

Clinical Report

Velocardiofacial Syndrome in an Unexplained XX Male

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We report the unusual finding of velocardiofacial syndrome (VCF) in an unexplained 46,XX male. A microdeletion of 22q11.2 was confirmed by fluorescence in situ hybridization (FISH) analysis. Routine G-banded chromosome analysis revealed an XX sex chromosome constitution. FISH was performed using the SRY probe and failed to detect hybridization. The sex chromosome status of the patient was further investigated by PCR testing to screen for the presence of 24 distinct loci spanning the Y chromosome. PCR screening failed to detect any apparent Y chromosome material.

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INTRODUCTION

Velocardiofacial syndrome (VCF) is a well-described entity characterized by cleft palate, cardiac defects, speech and learning deficiencies, and typical facial appearance [Kelly et al., 1993]. Microdeletions of chromosome 22q11.2 are observed by high-resolution chromosome analysis in only about 20% of cases, but can be detected by fluorescence in situ hybridization (FISH) in over 75% of affected individuals [Driscoll et al.,

1993]. Although most microdeletions associated with VCF occur sporadically, it is estimated that from 8% to 28% of deletions are inherited from a carrier parent [Driscoll et al., 1993; Hall, 1993; Ryan et al., 1997].

In the present case, a six-year-old black male with hypernasal speech was referred for chromosome analysis to rule out VCF. A microdeletion of 22q11.2 consistent with velocardiofacial syndrome was confirmed by FISH. Analysis of G-banded chromosomes revealed the unexpected finding of an XX sex chromosome constitution. Attempts to detect Y chromosome material in this individual using molecular genetic techniques were unsuccessful; hence he is considered an "unexplained" XX male.

CLINICAL REPORT

MM was the 2,665 gram product of a term pregnancy delivered by C-section due to decreased fetal heart rate. Pregnancy was otherwise uncomplicated. MM walked at 11 months but did not say his first clear words until three years of age. At six years of age, his head circumference was 50.7 cm (50th centile). He had mild hypertelorism, mild epicanthal folds, and a rather broad nasal root. His ears were small and cupped. His palate was arched with no submucous cleft by palpation. He had severe hypernasality.

Genitalia were normal for age with both testes descended. The extremities revealed mild joint laxity. There was no apparent heart defect.

Cytogenetic Studies

A peripheral blood sample was obtained for chromosome analysis and fluorescence in situ hybridization studies to rule out velocardiofacial syndrome. Chromosomes were examined by G-banding and revealed a 46,XX chromosome constitution. Fluorescence in situ hybridization was performed on metaphase cells using the D22S75 probe (Oncor #P5140, Gaithersburg, MD) and revealed a deletion of the locus detected by the probe. Because of the discrepancy between the phenotypic sex and the chromosomal sex of MM, a repeat blood

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sample was obtained and confirmed an XX sex chromosome constitution with deletion at 22q11.2. To determine if a cryptic translocation involving the male determining region of the Y chromosome was present, metaphase FISH was performed using the *SRY* probe. No evidence of hybridization was observed, suggesting that the *SRY* region was not present. While no evidence of mosaicism for a cell line containing a Y chromosome was observed in either of the two blood samples, fibroblasts or other tissues were not available for study. Parental karyotypes were normal with no evidence of a microdeletion at 22q11.2.

Molecular Studies

DNA studies were performed using 24 Y specific sequences spanning the Y chromosome. The loci tested were *SRY* (G38356), *RPS4Y* (G38351), *ZFY* (G38352), *DYS252* (G12010), *sY211* (G38342), *DYS257* (G38358), *RBMY1A1* [Ma et al., 1992], *TSPY* (G38360), *DYS260* (G66515), *PRKY* [Schiebel et al., 1997], *AMELY* (G38362), *DYZ3* (G38359), *DYS274* (G49205), *KALP* (G38357), *STSP* (G38361), *SMCY* [Agulnik et al., 1994], *DYS212* (G38341), *DYS231* (G38347), *DAZ* (G38349), *sY148* (G66539), *sY202* (G38340), *DYS241* (G12006), *DYZ2* (G38354), and *DYZ1* (G38343). MM was negative for all of the loci tested, suggesting that he does not carry Y chromosome material.

