

Presence of an expressed β -tubulin gene (TUBB) in the HLA class I region may provide the genetic basis for HLA-linked microtubule dysfunction

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Abstract. An expressed β -tubulin gene (TUBB) has previously been localized to chromosome region 6pter–p21 in man. By using a panel of deletion mutant cell lines and radiation-reduced hybrids containing fragments of chromosome 6, the TUBB locus could be mapped to the HLA class I region at 6p21.3. A long range restriction map including TUBB and several HLA class I genes was then generated by rotating field gel electrophoresis. The results show that TUBB maps to a segment 170–370 kb telomeric of HLA-C. This location suggests that a mutation at the TUBB locus could be the cause for certain forms of HLA-linked microtubule dysfunction, including immotile cilia syndrome.

Introduction

Immotile cilia syndrome (ICS) is characterized by dysmotility or even complete immotility of spermatozoa and cilia in ciliated epithelia. It is inherited as an autosomal recessive trait; several loci are responsible for the different forms of the disease, depending on which structure of the cilia is altered (Afzelius et al. 1981; Palmblad et al. 1984). A common cause is attributable to structural alterations of the microtubules (Sturgess et al. 1980). The most frequent consequences include recurrent airway infections from early childhood on, and male sterility; females may be either fertile or infertile. Furthermore, a dramatic consequence in about 50% of the affected individuals is *situs inversus* (Mc Kusick 1987), in which case ICS is also referred to as Kartagener's syndrome.

Microtubules are required by most eukaryotic cells to perform essential functions, such as chromosome segregation in mitosis or cellular movement and the maintenance

of cell shape during interphase. They consist of α/β tubulin heterodimers, which are closely related polypeptides. Four classes of β -tubulins, characterized by their amino acid sequence, have been described; the class I isotype is the major constitutively expressed β -tubulin (Lopata and Cleveland 1987). The human β -tubulin class I gene, TUBB (originally named M40 or h β 1), has previously been mapped to chromosome region 6pter–p21 (Floyd-Smith et al. 1986). This region covers about 50 Mb and encompasses the major histocompatibility (HLA) complex, localized at 6p21.3 (Ziegler et al. 1993). Bianchi and coworkers (1992) have recently presented evidence for the possible involvement of an HLA-linked gene in ICS. They described four children, with typical clinical symptoms of ICS, from two families. The affected individuals within a given family were HLA identical and were found to have abnormalities associated with microtubules in the cilia from the nasal mucosa. Based on the sib-pair test, Bianchi et al. (1992) have suggested linkage between this form of ICS and the TUBB locus. We demonstrate here that TUBB is indeed part of the HLA class I region, that it maps between HLA-C and HLA-E and that it may therefore be a candidate disease gene in certain forms of ICS.

Materials and methods

Cell lines

The deletion mutant cell lines employed were derived from the cloned Epstein-Barr-virus-infected B lymphoma line BJAB-B95.8.6 (HLA haplotypes A1, Cw4, B35; A2, C-, B13) (see Volz et al. 1992 for references). They exhibit interstitial deletions of the short arm at one of the two chromosomes 6 present.

The radiation hybrid cell lines used were derived from the human/hamster somatic cell hybrid R21B1, which contains one human chromosome 6 with an interstitial deletion of 6q21–q25.2 (Meese et al. 1992) and a Y chromosome (Ragoussis et al. 1991). R21B1 cells were irradiated, fused with the hamster cell lines A23N or W3GH, and cloned as described previously (Ragoussis et al. 1991).

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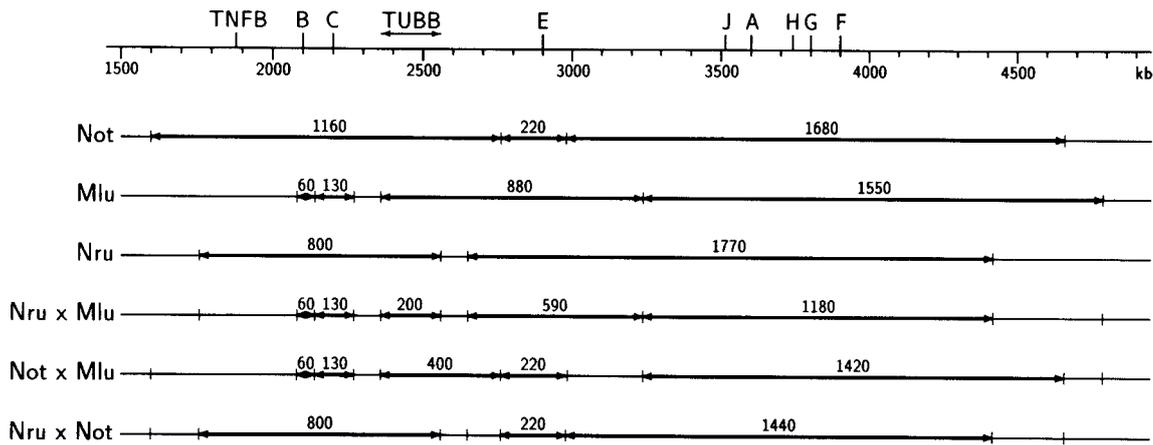


