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Kousseff Syndrome Caused by Deletion of Chromosome 22q11-13

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Kousseff syndrome was originally described by Boris Kousseff in 1984 in three siblings whose main features were conotruncal heart defects, neural tube defects, and dysmorphic features. The proband is a white male who has spina bifida, shunted hydrocephalus, cleft palate, short stature, cognitive impairment, and the typical craniofacial features of velo-cardio-facial syndrome (VCFS), including low-set and dysplastic ears, broad base of the nose, narrow alae nasi, and retrognathia. The family history is significant for a brother who died at 2 weeks of age with myelomeningocele, hydrocephalus, transposition of the great vessels, and unilateral renal agenesis, and a sister who died at 11 days of age with myelomeningocele, truncus arteriosus, hypocalcemia, and autopsy findings of absent thymus and parathyroid glands, consistent with DiGeorge anomaly. Given the clinical findings, family history, and recent knowledge that open neural tube defects can occur in VCFS/DiGeorge anomaly, FISH analysis for 22q11-13 deletion was performed on the proband. A deletion was detected in him and subsequently confirmed in his father. Molecular analysis on autopsy material confirmed the deletion in the proband's deceased brother. We suggest that individuals with neural tube defects associated with other anomalies such as congenital heart defects or cleft palate be evaluated for 22q deletions. © 2002 Wiley-Liss, Inc.

KEY WORDS: **Kousseff syndrome; velo-cardio-facial syndrome/DiGeorge anomaly; deletion 22q11-13; neural tube defect**

INTRODUCTION

We report further studies in a family originally described by Boris Kousseff [1984] as a possible new autosomal recessive disorder associated with conotruncal heart defects and neural tube defects in two siblings who died in the neonatal period. A subsequent evaluation by fluorescence in situ hybridization of a sibling with a neural tube defect, cleft palate, and dysmorphic features revealed a 22q11-13 microdeletion which he inherited from his father. Molecular analysis using autopsy material confirmed this deletion in one of the older deceased siblings. This report adds to the clinical spectrum of velo-cardio-facial syndrome/DiGeorge anomaly and recommends consideration of this diagnosis in any individual with a neural tube defect.

CLINICAL REPORTS

Patient II-4 (The Proband)

This was the couple's fourth child. Amniotic fluid alpha fetoprotein levels were elevated in the pregnancy, but sonograms at 15, 17, and 22 weeks failed to demonstrate the suspected open neural tube defect. Prenatal karyotype was normal 46,XY. The infant was born at term and weighed 3,150 grams (50th centile). Birth length was 48.5 cm (25–50th centile) and occipitofrontal circumference (OFC) 34 cm (25th centile). Apgars were 5 at one and five min and 6 at ten min. He was found to have a lumbosacral myelomeningocele, cleft palate, and dysmorphic features consisting of low-set and posteriorly rotated ears, retrognathia, and clinodactyly of the fifth toes. Placement of a ventriculoperitoneal shunt for hydrocephalus and closure of the myelomeningocele was performed on day three. Developmental milestones were delayed, with sitting at 2 years, walking at 5 years, and using two-word sentences at 4 years. He has required speech and

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physical therapy in addition to special education classes throughout school. He had pharyngeal flap surgery at age 12 years and a spinal fusion for scoliosis at age 13 years. The scoliosis has recurred. He also has myopia and moderate hearing loss. Upon evaluation in the Genetics Clinic at the age of 15 years, height was 129.5 cm (<5th centile), weight 27.5 kg (<5th centile), and OFC 50.8 cm (<5th centile). Dysmorphic features included bifrontal bossing, dysplastic and low-set ears, broad base of the nose, hypoplastic nares, a high arched and narrow palate, retrognathia, short neck with low hairline, and severe scoliosis (Fig. 1). VCFS was suspected. Fluorescence in situ hybridization (FISH) analysis indicated that he has a 3-megabase deletion of the VCFS/DiGeorge Critical Region (DCR).

