Role of CCG interruptions on disease penetrance in families with spinocerebellar ataxia type 8

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Background
Spinocerebellar ataxia type 8 (SCA8) is a dominantly inherited, slowly progressive disease that causes neurons in the cerebellum to die. Patients develop impaired gait, slurred speech, and abnormal eye movements. The average age of disease onset is approximately 43 years old, but has presented in juveniles (<5 years). SCA8 is caused by a CAG·CTG trinucleotide expansion mutation on chromosome 13q21. A puzzling feature of SCA8 is the reduced disease penetrance, in other words, in many cases people who inherit expansion mutations do not develop the disease. The reduced penetrance results in most cases of SCA8 presenting with no obvious family history and only occasional families in which 2, 3 or more individuals in the same family are affected. Additionally, while most expansion mutations contain pure CAG·CTG repeat tracts, expansions with CCG·CGG interruptions are found in families with three or more affected members. Both the number and position of the interruptions as well as repeat length can change when the expansion mutation is transmitted from parent to child. Our hypothesis is that these interruptions increase disease penetrance and will be found more frequently in families with multiple affected individuals.

Results

a. Spinocerebellar ataxia type 8 (SCA8) is a dominantly inherited, slowly progressive disease that causes neurons in the cerebellum to die. Patients develop impaired gait, slurred speech, and abnormal eye movements. The average age of disease onset is approximately 43 years old, but has presented in juveniles (<5 years). SCA8 is caused by a CAG·CTG trinucleotide expansion mutation on chromosome 13q21. A puzzling feature of SCA8 is the reduced disease penetrance, in other words, in many cases people who inherit expansion mutations do not develop the disease. The reduced penetrance results in most cases of SCA8 presenting with no obvious family history and only occasional families in which 2, 3 or more individuals in the same family are affected. Additionally, while most expansion mutations contain pure CAG·CTG repeat tracts, expansions with CCG·CGG interruptions are found in families with three or more affected members. Both the number and position of the interruptions as well as repeat length can change when the expansion mutation is transmitted from parent to child. Our hypothesis is that these interruptions increase disease penetrance and will be found more frequently in families with multiple affected individuals.

*b) Reduced Penetrance: many gene carriers do not develop ataxia.

Table: Disease penetrance

<table>
<thead>
<tr>
<th>Number of Affected Individuals</th>
<th>Number of Families</th>
<th>Penetrance Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>2</td>
<td>3%</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>3%</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>15%</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>79%</td>
</tr>
</tbody>
</table>

Increased penetrance: defined here as 3 or more affected individuals in one family.

The goal of this project is to test if CCG·CGG interruptions increase disease penetrance. This will be done by studying the frequency at which interruptions are found in newly identified SCA8 families correlating the results with the number of affected individuals within the family.

Materials and Methods

Patient Samples: SCA8 patient DNA was previously extracted from venous blood.

Polymerase Chain Reaction. The SCA8 repeat region was amplified by PCR using Finnzyme Phusion® Hot Start High-Fidelity DNA Polymerase. Results were visualized on a 1% agarose gel and stained with 0.1μL ethidium bromide per 10mL gel solution.

PCR Purification and Sequencing. Expanded allele bands were cut out of the agarose gel and purified using a Promega Wizard® SV Genomic DNA purification kit. Samples were then submitted to the University of Minnesota’s Biomedical Genomics Center for DNA sequencing.

Continuing Research

• Sequence data will be obtained for the rest of the SCA8 families. A new reverse primer will be used to improve accuracy at the CAG – TAG border of the repeat.

• Southern assays will be used to estimate size of repeat tracts too large for PCR.

• Digestion assays using MspA1I enzyme will allow for interruption detection on large repeat carriers. MspA1I digests PCR products containing CCG interruptions in the CAG direction.

• Mouse models previously developed in the Ranum lab will be used to study whether the number of CCG interruptions and the length of the repeat change in mice as they do in humans from generation to generation.

Acknowledgements

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References