Fig. 1. Restriction map of the HLA class I region from the monosomy 6 mutant cell line BM19.7 (HLA-A2,C-,B13 haplotype)

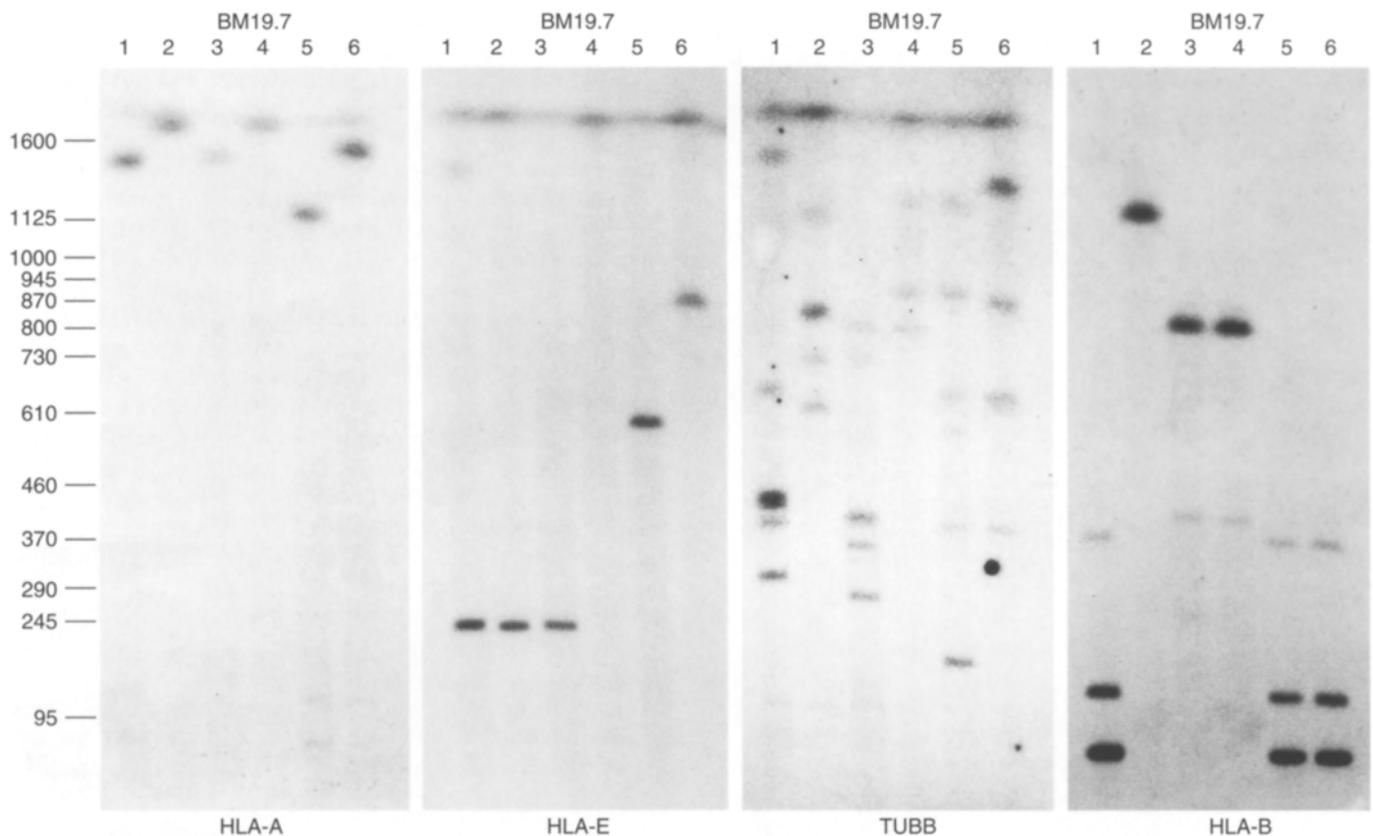


Fig. 2. Sequential southern hybridizations after ROFE with genomic DNA of the cell line BM19.7. DNA was digested with: lanes 1, *MluI* × *NotI*; lanes 2, *NotI*; lanes 3, *NotI* × *NruI*; lanes 4, *NruI*; lanes 5, *NruI* × *MluI*; lanes 6, *MluI*; fragment lengths in kb

The HLA-A probe revealed long, largely overlapping *NotI*, *NruI* and *MluI* fragments characteristic of the HLA-A2, B13 haplotype (Ragoussis et al. 1989). HLA-E was not present on any of the fragments detectable by the HLA-A probe, except on a large *NruI* fragment that contained HLA-A,-J,-H,-G and F (results not shown) (Gruen et al. 1992). This fragment showed overlapping with an

