Chorea Detection for the Diagnosis of Chorea-Acanthocytosis

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Chorea-acanthocytosis (ChAc) is a severe, neurodegenerative disorder that shares clinical features with Huntington’s disease and McLeod syndrome. It is caused by mutations in VPS13A, which encodes a large protein called chorein. Using antichorein antisera, we found expression of chorein in all human cells analyzed. However, chorein expression was absent or noticeably reduced in ChAc patient cells, but not McLeod syndrome and Huntington’s disease cells. This suggests that loss of chorein expression is a diagnostic feature of ChAc.

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Chorea-acanthocytosis (ChAc, OMIM 200150) is an autosomal recessive neurodegenerative disorder characterized by progressive onset of hyperkinetic movements and unusual spiny erythrocyte morphology (acanthocytosis).1 ChAc diagnosis can be challenging, due to interlaboratory variability in acanthocyte detection efficiency,2 and the clinical similarity of ChAc to Huntington’s disease (HD) and McLeod syndrome (MLS, OMIM *314850), an X-linked neuroacanthocytosis.3

CHAC, encoding the large (>3,000 amino acids) protein chorein, is the gene mutated in chorea-acanthocytosis.4,5 CHAC, now renamed VPS13A,6 is organized into 73 exons and has two main splice forms: isoform 1A (exons 1–68, 70–73) and isoform 1B (exons 1–69).4 Because of the size of the gene and the allelic heterogeneity of ChAc,7 mutation screening of VPS13A is a cumbersome process, impeding diagnostic progress. Herein, we report the generation of antichorein polyclonal antisera. We note the apparently ubiquitous distribution of chorein and demonstrate that loss of chorein expression in erythrocyte membranes and other cells is a diagnostic feature of ChAc.

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Subjects and Methods

Subjects

Informed patient consent for molecular analysis of blood samples was obtained according to local guidelines. Samples from 14 patients with a clinical diagnosis of chorea-acanthocytosis (10 men, 4 women) were studied. Patients 1 to 4 correspond to CHAC2IV5, CHAC6II1 and a newly diagnosed brother, and CHAC11II2 in previous studies4,8; Patients 5, 6, 7, and 14 correspond to probands 24, 11, 2, and 23, respectively, in a previous study.7 All displayed characteristic clinical features of chorea-acanthocytosis5 that included elevation of serum creatine kinase (14 of 14 patients), ankle areflexia (10/14), cognitive or neuropsychiatric changes (12/14), limb chorea (13/14), oralofacial dyskinesia (including tongue dystonia and lip biting, 12/14), dysarthria (11/14), involuntary vocalizations (7/14), and parkinsonian features (2/14). Nine of the 14 patients had experienced seizures. Blood samples from two men with MLS were also studied (Patients 3 and 13 from Danek and colleagues3).

Mutation Detection

ChAc patient DNA was screened for VPS13A mutations as described previously.7 At least one heterozygous VPS13A mutation likely to cause disease was found in each ChAc patient, as shown in the Table.

Production of Polyclonal Antiserum Anti-chor1

A fragment of chorein comprising amino acids 27 to 326 was expressed as a fusion protein with glutathione S-transf erase (GST-chor1). Bacterial expression and purification of GST-chor1 was performed as described by Frangioni and Neel,9 using glutathione Sepharose 4B beads (Amersham Biosciences UK Ltd, Chalfont St. Giles, UK). Immunization of rabbits with the chor1 moiety was performed by Eurogentec Bel SA (Herstal, Belgium); the serum collected 87 days after immunization was designated anti-chor1.

Western Blotting of Cell Lysates

Cells were harvested by trypsinization and centrifugation at 3,400g for 5 minutes. Cell pellets were washed with ice-cold phosphate-buffered saline then lysed in 10 × volume of ice-
Table. ChAc and McLeod Syndrome Patients Analyzed in This Study