DISCUSSION

Velocardiofacial syndrome is characterized by cleft palate or velopharyngeal insufficiency (40–60%), conotruncal heart defects (75–85%), speech and learning difficulties (90–100%), and typical facial appearance including straight nose with narrow alar flare (75%), malar flattening (70%), narrow palpebral fissures (68%), and minor ear anomalies (60–70%) [Ryan et al., 1997; Thomas and Graham, 1997; Leana-Cox et al., 1996]. VCF was first described by Shprintzen et al. in 1978 but it was not until 1992 that the association with microdeletions of chromosome 22 was reported [Shprintzen et al., 1978; Driscoll et al., 1992; Scambler et al., 1992]. The incidence of microdeletions of 22q is estimated to be about 1/4,000 to 1/5,000 individuals [Wilson et al., 1994; Swillen et al., 2000]. Although most cases are sporadic, it is estimated that at least 8% of deletions are inherited from a carrier parent [Driscoll et al., 1993]. An unexpectedly high deletion rate of 28% among parents was reported by Ryan et al. [1997]. In their series of 558 patients with the deletion, 204 of 285 parents tested also had the deletion. The high deletion rate among the parents in this series may result from selection bias, since all parents were not tested and some parents were chosen for testing based on clinical suspicion [Ryan et al., 1997].

XX maleness occurs in about one in 20,000 males [Page et al., 1985]. XX males are classified as Y-positive or Y-negative depending upon the presence or absence of the sex-determining region (*SRY*) of the Y chromosome [Ferguson-Smith et al., 1990]. In Y-positive XX males, Y chromosome-specific material is present, typically on

the distal short arm of the X chromosome [Anderson et al., 1986; Page et al., 1987]. During male meiosis, obligatory crossing-over occurs between the pseudoautosomal regions on the distal short arms of the X and Y chromosomes. In Y-positive XX males, an abnormal exchange takes place such that the Y-specific region containing the *SRY* gene is involved in the unequal recombination. This abnormal interchange results in the translocation of both the pseudoautosomal region and the *SRY* gene to the distal short arm of the X chromosome [Wang et al., 1995].

About 90% of XX males are Y-positive [Zentano et al., 1997]. These males are sterile with small testes and they may have some degree of feminization, such as mild breast enlargement similar to that seen in Klinefelter syndrome. They do not generally have ambiguous genitalia or other congenital anomalies. Although most cases of Y-positive maleness are sporadic, affected brothers have been reported [Page et al., 1985].

In Y-negative XX males, no Y-chromosome specific material can be detected. It is speculated that autosomal or X-linked mutations may account for testicular determination in the absence of *SRY*, although the possibility of mosaicism or chimerism for an XY cell line must be considered [Ferguson-Smith et al., 1990]. About 10% of males with a 46,XX karyotype and most 46,XX hermaphrodites appear to carry no part of the Y chromosome. These males are invariably sterile. Many of the Y-negative XX males and XX hermaphrodites have ambiguous genitalia although others show normal masculinization. Congenital anomalies are generally limited to abnormalities in sexual development. The recurrence risk for Y-negative XX maleness is significant with several reports of affected brothers [Abbas et al., 1990; Numabe et al., 1992; Zentano et al., 1997].

Recently, Velagaleti et al. [2000] described deletion of 22q11.2 and patent ductus arteriosus in an individual with Klinefelter syndrome (47,XXY). The present case is the first report of an unexplained XX male with the velocardiofacial syndrome. The occurrence of these two disorders in a single individual raises the possibility that the two phenotypes might be etiologically related. That the deletion of chromosome 22 could also affect a gene that results in XX sex reversal seems unlikely. Numerous cases of VCF and del(22)(q11.2) have been reported, yet no previous cases with sex reversal have been described.

Based on an incidence of 1/5,000 for VCF and 1/20,000 for XX maleness, one would predict a one in 10,000,000 chance that these two conditions would occur concomitantly in a single individual. Although certainly a rare occurrence, it raises an interesting point regarding FISH testing: if the phenotype of an individual strongly suggests a microdeletion that is easily detectable by FISH but infrequently detected by G-banded analysis, is G-banded analysis warranted? In the present case, FISH studies alone would have detected the microdeletion of 22q11.2, but the sex chromosome abnormality would have gone undetected. Thus, although a particular phenotype is strongly suggestive of a specific microdeletion syndrome, performing FISH without a G-banded analysis could result in the misdiagnosis,

or missed diagnosis, of other clinically significant disorders.

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