Patient II-2

This male child was the second pregnancy of his then 29-year-old mother. He was born at term by spontaneous, vaginal delivery after an uncomplicated pregnancy with no teratogenic exposures. The infant was cyanotic at birth with Apgar scores of 6 and 7 at one and five min, respectively. Birth weight was 3,317 grams (50–75th centile), length 49.5 cm (25–50th centile), and OFC 33.5 cm (25th centile). Examination at birth revealed a one-centimeter lumbosacral myelomeningocele. Heart disease was suspected because of cyanosis and a heart murmur later diagnosed as transposition of the great vessels with a ventricular septal defect. Minor dysmorphic features noted were low-set and posteriorly rotated ears, retrognathia, short neck, low hairline, and bilateral single palmar creases. Ultrasound of the kidneys revealed agenesis of the left kidney and ureter and right hydronephrosis with hydroureter. At 72 hr of age, a ventriculoperitoneal shunt was placed and the neural tube defect repaired. Postoperatively he developed seizures and continued to suffer from congestive heart failure which led to death at 14 days of age. The autopsy report confirmed these congenital anomalies but did not comment on the thymus. Karyotype was 46,XY.

Patient II-3

This female was born approximately 1 year after the death of II-2. Pregnancy, labor, and delivery were uncomplicated. Apgars were 8 at one and five min. Birth weight was 3,200 grams (50th centile), birth length 51 cm (75th centile), and OFC 34.5 cm (50–75th centile). The infant had a two-by-two-centimeter sacral myelomeningocele in addition to minor dysmorphic features

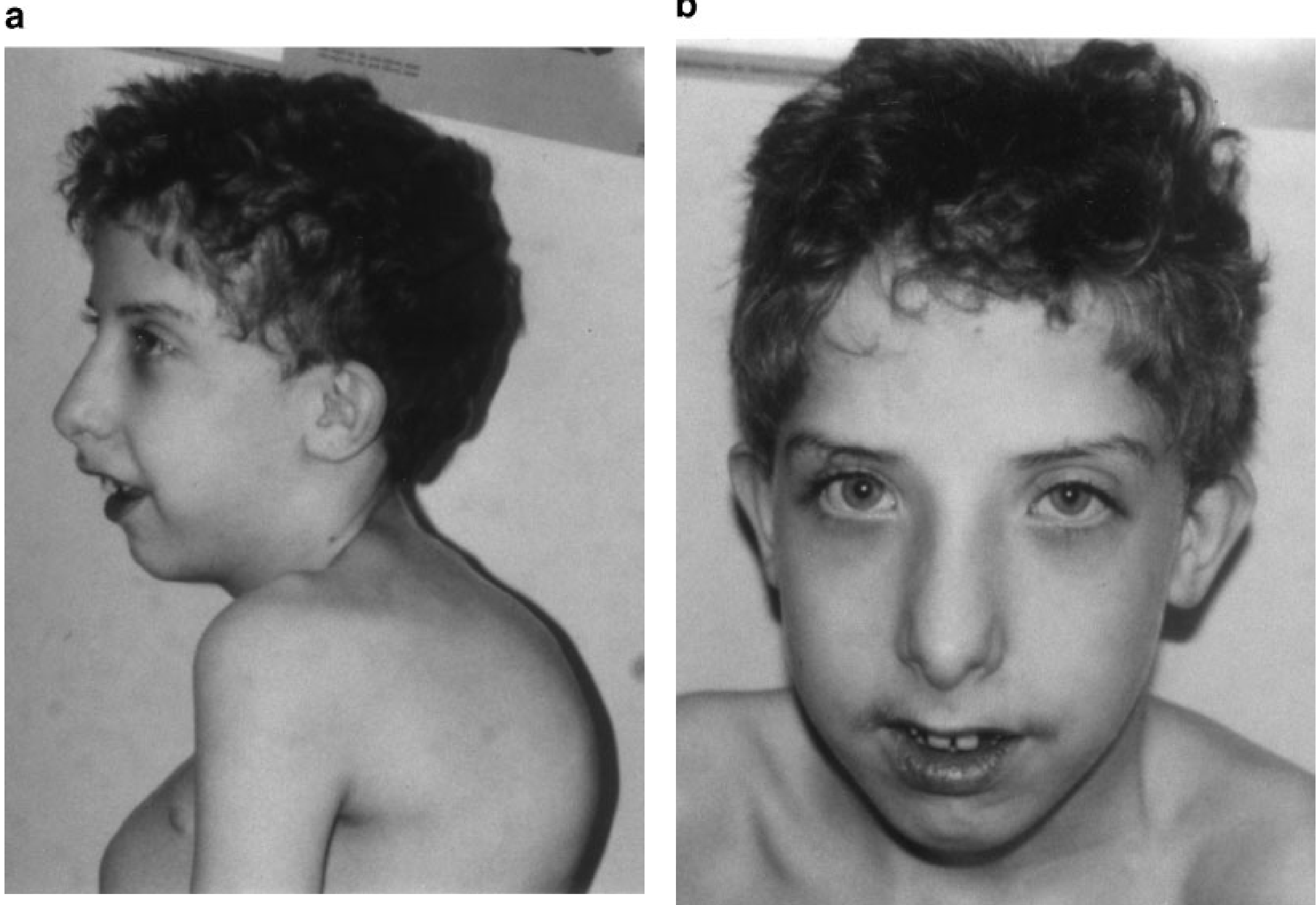

Fig. 1. Proband at age 15 years showing broad nasal bridge, low-set dysplastic ears, and micrognathia.

