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SHORT COMMUNICATION

Chromosomal abnormality at 6p25.1—25.3 identifies a susceptibility locus for hypothalamic hamartoma associated with epilepsy

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Summary The pathogenesis of hypothalamic hamartoma (HH) associated with epilepsy is unknown. We have identified an individual with HH and refractory epilepsy exhibiting subtle dysmorphic features. High-resolution karyotype identified a duplication of the terminal end of 6p (6p25.1—25.3), confirmed by fluorescent in situ-hybridization (FISH). Copy number analysis with high-density (250K) single nucleotide polymorphism (SNP) genotyping microarrays characterized the abnormality as a series of amplified regions between 1.4 Mb and 10.2 Mb, with a small tandem deletion from 8.8 Mb to 9.7 Mb. There are 38 RefSeq genes within the duplicated regions, and no known coding sequences within the deletion. This unique patient helps identify 6p25.1—25.3 as a possible susceptibility locus for sporadic HH.

Introduction

Hypothalamic hamartoma (HH) associated with epilepsy is an uncommon and usually sporadic disorder. In a recent Scandinavian population-based study, its prevalence was estimated at 1 in 200,000 children and adolescents (Brandberg et al., 2004). The intrahypothalamic subtype of HH typically manifests with gelastic seizures, often during infancy. These seizures are usually refractory to medical management. Other seizure types may occur later in

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childhood, although there is considerable variability in the natural history of individual cases (Berkovic et al., 1988).

A small number of cases with HH and epilepsy are associated with Pallister-Hall syndrome (PHS), an autosomal dominant dysmorphology syndrome associated with HH, hypopituitarism, postaxial polydactaly, bifid epiglottis, imperforate anus, and other somatic malformations (Biesecker et al., 1996). PHS is caused by haploinsufficiency of the *GLI3* gene at chromosome 7p13 (Kang et al., 1997).

The cause of sporadic HH, not associated with PHS, is unknown. We have identified an individual patient from our series of patients with HH and refractory epilepsy with a segmental chromosomal abnormality involving the telomeric region of 6p. This case identifies a possible candidate region associated with the sporadic pathogenesis of HH.

Methods

One patient in our series of HH associated with refractory epilepsy ($N = 136$) was identified as having subtle dysmorphic features that do not correspond to a previously identified dysmorphic syndrome. Informed consent for database entry was obtained using a protocol approved by the Institutional Research Board (IRB) of St. Joseph's Hospital and Medical Center.

The proband and both parents were karyotyped with high-resolution banding. Fluorescent in situ-hybridization was performed using a multiprobe panel specific for chromosome 6 (Chromoprobe Multiprobe System, Cytocell Technologies, Cambridge). To further characterize the chromosomal abnormality for the proband, copy number was measured across the chromosomal abnormality using Affymetrix high-density SNP genotyping microarrays.

DNA extracted from blood was genotyped on Affymetrix 10K and 500K EA GeneChip platforms (Affymetrix, Santa Clara, CA). The Affymetrix 500K array set consists of a 250K Nsp array and a 250K Sty array. The copy number analysis package within CNAT 4.0 (Chromosome Copy Number Analysis Tool, http://www.affymetrix.com/) was used to analyze the signal intensity values of single nucleotide polymorphisms (SNP). Briefly, the proband sample was analyzed using an un-paired analysis comparison to pooled normal control (PNC) consisting of 100 healthy individuals, replicated on 32 chips, as a reference dataset. PNC samples should exhibit an average copy number of two across the genome, masking any of the recently reported common copy number polymorphisms (Slater et al., 2005). Samples were normalized by quantile normalization on perfect match probes, following allele summarization utilizing the BRLMM software component within GTYPE (both available from http://www.affymetrix.com), per the default CNAT 4.0 protocol. Similar results were also obtained with dCHIPSNP, implemented as previously described (Beroukhim et al., 2006). Results were correlated with the published gene map from Genome Build 35.1. Individual genes thus identified were explored for possible clinical relevance using Web-based tools including the Online Mendelian Inheritance in Man (OMIM) (http://www.ncbi.nlm.nih.gov/omim/).

Case report

The proband, an 8.6-year-old girl, was evaluated at our institution for possible HH resection to treat her refractory epilepsy. She was born at 38 weeks gestational age after labor was induced due to maternal preeclampsia. She was small for gestational age (birth weight, 2270 g) and microcephalic (head circumference, 31.8 cm). Mild craniofacial anomalies included a tall forehead; broad nasal tip; hemangioma on the right side of the nose; and a short, deep philtrum with excessive bowing to the upper lip. She also exhibited mild left-sided ptosis, mild hypotonia, and excessive joint laxity.

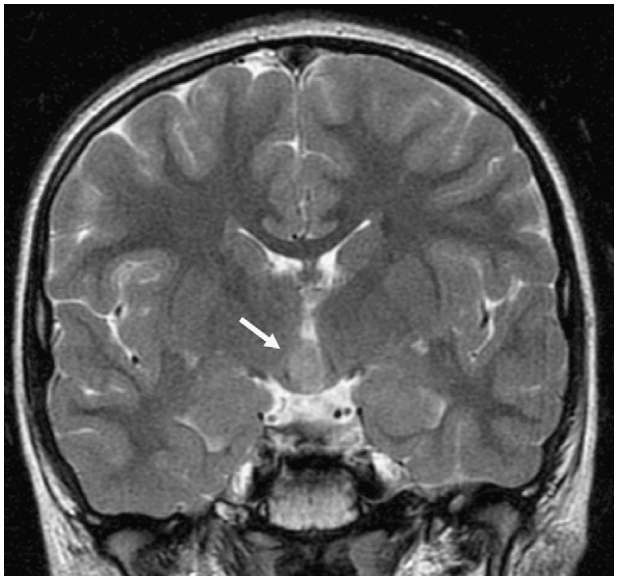

Figure 1 Coronal T2-weighted fast spin-echo magnetic resonance image (TR 3500, TE 83) of the girl shows the HH (arrow). No other MR imaging abnormalities were observed.

The mother reported brief peculiar laughing episodes that had occurred since birth. The episodes occurred multiple times daily and initially had been attributed to ''anxiety.'' Genetics consultation at the age of 1.6 years revealed mild developmental delay and mild generalized hypotonia. Karyotyping of peripheral blood at the 650—800 band level of resolution demonstrated a small duplication on the distal short arm of chromosome 6, corresponding to 6p25.1—25.3, immediately adjacent to the native segment, which was confirmed by the use of multiprobe fluorescent in situ-hybridization (FISH) specific for chromosome 6. The full karyotype result was 46,XX,dup(6)(p25.1—p25.3). The karyotype for both parents was normal.

A neurologist saw the patient for the first time when she was 8.1 years old for recurrent laughing spells. Magnetic resonance imaging of the brain showed an HH within the third ventricle and no other cerebral abnormalities (Fig. 1). Routine EEG showed mildly disorganized background activity for her age, with frequent left parietal spikes and a single right frontal spike. Her pituitary function was normal. Treatment with multiple antiepilepsy drugs failed to ameliorate her gelastic seizures, which occurred multiple times each day. No other seizure types were observed.

Results

Mutation analysis with SNP microarrays

Affymetrix® SNP microarray analysis, using the 10K (Xba) and 500K platforms (250K Nsp and 250K Sty), demonstrated a series of copy number abnormalities, corresponding to two small duplications ranging from about 1.4 Mb (rs6925228)