Disruption of Sodium Bicarbonate Transporter SLC4A10 in a Patient With Complex Partial Epilepsy and Mental Retardation

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Objective: To determine gene(s) disrupted in a patient with partial frontal lobe epilepsy and cognitive impairment with concomitant de novo balanced chromosomal translocation t(2;13)(q24;q31).

Design: Fluorescence in situ hybridization and array comparative genomic hybridization were used to map the locations of chromosomal translocation breakpoints.

Results: SLC4A10 (OMIM 605556), a sodium bicarbonate transporter gene with high expression in the cerebral cortex and hippocampus, was disrupted by the translocation breakpoint on chromosome 2q24. The breakpoint on chromosome 13q31 was in a 1-megabase (Mb)–gene desert. Genomewide array comparative genomic hybridization confirmed the absence of additional chromosomal abnormalities.

Conclusion: SLC4A10 is the third SLC4 base transporter family member to be implicated in human cognition and epilepsy.

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Neuronal excitability is affected by alteration of acid-base homeostasis in the central nervous system. Hyperventilation, resulting in respiratory alkalosis, increases the frequency of generalized epileptiform discharges and absence seizures. Fever-induced respiratory alkalosis may also trigger seizures, an effect that can be modeled by direct application of sodium bicarbonate to the brain. Further evidence of the importance of acid-base balance in neuronal excitability is demonstrated by the use of carbonic anhydrase inhibitors to treat epilepsy. Changes in pH that may contribute to the epileptic phenotype include effects on ion channel activity, transporter function, and neurotransmission.

Methods

Patient: A 13-year-old girl with moderate mental retardation and partial complex epilepsy was examined. She weighed 4710 g at birth following an uncomplicated pregnancy and delivery. She did not walk until 30 months and had delayed speech. No dysmorphic features were noted. Growth was normal. There was no family history of seizures, mental retardation, or autism. The patient’s first seizure occurred at age 7 years. Seizures were brief (<60 seconds) and consisted of right arm movements with head and eye deviation and alteration of consciousness. Seizures were well controlled with zonisamide therapy but had not responded to lamotrigine therapy.
An electroencephalogram demonstrated slowing of the posterior dominant rhythm, as well as left frontal spike discharges (Figure 1A). Brain magnetic resonance imaging was normal. As a part of the workup for developmental delay, karyotyping was performed showing a de novo balanced translocation t(2;13)(q24;q31).

Neuropsychological testing with the Stanford-Binet Intelligence Scales revealed deficits in all areas (Figure 1B). At age 6 years, the patient experienced a significant slowing in the rate of cognitive development, which was reflected by declines in intelligence (z scores). Result of testing using the Childhood Autism Rating Scale was in the nonautistic category (raw score, 18.5).

CONSENT AND SAMPLE COLLECTION
Research was approved by the Washington University Human Subjects Committee, St Louis, Missouri. After obtaining informed consent, DNA and RNA were extracted from lymphoblastoid cell lines using standard methods.

FLUORESCENCE IN SITU HYBRIDIZATION
Bacterial artificial chromosome (BAC) clones flanking the presumed translocation breakpoints were obtained from BACPAC Resources (Children’s Hospital Oakland Research Institute, Oakland, California) and used as probes for fluorescence in situ hybridization (FISH) as described elsewhere.

COMPARATIVE GENOMIC HYBRIDIZATION
Patient DNA was evaluated by whole genome array comparative genomic hybridization (385,000 probes) (NimbleGen Systems, Inc, Madison, Wisconsin) to detect DNA copy number variations. Data were analyzed with SignalMap browser (NimbleGen Systems, Inc). Copy number variations were screened against databases of known variants.

REAL-TIME QUANTITATIVE POLYMERASE CHAIN REACTION
Primers designed to SLC4A10 RNA (GCTGCTGCTAC-GAGAATG and CTGTGTTATGACGATG) were used for amplification with a cycler (model 7000; Applied Biosystems, Foster City, California) using SYBR Green dye (Applied Biosystems). Beta-actin was used as the reference. Control RNA was obtained from CEPH lymphoblastoid cell lines. The delta-delta Ct method was used for quantification.

RESULTS
FISH MAPPING OF TRANSLLOCATION BREAKPOINTS
FISH was performed to localize translocation breakpoints using a series of bacterial artificial chromosome (BAC) clones near the cytogenetically detected breakpoints on chromosomes 2q24 and 13q. Beta-actin was used as the reference. Control RNA was obtained from CEPH lymphoblastoid cell lines. The delta-delta Ct method was used for quantification.
letion detected in a pair of identical autistic twins (Figure 2B). A cluster of sodium channels relevant to human epilepsy (SCN1, SCN7, and SCN9) are located more than 4 Mb distal to the breakpoint (Figure 3).

On chromosome 13q, the breakpoint was narrowed to a 422-kb region within a 1-megabase (Mb)–gene desert by identifying BAC clones located above (RP11-206K5) and below (RP11-19D22) the breakpoint (data not shown). The nearest gene proximal to the breakpoint on chromosome 13q is LMO7, located at least 200 kb from the breakpoint (Figure 3). KCTD12 is located at least 400 kb distal to the breakpoint on chromosome 13q.

ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

Because recent evidence suggests that additional genomic imbalances may occur in patients with apparent balanced chromosomal translocations, DNA copy number changes were evaluated using genomewide comparative genomic hybridization. Although several known and nondisease-related copy number polymorphisms were detected on other chromosomes (data not shown), no deletions were detected near the breakpoints on chromosome 2 or 13 (Figure 3) nor anywhere else in the patient's genome.

EVALUATION OF SLC4A10 TRANSCRIPTS

Because brain tissue was unavailable from this patient, immortalized lymphoblast RNA was evaluated. SLC4A10 is minimally expressed in lymphoblasts as evidenced by a lack of expression by either Northern or Western blot analysis (data not shown). Quantitative real-time polymerase chain reaction demonstrated a 48% reduction in SLC4A10 transcript in the patient's lymphoblasts compared with reference gene beta-actin when normalized to a calibrator consisting of control lymphoblasts RNA.

COMMENT

To our knowledge, this is the first article to describe a role for the sodium bicarbonate transporter, SLC4A10, in epilepsy and human cognition. SLC4A10 is a member of the SL4 family of base transporters that consists of 10 family members. SLC4A10 is present in multiple tissues but is highly expressed in the cerebral cortex and hippocampus, 2 regions commonly implicated in epilepsy. To date, 5 members of the SLC4 family of base transporters have been implicated in human disease (Table). SLC4A3 polymorphisms are associated with generalized epilepsy in humans, and mice with targeted disruption of this gene have reduced seizure thresholds.

Although chromosomal translocations may have long-distance effects on genes due to disruption of regulatory sequences or chromatin structure, there are few examples of effects occurring from distances greater than 1 Mb. Therefore, a cluster of sodium channel genes relevant to human epilepsy (SCN1, SCN7, and SCN9) that is located more than 4 Mb distal to the chromosomal 2 breakpoint (Figure 3) is unlikely to be affected by this patient's translocation.

A chromosomal deletion involving SLC4A10 was previously detected in both members of a pair of identical twins from a large cohort of patients with autism screened for the presence of DNA copy number mutations. However, these patients were not described in detail, and it is unknown whether these twins had cognitive dysfunction or epilepsy. Many genetic diseases, including fragile X and tuberous sclerosis, give rise to a spectrum of cognitive phenotypes that may manifest as autism, mental retardation, or both, reflecting the polygenic inheritance of these traits. The patient described herein had an unusual decline in cognitive function despite having seizures that were well controlled with a single medication. Additional pa-
patients with mutations or deletions of SLC4A10 will need to be identified to determine the frequency of cognitive deficits, cognitive decline, autism, and epilepsy.

Disruption of SLC4A10 occurring through either a chromosomal translocation or deletion (as described in the identical twins concordant for autism described in Sebat et al 21) may result in disease through haploinsufficiency. Haploinsufficiency of several gene products critical to brain function results in epilepsy, including the sodium channel gene SCN1A and the glucose transporter GLUT-1. 21 Although autosomal dominant hereditary spherocytosis resulting from SLC4A1 mutations may result from haploinsufficiency, some mutations may cause disease via gain of function effects through misfolding and heterodimer formation. 22 However, SLC4A10 is not expressed sufficiently in lymphoblasts to determine whether any aberrant truncated forms are expressed. Pheno-

totypes resulting from mouse SLC4A10 gene knockouts have not yet been described but may allow the mechanism by which sodium bicarbonate transporter disruption leads to epilepsy and cognitive dysfunction to be elucidated.

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Table. SLC4 Base Transporters Associated With Human Disease

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein Name</th>
<th>Phenotype</th>
<th>Inheritance</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC4A1</td>
<td>AE1, Band 3</td>
<td>Anemia, spherocytosis, distal RTA, hypokalemia, hypercalciumia, nephrocalcinosis, and nephrolithiasis</td>
<td>AR, AD</td>
<td>Schofield et al 13</td>
</tr>
<tr>
<td>SLC4A3</td>
<td>AE3</td>
<td>Generalized seizures</td>
<td>Polymorphism</td>
<td>Sander et al 14</td>
</tr>
<tr>
<td>SLC4A4</td>
<td>NBC1, NBCe1</td>
<td>Short stature, basal ganglia calcifications, mental retardation, cataracts, band keratopathy, proximal renal tubular acidosis, and hypokalemia</td>
<td>AR</td>
<td>Igarashi et al 15</td>
</tr>
<tr>
<td>SLC4A5</td>
<td>NBC4, NBCe2</td>
<td>Mental retardation, autism, and seizures</td>
<td>Polymorphism</td>
<td>Barkley et al 16</td>
</tr>
<tr>
<td>SLC4A10</td>
<td>NCBE</td>
<td>Hypertension</td>
<td>AD</td>
<td>This report and Schopler et al 17</td>
</tr>
</tbody>
</table>

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; RTA, renal tubular acidosis.

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