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ORIGINAL INVESTIGATION

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Anesthesiologic problems in Williams syndrome: the CACNL2A locus is not involved

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Abstract We present the case of a patient affected with Williams syndrome (WS), who developed a suspected malignant hyperthermia (MH) reaction to general anesthesia. The proximity to the WS region of the gene encoding the L-type voltage-gated calcium channel α_2/δ-subunit (CACNL2A) on 7q11.23–q21.1, previously shown to be closely linked to some forms of MH susceptibility, prompted us to investigate whether this gene is deleted in WS. Linkage studies and fluorescence in situ hybridization analysis demonstrated that the CACNL2A locus is localized outside the WS deleted region.

Introduction

Williams syndrome (WS) is a well-known genetic disorder with an estimated incidence of 1 in 20000–50000 live births (Greenberg 1990). It is characterized by facial dysmorphism, moderate growth deficiency, cardiovascular anomalies (most frequently supravalvular aortic stenosis), and general cognitive impairment associated with a consistent behavioral feature, such as friendly loquacious personality. Furthermore, some WS patients have disturbed calcium homeostasis with hypercalcemia or symptoms compatible with hypercalcemia (Morris et al. 1988). Although extensively studied, the cause of this last finding is unknown. Recent genetic studies carried out to gain a better understanding of the variability in WS presentation at the molecular level have demonstrated hemizygosity at the elastin locus (ELN) on the proximal long arm of human chromosome 7 in the majority of WS cases (Lowery et al. 1995). However, since mutations within ELN are associated with familial nonsyndromic supravalvular aortic stenosis (Ewart et al. 1994), it has been suggested that WS with its composite phenotypic spectrum is in fact a contiguous gene deletion syndrome. It had previously been proposed that disturbances in the synthesis or release of calcitonin may be involved in WS (Culler et al. 1985), although involvement of the human calcitonin receptor gene (CALCR) at chromosome band 7q21.3 has recently been excluded (Perez Jurado et al. 1995). At present, other genes involved in the clinical presentation of WS remain to be identified. We describe the case of a classical WS patient with an aspecific fiber-type disproportion myopathy, whom we have followed up for 12 years, who developed high serum levels of muscle enzymes after general anaesthesia. The proximity to the WS region of the gene encoding the L-type voltage-gated calcium channel α_2/δ-subunit (CACNL2A) on 7q11.23–q21.1, previously shown to be closely linked to some forms of susceptibility to malignant hyperthermia (MHS) (Iles et al. 1994), prompted us to investigate whether this gene is also deleted in WS and whether its deletion may underlie the disturbances in calcium homeostasis observed in some WS patients.

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Materials and methods

Patient

FF is the second-born, 16-year-old son of healthy, unrelated parents. He was born vaginally at term after an uneventful pregnancy. Because neonatal weight and length were at the 3rd centile, he was admitted at birth to a neonatal care division, where a ventricular septum defect was detected. Routine laboratory tests, including serum calcium concentration, were normal. His growth was delayed in the first 2 years of life and psychomotor development was retarded.

The clinical diagnosis of WS was made at the age of 4 years 10 months, on the basis of characteristic dysmorphic features (epicanthal folds, flat nasal bridge, antiverted nostrils, opened mouth with

