Analysis of human sarcospan as a candidate gene for CFEOM1

ABSTRACT

In this report we evaluate sarcospan as a candidate gene for CFEOM1. We have found that it is highly unlikely that sarcospan is involved in the pathogenesis of this disease. As of yet no sarcospan gene mutations have been found to cause muscular abnormalities.

INTRODUCTION

Background CFEOM1 is an autosomal dominant disorder that has been linked to the pericentromere of chromosome 12, flanked by marker D12S1584 on the p arm and D12S1668 on the q arm. The clinical phenotype consists of congenital, bilateral ptosis and external ophthalmoplegia, with the eyes partially or completely fixed in a hypotrophic or downward position. On autopsy, CFEOM1 patients appear to be lacking the superior division of cranial nerve III, which innervates the levator and superior rectus muscles. Whether this disease is caused by a primary defect in the nerve or the muscle remains unclear. The disease was initially linked to an 8 centiMorgan region spanning the centromere of chromosome 12, and then further refined to a critical region of 3 cM. Yeast and bacterial artificial chromosome (YAC and BAC) contigs have been generated and a positional cloning approach to identify the CFEOM1 causative gene is ongoing. Sarcospan is a member of the dystrophin associated protein complex present in skeletal and extraocular muscle. Sarcospan is most tightly associated with the transmembrane sarcoglycan subcomplex, mutation of which causes autosomal recessive limb girdle muscular dystrophy (LGMD2C-2F). Primary mutation of α - δ sarcoglycan leads to a variable degree of secondary instability of sarcospan and the non-mutant sarcoglycans. Sarcospan is homologous to the tetraspanin superfamily, members of which have been shown to facilitate both integral-membrane and membrane-proximal protein interactions involved in many different cellular processes. Sarcospan had previously been identified as Krag, a gene that is co-amplified with Ki-ras in the Y1 murine adrenal carcinoma cell line. Portions of the gene's genomic structure were elucidated at that time, and the gene was localized to chromosome 12p11.2. Given the genomic localization of sarcospan in the critical region defined for CFEOM1, and its association with other proteins known to be involved in muscular diseases, sarcospan has been proposed as a candidate disease gene for CFEOM1. We have refined the previously published genomic structure of sarcospan more fully and screened for mutations in six families with CFEOM1. We have also generated antibodies that recognize human sarcospan and examined extraocular muscle samples from CFEOM1 patients. We find sarcospan to be unmutated in all six CFEOM1 families studied and sarcospan immunoreactivity to be identical in control and CFEOM1 extraocular muscle. Sarcospan is also shown to map electronically to BACs that are considered to be outside of the CFEOM1 critical region. These data make it unlikely that sarcospan, or other dystrophin associated proteins, are involved in the pathogenesis of CFEOM1.

CONCLUSION

Conclusions The DNA sequence analysis and protein immunofluorescence results that show sarcospan to be normal in CFEOM1 patients, combined with the localization of sarcospan to BACs that are outside of the CFEOM1 critical region, make it unlikely that sarcospan is

involved in CFEOM1.