<table>
<thead>
<tr>
<th>Patient</th>
<th>DNA Changeb</th>
<th>Protein Changeb</th>
<th>Type of Mutation</th>
<th>Tissue analyzed</th>
<th>Chorein Expressiona</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPS13A mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (4)</td>
<td>[1592del]</td>
<td>[1592del]</td>
<td>Frameshift Frameshift</td>
<td>Lb</td>
<td>−</td>
</tr>
<tr>
<td>2, 3 (4)</td>
<td>[3597T&gt;C]</td>
<td>[1531fsX]</td>
<td>Mis sense Mis sense</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>4 (4)</td>
<td>[237del]</td>
<td>[1531fsX6]</td>
<td>Frameshift Frameshift</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>5 (7)</td>
<td>[6419C&gt;G]</td>
<td>[9190del]</td>
<td>None None</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>6 (7)</td>
<td>[1125_1128del]</td>
<td>[52140X]</td>
<td>Frameshift Frameshift</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>7 (7)</td>
<td>[2288 + 2&gt;T&gt;C]</td>
<td>[52140X]</td>
<td>Frameshift Frameshift</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>8 (5)</td>
<td>[9556_2A&gt;C]</td>
<td>[V3064fsX16]</td>
<td>None None</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>9 (5)</td>
<td>[1596 – 2A&gt;C]</td>
<td>[V3064fsX16]</td>
<td>Frameshift Frameshift</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>10 [EX46_EX50del]</td>
<td>[SA]</td>
<td>[S1452P]</td>
<td>Missense Missense</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>11 [5909_5910del]</td>
<td>[SA]</td>
<td>[S1452P]</td>
<td>None None</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>12 [188-5T&gt;G]</td>
<td>[SA]</td>
<td>[S1452P]</td>
<td>None None</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>13 [495 + 1G&gt;A]</td>
<td>[SA]</td>
<td>[S1452P]</td>
<td>None None</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>14 (7) [1208_1211del]</td>
<td>[SA]</td>
<td>[S1452P]</td>
<td>None None</td>
<td>Lb</td>
<td>+</td>
</tr>
<tr>
<td>XK mutations</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1 (13)</td>
<td>508 + 1G&gt;A</td>
<td>SD</td>
<td>Splicing Splicing</td>
<td>Lb</td>
<td>++</td>
</tr>
<tr>
<td>M2 (3)</td>
<td>EX1del</td>
<td>unknown</td>
<td>Deletion Deletion</td>
<td>Ec</td>
<td>++</td>
</tr>
</tbody>
</table>

aReferences for patients who were screened for mutations in a previous study are given in parentheses.
bNucleotides and amino acids are numbered according to the cDNA sequence of VPS13A/chac1 isoform A reported by Rampoldi and colleagues (GenBank accession no. NM_033305) or that of XKX (GenBank accession no. Z32684). Mutation nomenclature is as recommended by the Human Genome Variation Society (http://www.genomic.animalb.edu.au/midi/mutnomen/index.html).

Results

Detection of Endogenous Chorein

Western blot analysis of ChAc Patient 1 and control lymphoblastoid cell lysates shows that anti-chorein antibody recognizes chorein (see Fig 1A). A band similar in size to huntingtin (350kDa, lanes 7 and 8) was detected in control cells (lane 5); this is consistent with the predicted molecular weight of chorein (360kDa). This band was absent in cells from Patient 1 (lane 6). The signal was not detected using preimmune serum (lanes 1 and 2) or when using serum depleted in chorein-binding antibodies (lanes 3 and 4), thereby confirming the detection specificity. To investigate chorein distribution, we analyzed lysates from a variety of cell lines routinely used in tissue culture techniques by Western blot (see Fig 1B). Anti-chorein antibody detected a high-molecular-weight signal in all cell lines analyzed.

Expression of Mutant Chorein in Chorea-Acanthocytosis Cell Lines

Western blot analysis of lymphoblastoid cell lysates shows that ChAc Patients 2 to 4 have markedly reduced chorein levels compared with the control (Fig 2A, see Table), in contrast with unaffected family members (lanes 3, 5, and 6) and MLS Patient 1 (lane...
The affected son in Family CHAC11 (Patient 4) has inherited a maternal 237del mutation in exon 4 and a paternal 9429_9432del mutation in exon 72. Any protein that is produced from the 237del allele will be severely truncated (predicted size 10kDa). The weak signal seen for Patient 4 therefore must derive from truncated protein generated by the 9429_9432del allele (357kDa), and/or other chorein isoforms, such as isoform 1B (345kDa), which is theoretically unaffected by the exon 72 mutation. Chorein expression in primary skin fibroblasts from ChAc Patients 5 to 7 was undetectable or markedly reduced (see Table).