consisting of low-set ears, mild retrognathia, and short neck. The infant became tachypneic and cyanotic and congestive heart failure ensued. Cardiac catheterization demonstrated a truncus arteriosus type I. Additionally, the infant developed hypocalcemia and absence of T cells was established. The infant died 11 days after surgery to repair her cardiac defect. Autopsy revealed absent parathyroid and thymus. Although DiGeorge anomaly was taken into consideration, the autopsy report concluded that there was insufficient evidence to confirm this diagnosis. Karyotype was normal 46,XX. A sample of brain tissue in formalin was subjected to FISH, unsuccessfully.

FAMILY HISTORY

The family pedigree is shown in Figure 2. Both parents (I-1 and I-2) are healthy. The father has a learning disability and no physical features suggestive of VCFS. The mother tested negative and the father tested positive by FISH for the 22q11deletion. Molecular studies confirmed the same size deletion identified in his son (II-4). II-4 has three healthy siblings with cognitive impairment and another sister with androgen insensitivity. There is a paternal half-aunt and half-uncle who have cognitive impairment, but they have not been tested. Otherwise the family histories are negative for cleft lip, cleft palate, neural tube defects, and congenital heart defects.

MOLECULAR ANALYSIS

Buccal scrapings were collected from participating family members and processed using standard methods. For individuals II-2 and II-3, autopsy material provided the source of DNA used in genetic analysis. Specifically, genomic DNA was extracted from 4-micron histology slides. A modified extraction protocol was developed, using a combination of published tissue extraction protocols [Diaz-Cano et al., 1997; Howe et al., 1997; Weirich et al., 1997]. Briefly, tissue sections were deparaffinized with two 10-min extractions in fresh xylene. The samples were then dehydrated in serial washes of 100% ethanol, 10 min each, and allowed to dry. The cells were collected from the slides with 200 μL of proteinase K digestion buffer [10 mM Tris-HCl (pH 7.8), 5 mM EDTA, 0.5% SDS] containing 8 μL of proteinase K (10mg/mL). Proteinase K digestion proceeded for 5 days at 55°–65°C, during which 8 μL of fresh proteinase K was added daily. At the end of the digestion period, the proteinase K was inactivated by heat denaturation at 94°C for 15 min. At this time, soluble protein was precipitated by the addition of 50 μL saturated NaCl solution. The sample was vortexed for 5 min, followed by centrifugation at 4,000 rpm for 15 min at room temperature. The DNA fraction contained in the supernatant was precipitated with ethanol using 10 μg yeast tRNA as carrier nucleic acid followed by resuspension in a final volume of 75 μL water.

DNA was amplified by using PCR with the following microsatellite markers: D22S420, D22S427, D22S941, D22S944, D22S264, and D22S425. Markers D22S427, D22S941, D22S944, and D22S264 map to the VCFS/DiGeorge loci on chromosome 22 with areas corresponding to markers D22S941 and D22S944 being the most frequently deleted [Bonnet et al., 1997]. Markers D22S 420 and D22S425 map proximal and distal, respectively, to the 22q deletion and were chosen as controls. Amplification conditions for markers D22S420, D22S427, D22S941, D22S425 were 1) 95°C for 45 sec; 2) 55°C for 45 sec; and 3) 72° C for 45 sec for a total of 35 cycles. Markers D22S944 and D22S264 were amplified using 35 cycles at 1) 95°C for 45 sec; 2) 60°C for 45 sec; and 3) 72°C for 45 sec. Amplification products prepared in a denaturing formamide loading solution were analyzed on a 6% denaturing polyacrylamide gel by electrophoresis for 1 to 2 hr. The results were then visualized with an FMBIO-100 fluorescent image-scanning unit [Hitachi; South San Francisco, CA].