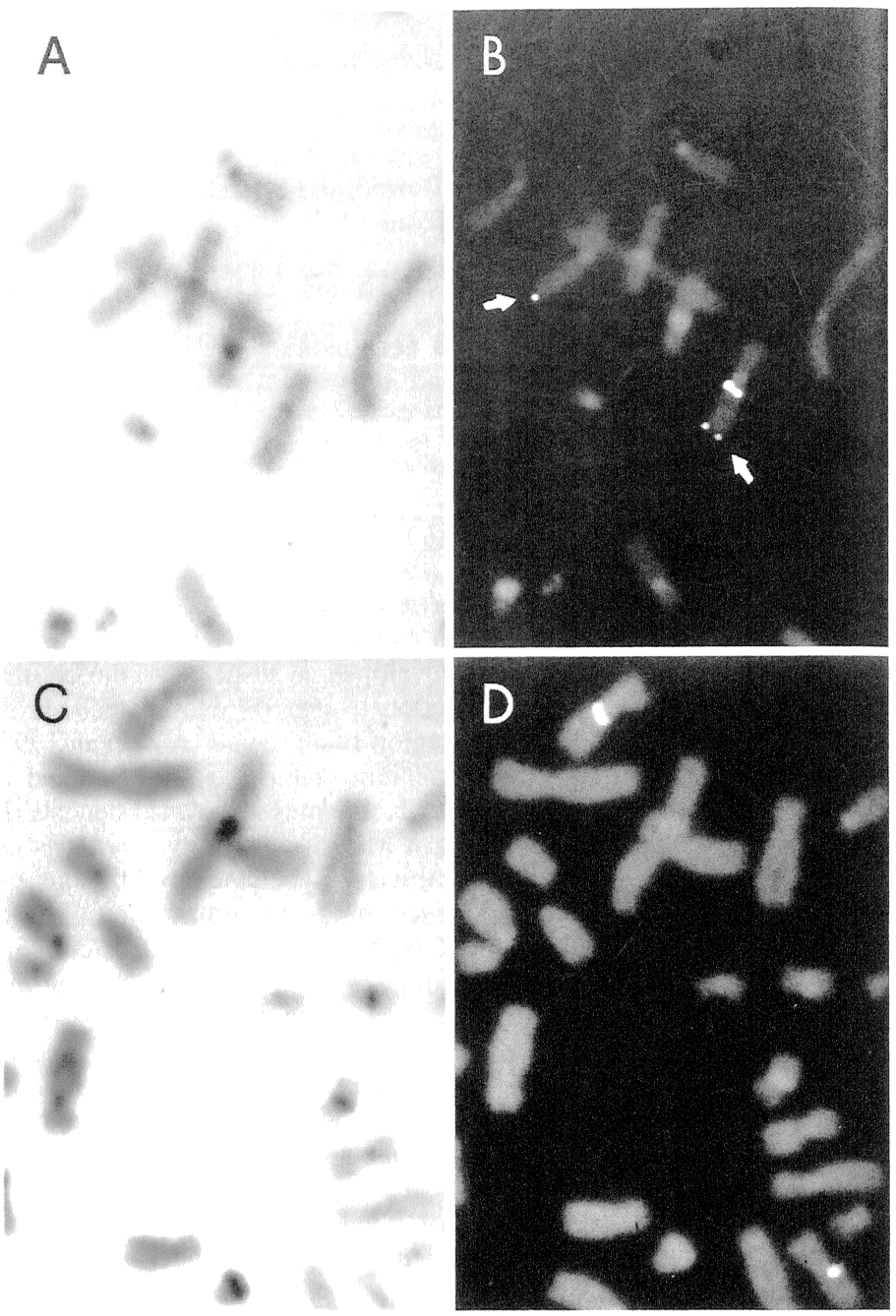

Fig. 1A–D Fluorescence in situ hybridization of probes P5155 (ELN), D7S427 and HSCE520 (CACNL2A) to metaphase spreads from the Williams syndrome patient. **A, C** 4′6-diamidino-2-phenylindole staining of metaphase spreads for chromosomal identification. **B** Fluorescence signals from P5155 and D7S427 on the same metaphase spread as shown in **A**. Note the absence of P5155 hybridization on one of the chromosome 7 chromatid pairs. The presence of two chromosome 7 chromatid pairs in this spread is confirmed with probe D7S427, which hybridizes close to the telomeres (*arrows*). **D** Fluorescence signals from hybridized yeast artificial chromosome clone HSCE520 are clearly seen on both chromosome 7 chromatid pairs in the metaphase spread depicted in **C**, thus placing CACNL2A outside the region deleted in this WS patient

prominent full inferior lip), mild pectus excavatum, and clinodactyly of the fifth fingers. Neurological findings were mild dysmetria and ataxia, and moderate mental retardation; he had a friendly personality and the characteristic hoarse voice. Echocardiography at the age of 4 years revealed a spontaneous closure of the ventricular septum. High resolution chromosomal analysis was normal.

