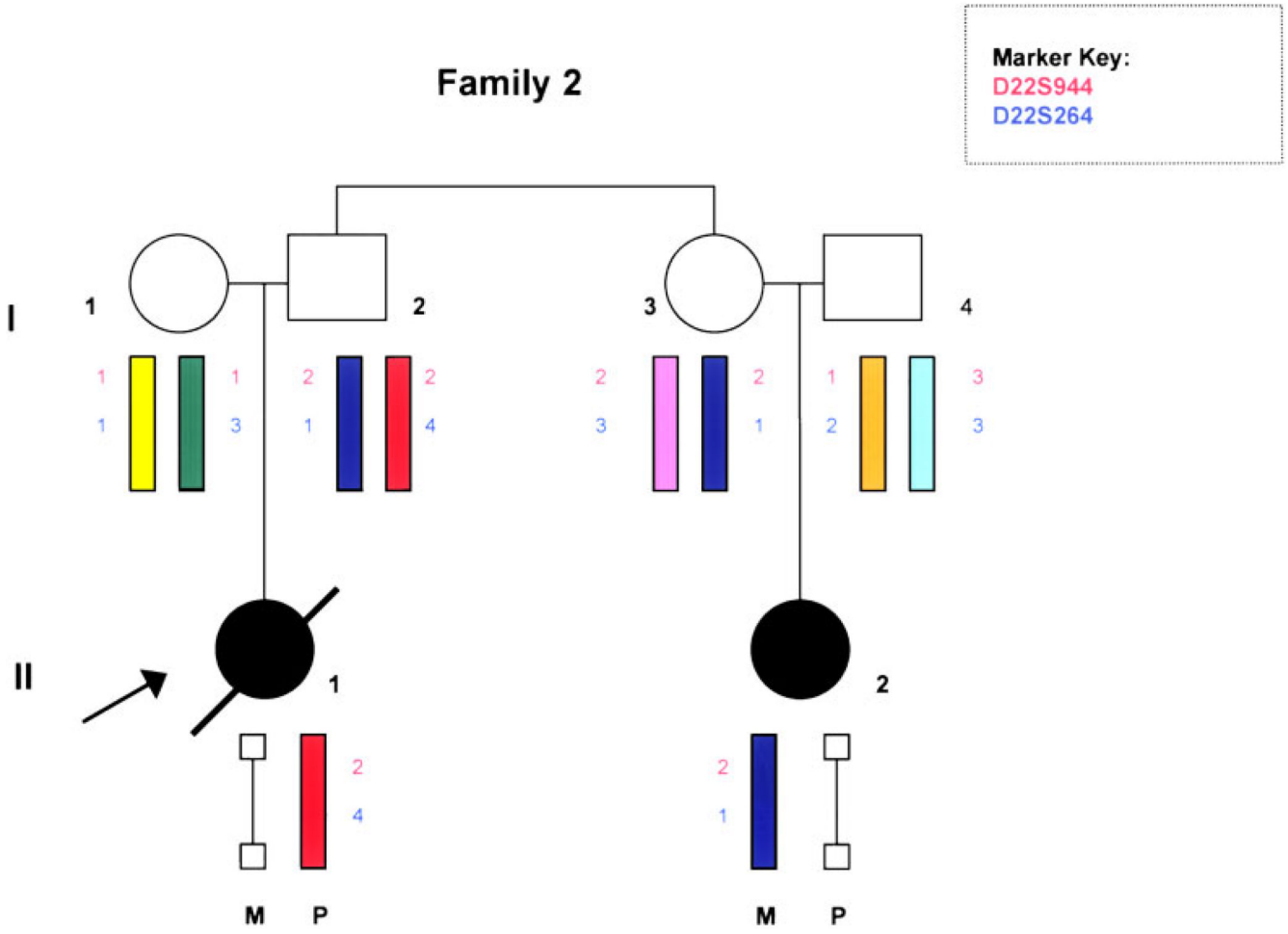

Fig. 2.

Haplotype analysis and pedigree of Family 2, with numbers designating the inherited alleles. The proband is designated by an arrow, and maternal (M) or paternal (P) origin of a chromosome is noted. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]