880-kb *MluI* fragment and gave rise to a 590-kb fragment after double digestion. Both the 880-kb and the 590-kb bands contained HLA-E. The IFGT-hybrid mapping results had indicated that TUBB might be located close to HLA-E. However, the 590-kb *MluI* × *NruI* double digestion product did not hybridize to TUBB, showing that, if this gene was part of the 880-kb *MluI* fragment, then it mapped centromeric of HLA-E in the other fragment generated by the double digestion, with a predicted maximal length of 290 kb. The only fragment with this maximal length in the double digest had a length of 200 kb and was not present in the *MluI* or *NruI* single digests. It hybridized to the TUBB probe but not to any other HLA

class I gene probe employed here. The HLA-B and HLA-C loci were found on a *NotI* fragment with a length of 1160-kb and on an 800-kb long *NruI* fragment. Both these fragments also hybridized to the TUBB probe and a double digest with these enzymes gave the expected (Ragousis et al. 1989) unchanged *NruI* pattern with HLA-B and with TUBB. The known distance between HLA-C and HLA-E (Gruen et al. 1992; Bronson et al. 1991) implied that the TUBB gene was on a 400-kb *NotI* × *MluI* double digestion fragment. This was indeed the case, and demonstrated, when combined with results from all other digestions, that, in the HLA-A2,B13 haplotype present within BM19.7 cells, TUBB maps between HLA-C and HLA-E, 170–370-kb telomeric of HLA-C and 320–520-kb centromeric of HLA-E.

Additional experiments with the R21B1 hybrid cells confirmed these observations: the loss of all human chromosomes, except the Y and the partially deleted single chromosome 6 in this hybrid cell line, gave a simplified hybridization pattern with TUBB. A comparison with BM19.7 using the TUBB probe showed that the *NruI* fragment predicted to contain TUBB in the HLA-A2, B13 haplotype of BM19.7 was the only one observed in R21B1. An analogous situation was observed for *NruI* × *MluI* double digestions, although the *MluI* single digest fragment of BM19.7 hybridizing with TUBB and with HLA-E was 400 kb longer than the equivalent R21B1 fragment. The long *NotI* fragment containing HLA-B,C and TUBB in BM19.7 cells also occurred in R21B1, further confirming the HLA-A2, C-, B13 restriction map (Fig. 1).

Discussion

All the results from the monosomy 6 mutant and the R21B1 human/hamster hybrid are in concordance and lead to the conclusion that TUBB is part of the HLA complex and that it maps between HLA-C and HLA-E. No locus unrelated to histocompatibility antigens has been mapped to the HLA class I region to date, apart from the gene for a putative G-protein (Denizot et al. 1992). Since this gene is localized to a 210-kb *NotI* fragment that also hybridizes with an HLA-E probe (determined to be 220 kb long in this study), it must map telomeric of TUBB.

Our data are compatible with the involvement of the TUBB gene in those forms of HLA-linked ICS in which microtubular function is impaired. Bianchi and coworkers (1992) have reported that all four children with ICS from the two investigated families expressed HLA-DR7 and HLA-DQ2, either in a heterozygous or homozygous manner. Since the distance between HLA-DRA and TUBB must be about 1500 kb, we anticipate that linkage disequilibrium within the HLA complex might account for the observed association. In one family, HLA-A29, C-, and B44 were also part of the DR7, DQ2-haplotype carried by the diseased children; the other haplotype was in both cases identical (HLA-A11, Cw4, B35, DR1, DQ1). In the other family, the two patients were HLA-identical (A26, Cw6, B13, DR7, DQ2; A30, Cw6, B13, DR7, DQ2). Both HLA-B13 and HLA-B44 are in strong linkage disequilibrium with HLA-DR7, and the same has been

observed for HLA-A30 and B13. Extended haplotypes involving other HLA class I and class II alleles, such as HLA-A1, Cw7, B8 and DR3, have been known for many years (Tiwari and Teraski 1985). The significant association of ICS with the HLA-DR7 antigen observed by Bianchi and coworkers (1992) is thus conceivably the consequence of haplotype conservation by linkage disequilibrium.

Since the mouse class I β -tubulin gene (M β 5) resembles TUBB (Lopata and Cleveland 1987), the apparently ubiquitous expression of the murine gene suggests that this may also be the case for TUBB. Thus, certain forms of ICS are consistent with a mutation in TUBB. Although it has been reported that different tubulin- β chains are co-assembled in functionally distinct microtubules in vivo (Lewis et al. 1987), it has been speculated that specialized "microtubule-associated proteins" might need the "right" $\alpha\beta$ tubulin for interaction. Additionally, the expression of β -tubulin isotypes is tissue specific and therefore substitution of the TUBB product by other isotypes may fail. To our knowledge, mutations affecting the TUBB locus have not been described to date. The reason for this could be that inactivation of the TUBB gene may be lethal because of its essential function in mitotic chromosome segregation in the embryo. Therefore, the presence of TUBB in the HLA region might be one of the factors responsible for the prevention of heritable losses of chromosomal material from the HLA complex. It will be interesting to investigate the conservation of this localization in different species, in addition to searching for mutated TUBB genes in individuals with HLA-linked forms of ICS.

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