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.

"Disease penetrance" refers to the likelihood a given gene will result in disease. SCA8 is characterized by Reduced Penetrance: many gene carriers do not develop ataxia.

>3 affected	2 families	3%
3 affected	2 families	3%
2 affected	10 families	15%
1 affected	52 families	79%

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.

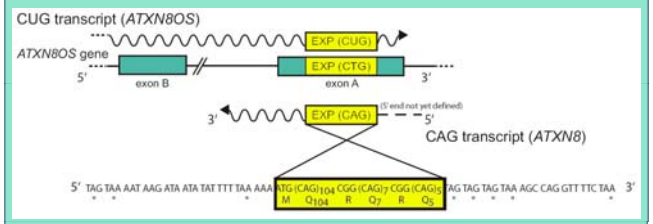

Figure 1. Diagram of the two genes expressed in SCA8: ATXN8 and ATXN8OS. ATXN8 is expressed in the CAG direction and encodes a nearly pure polyglutamine protein. ATXN8OS is expressed in the CTG direction and its transcript is non-protein coding. Repeat expansions are represented in yellow boxes and transcription direction is shown with wavy lines. Adapted from Moseley et al., Nature Genetics 38:758–769 (2006).

Hypothesis

- CCG·CGG interruptions in CAG·CTG repeat tracts of SCA8 patients will be found in higher frequency in families with multiple affected individuals.
- These or other sequence interruptions will also be found in a greater percentage of juvenile onset patients.

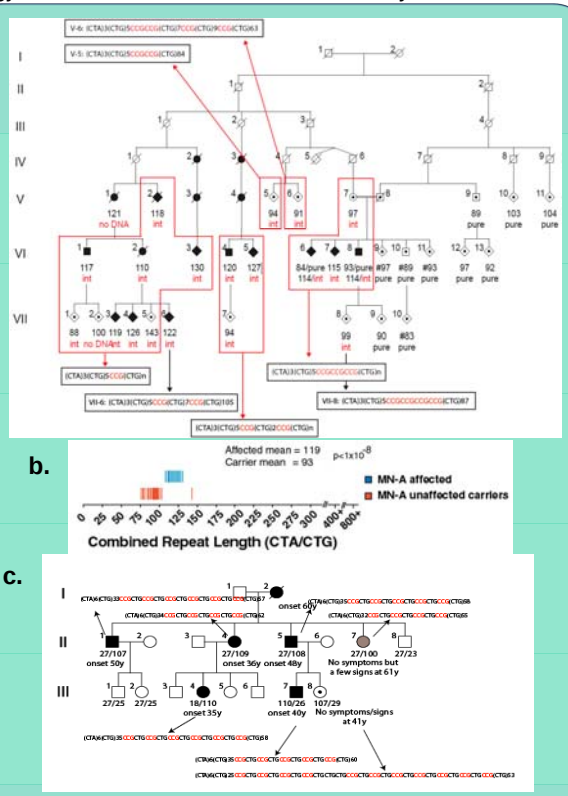

Figure 2. a) Section of the MN-A family pedigree, the largest SCA8 affected family to date, with a high degree of disease penetrance. V7 and V8 produced homozygous offspring. **b)** MN-A SCA8 affected individuals tend to have longer repeat tracts than asymptomatic expansion carriers. **c)** Danish SCA8 Family 1 – Large number of repeat interruptions and increased penetrance.

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.

Results

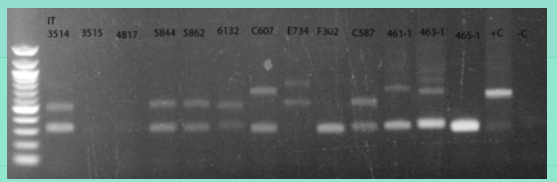

Figure 3. Bands at ~350 bp are the normal alleles found in heterozygous patients. Bands at ~500bp to ~700 bp are the expanded alleles. Lane 8 shows a homozygous individual. Lanes with the normal allele only indicate the expansion is too large for PCR amplification. Positive control was an SCA8 expansion transgenic mouse.

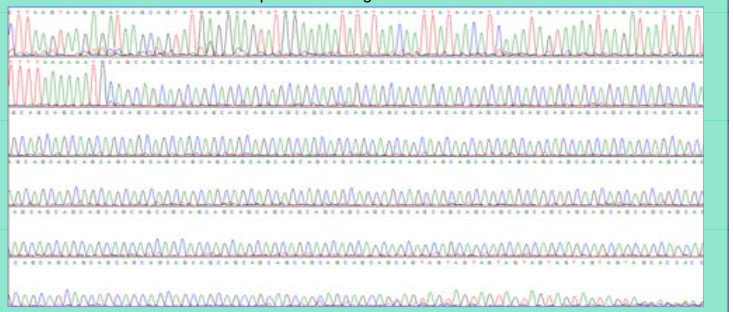

Figure 4. Chromatogram example of an expanded allele in the CAG (Forward primer) direction.

Samples obtained and preliminary genotype information

	# of families	# of samples	# of Expansion Carriers	# with no expansion	# undetermined
SFX Families	52	272	125 (39 sequenced)	106	41
Outside Samples	25	73	51 (7 sequenced)	8	13
Total	77	345	176 (46 sequenced)	114	54

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

- The entire Ranum lab
- University of Minnesota, Undergraduate Research Program

References

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