At the age of 6 years, he underwent an endoscopical extraction of a chicken bone from the esophagus, performed under general anesthesia, without any clinical complication.

After the operation, elevated serum concentrations of muscle enzymes were observed: creatine kinase (CK), 2565 U/l (normal range 24–195); glutamic-oxalacetic transaminase (GOT), 117 U/l (15–40); and lactate dehydrogenase (LDH), 552 U/l (230–460). Electromyography at the age of 8 years was normal, and serum CK and LDH levels at the ages of 9, 10, and 11 years were also normal. MH was therefore suspected, and muscle biopsy was performed at the age of 14 years. The results of caffeine and halothane in vitro contracture tests were normal. Histological examination showed mild atrophy of the type-I fibers with average diameter of type-I and type-II fibers of 47μ and 66μ, respectively, suggesting a fiber-type disproportion myopathy. Despite his MHN status, it was decided not to use succinylcholine for future surgery in this patient in case of defects in muscle metabolism or cell membranes. The child underwent orchidopexy surgery at the ages of 8 and 9 years in another hospital, without any reported problem.

Molecular studies

Inclusion of the CACNL2A locus in the WS deleted region was investigated using genetic analysis and fluorescence in situ hybridization (FISH). The markers D7S634 (7q11.23), D7S660 (7q11.23–q21.1), D7S849 (7q11.23–q21.1), D7S524 (7q11.23–q21.1) and D7S644 (7q21.1), known closely to flank the CACNL2A locus (Iles et al. 1994) were used to construct haplotypes for the patient and his immediate family as described previously (Iles et al. 1994). FISH analysis was carried out essentially as described elsewhere (Suijkerbuijk et al. 1991, 1992) using yeast artificial clone (YAC) clone HSCE520, known to contain the CACNL2A gene (Iles et al. 1994), cosmid P5155 containing the ELN gene (Lowery et al. 1995), and probe D7S427 (Oncor, Gaithersburg, USA) as a marker for sequences located close to the chromosome 7q telomere. Briefly, cosmid or total YAC DNA (1 μg) was labeled with digoxygenin-11-dUTP (Boehringer) using a Bio-nick labeling system (Life Technologies), separated from unincorporated label by Sephadex G-50 column chromatography and ethanol precipitated in the presence of a 50-fold excess of Cot-1 DNA (Gibco, Life

Technologies). Probe diluted to a final concentration of 10 ng/μl in hybridization buffer (50% v/v deionized formamide, 10% dextran sulfate, 2 × SSC, 1% v/v Tween-20, pH 7.0) was denatured, preannealed and hybridized to metaphase spreads prepared from peripheral blood lymphocytes using standard procedures. After denaturation of the slides, hybridization was allowed to proceed under an 18 × 18 mm coverslip in a moist chamber for 45 h. Immunocytochemical detection of hybridized probe was achieved using fluoresceinated isothiocyanate (FITC)-conjugated sheep anti-digoxigenin (Boehringer Mannheim). For evaluation of the chromosomal preparations a Zeiss epifluorescence microscope was used, equipped with filters for the visualization of Texas Red, 4′,6-diamidino-2-phenylindole (DAPI) and FITC fluorescence. Digital images were acquired using a high performance cooled CCD camera (Photometrics, Tuscon, USA) interfaced with a Macintosh IICi computer. The acquiring, processing and analysis of all digital images was achieved using the BDS-Image FISH software package (Biological Detection Systems, Rockville, USA).