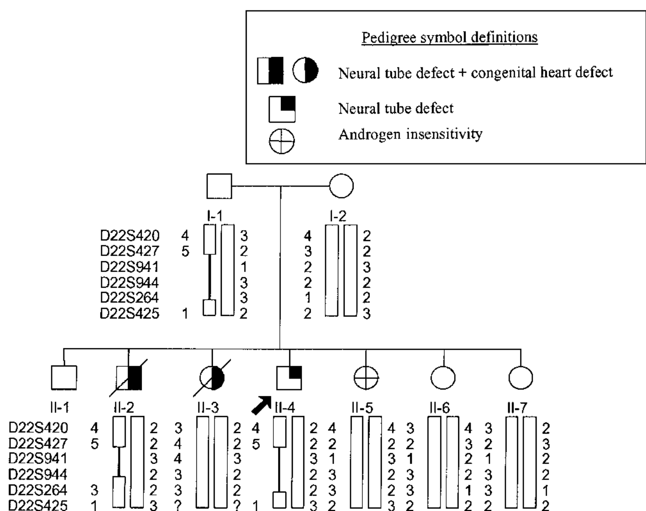

Fig. 2. Pedigree of family with chromosome 22q11-13 deletion showing genotype and haplotype analysis for markers within and surrounding the common deletion region of VCFS. Males are denoted by squares and females by circles. The proband is denoted with an arrow. Individuals I-1 and II-4 had the 22q deletion confirmed by FISH. Markers are listed in order from centromere to telomere on the long arm of chromosome 22. Haplotypes are represented by white bars. A gap in the haplotype, symbolized by a solid black line, represents deletions indicated by loss of heterozygosity identified in I-1, II-2, and II-4.

MOLECULAR RESULTS

Eight family members were genotyped with a series of microsatellite markers within the VCFS deletion region on chromosome 22q. A PCR based method for detecting chromosomal deletions was employed because two samples were not amenable to FISH analysis. The proband and his father were determined to have a chromosome 22q deletion by FISH analysis and served as controls in this study. These individuals showed a loss of heterozygosity for markers D22S941, D22S944, and D22S264. It is clear from this analysis that the proband inherited only his mother's alleles and inherited the father's allele with the deletion. Two siblings of the proband (II-2 and II-3) were suspected to have inherited the deletion from their father based on clinical findings and autopsy reports. Genotype analysis of DNA extracted

from archival material confirms the deletion in the older sibling (II-2) for markers D22S941 and D22S944. This individual however, was heterozygous for marker D22 S264, probably suggesting that the primers amplified another region in the genome since II-4 showed loss of marker D22S264. Analysis of individual II-3 gave unexpected results. She was heterozygous for all markers tested and did not appear to inherit the disease or non-disease allele from her father. These results indicate a possible inaccuracy in sample labeling or retrieval, non-paternity, or technical error in DNA extraction and analysis (however, these results were confirmed from three separate extractions and analyses of the same archival material). Molecular studies were done in autopsy samples obtained more than 20 years ago. Because of the clinical features suggestive of DiGeorge syndrome in her, non-paternity is considered less likely than the other possibilities.

DISCUSSION

We report a family previously described by Kousseff in 1984. At that time, it was thought that this family represented a possible new autosomal recessive condition consisting of sacral myelomeningocele, conotruncal heart malformations, and anomalies of the head and neck later to be termed “Kousseff syndrome”. The features of the proband along with the family history prompted testing for VCFS/DiGeorge anomaly, which was positive in the proband, father, and one deceased sibling. Toriello et al. [1985] subsequently published a report of a child with features of Kousseff syndrome including a neural tube defect, hydrocephalus, truncus arteriosus type I, and dysmorphic features consisting of depressed nasal tip, retrognathia, low-set and posteriorly rotated ears, and long fingers. It is possible that this also represents a case of VCFS/DiGeorge anomaly due to deletion 22q11.

VCFS/DiGeorge anomaly is known to be caused by a microdeletion on chromosome 22. Shprintzen originally described the syndrome in 1978 when he reported patients with a multiple malformation syndrome with main features being velopharyngeal insufficiency or cleft palate, conotruncal cardiac anomalies, learning disabilities or mental retardation, and a characteristic facial appearance [Shprintzen et al., 1978]. Minor dysmorphic features of VCFS include long face with a prominent nose with a squared nasal root, malar flatness, and long slender hands with tapering fingers. The affected children tend to be small and microcephaly is present in 40% [Nickel et al., 1994; Jones, 1997].

