...nts such as transposons. Moreover, the previous study of *MTS* did not include any brain specimens from patients with XDP. Therefore, the structure and expression of *MTS* transcripts are still unclear.

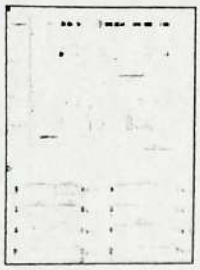

Figure 1.

Genomic sequencing analysis of the *DYT3* region. *a*, Physical map of the *DYT3* critical region on Xq13.1. Annotated genes in this region that have experimentally verified coding sequences (and their proteins) include *NLGN3* (neuroligin 3), *GJB1* (gap junction ...

We performed the following studies to reveal the disease-causative gene of XDP. To find all disease-specific mutations within the *DYT3* region, we first performed genomic sequencing analysis to accurately determine the complete DNA sequence of this region. We also performed detailed expression analysis of the gene in brain specimens obtained from patients with XDP, because the expression of disease genes can be tissue specific. To determine the complete structure of the disease gene, we used a library consisting of "full-length" cDNAs frequently containing their 5′ ends.[10]

Material and Methods

Go to:

Subjects and Samples

The study included 67 Filipino individuals (20 affected males) from 16 families residing in Panay. Information on all the patients is listed in table 1. All patients had the disease-specific haplotype between *DXS10017* and *DXS10018* in the *DYT3* region that had been defined elsewhere.[8,9] For construction of the BAC contig and portions of the RNA analysis, lymphoblastoid cells were immortalized by infection with the Epstein-Barr virus. This study involved a total of 137 healthy control subjects: 14 unrelated Filipinos, 44 Japanese, 38 African Americans, and 43 European Americans. Materials from the non-Filipino individuals were commercially provided by Coriell Cell Repositories. We used seven postmortem brains from seven male Filipino patients who had XDP. One of the seven brain specimens was frozen, and six were formalin fixed immediately at autopsy. The information on these seven patients with XDP is provided in table 2. We used the frozen brain from a single patient with XDP for long RT-PCR, northern analysis, quantitative RT-PCR, and in situ hybridization. In addition, we used the six formalin-fixed brain specimens for immunohistochemical staining. This study complied with the ethical guidelines of the institutions involved.

Table 1.

Information on All the Patients with XDP Who Were Studied by Southern Hybridization[Note]

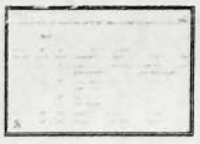

Table 2.

Information on All the Patients with XDP Who Were Studied by Expression Analysis[Note]

Genome Analysis

We constructed two series of BAC libraries, using genomic DNA from a patient with XDP who was aged 41 years and had generalized torsion dystonia without parkinsonism. Cultured lymphoblastoid cells from a patient with XDP were embedded in agarose plugs, and then high–molecular-weight DNA was partially digested with *Eco*RI and was size fractionated by pulse-field gel electrophoresis. Size-fractionated DNA was cloned into the CopyControl pCC1BAC vector (Epicentre) and was transformed into DH10B cells by use of an electroporator. The same procedure was repeated with *Hin*dIII. We identified BACs covering the *DYT3* region by hybridizing ^{32}P-labeled PCR probes to filters