Results

The results of FISH experiments using cosmid P5155 (containing the ELN locus) as probe clearly showed the presence of hybridization signals on only one pair of chromosome 7 chromatids (Fig. 1B). The presence of two chromosome 7 chromatid pairs in this metaphase spread was confirmed using probe D7S427, which hybridized close to the 7q telomere (arrows, Fig. 1B). In contrast, hybridization signals were clearly detected on both chromosome 7 pairs from the same patient using YAC clone HSCE520 (containing the CACNL2A locus) as probe (Fig. 1D). These results show that the ELN gene region has been deleted in this WS patient, while the chromosomal region around the CACNL2A locus is clearly present on both chromatid pairs, therefore placing the CACNL2A locus outside the WS deleted region.

Genetic analysis revealed the family to be informative for all the microsatellite markers tested but no loss of heterozygosity was observed in the WS patient (data not shown), suggesting that none of the markers tested are located within the WS deletion.

Discussion

Our WS patient developed abnormally high serum levels of muscle enzymes after general anesthesia, which spontaneously normalized. This episode was interpreted as possible MH, because only the presence of two or more adverse reactions (as generalized or masseter rigidity) is diagnostic for MHS (Larach et al. 1987).

MHS, a pharmacogenetic condition caused by a breakdown in the mechanism regulating sarcoplasmic calcium ion fluxes (Nelson 1988), has been associated with mutations in the gene (RYR1) encoding the calcium efflux channel of the skeletal muscle sarcoplasmic reticulum (ryanodine receptor) on chromosome 19q13.1 (MacKenzie et al. 1990). However, several non-chromosome 19q13.1-linked families have been reported, suggesting genetic heterogeneity in MHS (Ball and Johnson 1993). The ryanodine receptor forms a functional association with the L-type voltage-dependent calcium channel, also referred to as the dihydropyridine receptor (DHPR), which is essential for excitation-contraction coupling. The skeletal muscle DHPR is composed of five subunits (α_1, α_2, β_1, γ, δ), encoded by four distinct genes (Catterall 1991). Recently the gene encoding the α_2/δ-subunits (CACNL2A) has been localized to chromosome 7q11.23–q21.1 and linkage between markers flanking this region and MHS has been demonstrated in one of six MHS families tested (Iles et al. 1994).

This WS patient with suspected MH episode drew our attention, because of a possible connection between this self-limited anesthetic reaction and a disturbance in calcium regulation. Moreover, the reported localization of CACNL2A close to the WS chromosomal region made it a potential candidate for involvement in the Williams contiguous gene deletion syndrome. Alterations in CACNL2A gene expression could explain not only the reaction to anesthesia in our patient, but also the disturbances in calcium regulation observed in WS patients. However, our FISH results clearly show that while the ELN gene region is deleted in our WS patient, the CACNL2A locus lies outside the WS deletion. However, we cannot exclude the possibility that the genomic deletions causing WS alter chromosomal configurations or methylation patterns, thereby influencing the expression of neighboring genes, as suggested previously for the CALCR gene (Perez Jurado et al. 1995).

Our observation represents the second case of postanesthesia complications in a WS patient. A previous report described a 4-year-old WS boy presenting with laryngospasm and masseter spasm following anesthesia induced with halothane (Patel and Harrison 1991). There were no postoperative problems and the serum muscle enzyme levels were normal at 1 week after the operation. No muscle biopsy was carried out. A further report on WS in the literature on anesthesia describes a decreased difference of arterial pressure between arms (Coanda effect) induced by general anesthesia, performed for repair of undescended testis (Kato et al. 1989). The entire course of anesthesia was described as otherwise uneventful. However, underdiagnosis of postanesthesia complications is expected because the clinical features of the syndrome by themselves do not require surgery, and muscle enzymes are not generally being investigated.

Finally, the muscle biopsy from our WS patient showed the presence of mild myopathy, with histological aspects of fiber-type disproportion myopathy. Myopathic changes have been described previously in some WS patients (Voit et al. 1991), and episodes closely resembling MH have also been reported in several different neuromuscular disorders (Brownell 1988). Therefore, the high postoperative serum levels of muscle enzymes may have been due to the myopathic alterations in our patient, with muscle damage being triggered by the anesthetic drugs. For this reason we suggest that WS patients about to undergo surgery should be closely monitored and followed up.

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