Expression of Chorein in Erythrocyte Membranes
Western blot analysis of erythrocyte membrane fractions from ChAc Patients 8 to 14, MLS Patient 2, and two healthy individuals is shown in Figure 2B. Chorein expression was undetectable or markedly reduced in all ChAc patients analyzed. In contrast, a sig-

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Figure 1: Detection of chorein expression. (A) Anti-chor1 antiserum detects endogenous chorein. Twenty-microgram protein samples from lymphoblastoid cell lines (C = healthy control, P1 = ChAc Patient 1) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and analyzed by Western blot. Blots were analyzed with preimmune serum (lanes 1 and 2) or anti-chor1 antiserum (lanes 3–6). Anti-chor1 antiserum was either run through a column bound with chor1 antigen to remove chor1-binding antibodies (column-treated +, lanes 3, 4) or left untreated (column-treated –, lanes 5, 6). An anti-buntingtin blot (anti-Htt, lanes 7, 8) is provided for size comparison (Htt = 350kDa). Because of their similar size, it is not possible to distinguish between chorein isoforms 1A (360kDa) and 1B (345kDa). (B) Expression of chorein in different cell types. Human cell lines were derived from cervical carcinoma (HeLa), fetal lung (MRC5), embryonic kidney (293T), lymphoblasts (lymph), neuroglioma (H4), hepatocarcinoma (Hep3B), myelogenous leukemia (K562), and rhabdomyosarcoma (RD). Nonhuman cell lines were derived from African green monkey kidney (COS-7) and Chinese hamster ovary (CHO-K1). The much weaker signal in nonhuman cell lines is presumably caused either by a reduction in chorein ortholog expression or by limited cross-reaction with the anti-chor1 antiserum. Blot stripping and immunodetection of early endosome antigen 1 (EEA1) demonstrated equal loading of samples (data not shown).
Discussion

Mutations in the VPS13A gene are associated with the severe neurodegenerative disorder chorea-acanthocytosis. In this study, we report the first cellular detection of chorein, the VPS13A gene product. We found that chorein is expressed in cell lines derived from a wide variety of human tissues, as well as primary skin fibroblasts and erythrocytes. This is supported by previous analyses that showed ubiquitous expression of VPS13A mRNA.4

We have demonstrated that 19 different VPS13A mutations, including one missense mutation, lead to absence or marked reduction of chorein expression in ChAc patients. Even in a patient with an isoform 1A–specific mutation (9429_9432del), very little chorein expression was observed. We observed previously that several patients with ChAc harbored isoform 1A–specific mutations and speculated that exons 70 to 73 were essential for some functions of chorein.7 The relatively poor expression of isoforms lacking these exons also may explain their inability to compensate for full-length chorein in ChAc.

We have shown that chorein can be detected in association with the erythrocyte membrane (see Fig 2B). MLS and HD patient samples show normal chorein expression levels, in contrast with ChAc patients. This has obvious implications for ChAc diagnosis. Currently, if the VPS13A gene has not been screened, ChAc can be diagnosed only by excluding other clinically similar disorders. As VPS13A is a large gene with many exons, screening is costly and time consuming. Western blotting of patient erythrocyte membranes perhaps could give an early indication of the disorder before a precise diagnosis is given by a VPS13A gene screen.

Although chorein was absent or markedly reduced in all patient erythrocytes analyzed so far, one cannot exclude ChAc as a diagnosis if chorein is present in a sample. The missense mutation S1452P appears simply to affect chorein dosage (see Fig 2A); however, some substitutions may lead to apparently normal levels of chorein that is nevertheless functionally defective. Also, some mutations may allow almost normal expression of mutant chorein lacking only a few exons, which might not be resolved from wild-type chorein because of its large size. However, we anticipate that this would not be a significant problem, because missense changes compose less than 7% (5/75) of VPS13A mutations described so far (previous studies4,5,7 and this study), and most transcripts containing premature stop codons are believed to be rapidly degraded.12 In any case, if chorein is absent in a patient sample, the most likely diagnosis will be chorea-acanthocytosis.

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