The DiGeorge anomaly is a developmental field defect of the third and fourth pharyngeal pouches. Main features of this condition include absent or hypoplastic thymus, and parathyroid glands with decreased cell mediated immunity, hypocalcemia, and conotruncal cardiac malformations [Goldberg et al., 1993; Nickel et al., 1994; Wulfsberg et al., 1996]. Van Mierop and Kutsche [1986] report that the most common congenital heart defects in the DiGeorge anomaly are conotruncal defects. In 50 children with the DiGeorge anomaly, 38% had type B interrupted aortic arch, 26% had truncus arteriosus, 20% had tetralogy of Fallot, 8% had ventricular septal defect, and 6% had transposition of the great vessels. Dysmorphic features include small, posteriorly angulated ears, micrognathia, and hypertelorism [Goldberg et al., 1993; Jones, 1997]. Over 90% of cases are caused by a microdeletion on chromosome 22q11-13.

Neural tube defects have been previously reported in association with VCFS/DiGeorge anomaly. Nickel et al. [1994] reported a child with the DiGeorge anomaly and two children with VCFS, all of whom were unrelated and had meningomyelocele. All three have molecular deletions within the DiGeorge Critical Region (DCR). Palascios et al. [1993] additionally reported a case of the DiGeorge anomaly with meningomyelocele, but cytogenetic and molecular studies were not reported. McDonald-McGinn et al. [2001] reported a family where two siblings expired prior to the identification of the 22q11.2 deletion in a sibling and parent. One of the children was diagnosed with the DiGeorge anomaly on autopsy, and the other died secondary to complications of a neural tube defect. They also report an additional individual with VCFS and neural tube defect diagnosed at The Children’s Hospital of Philadelphia, PA [McDonald-McGinn, personal communication].

Many authors have commented on the intra- and interfamilial phenotypic variability seen in individuals with VCFS/DiGeorge anomaly. Vincent et al. [1999] reported female monozygotic twins with 22q11 deletions and dysmorphic features of VCFS and immunodeficiency of the DiGeorge anomaly; however, only one had a cardiac defect. The authors stated that this was the fourth report of a discrepancy between cardiac status in monozygotic twins with 22q11 deletions, suggesting that expression of the phenotype is influenced by nongenetic factors. The possible epigenetic influences contributing to the pleiotropy seen in the deletion 22q11 syndrome have yet to be elucidated. There have been reports of features of the DiGeorge anomaly in children exposed in utero to alcohol or retinoic acid [Ammann et al., 1982; Lammer et al., 1985].

Given the difficulty in assessing the contribution of genetic factors on the phenotypic variation seen in affected humans, Taddei et al. [2001] engineered a mouse model with a deletion (*Df1*) that encompasses 18 mouse homologs of genes deleted in deletion 22q11. As is seen in human patients, heterozygous mice (*Df1*/+) show reduced penetrance of 22q11 deletion-related cardiac defects. Through a series of complex matings between *Df1*/+ mice and inbred C57BL/6 and inbred 129SvEv mice, the authors were able to conclude that the penetrance of cardiovascular defects varies widely in different genetic backgrounds, since inbred 129SvEv *Df1*/+ embryos had a lower prevalence of cardiovascular anomalies (16.1%) than *Df1*/+ embryos on a mixed background (32%) and inbred C57BL/6 *Df1*/+ embryos (50%). Additionally, the authors state that while allelic variation within the haploid locus in *Df1*/+ mice and in heterozygous humans may be a genetic basis of phenotypic variability, it does not account entirely for the increased penetrance of cardiovascular defects in the C57BL/6 background given their results. Additionally,

the thymic and cardiovascular defects did not always occur together as is the case in affected humans, suggesting that the two phenotypes may be affected by independent genetic modifiers.

In conclusion, our family, in addition to these aforementioned ones, displays the intrafamilial variability seen with VCFS/DiGeorge anomaly due to deletion 22q11 and supports the suggestion that any patient with a neural tube defect and/or conotruncal heart defect should be tested for a microdeletion of 22q11-13 [Nickel and Magenis, 1996]. Since there is great clinical variability, one may also wish to consider testing individuals with a neural tube defect and first degree relative with features suggestive of VCFS/DiGeorge anomaly such as a conotruncal heart defect. This family demonstrates the need for accurate diagnosis for proper genetic counseling, since the recurrence risk for the parents of the affected children was changed from autosomal recessive to autosomal dominant mode of inheritance with an option of prenatal diagnosis in future pregnancies.

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