Genetic Variance in the Spinocerebellar Ataxia Type 2 (ATXN2) Gene in Children with Severe Early Onset Obesity

Karla P. Figueroa¹, Sadaf Farooqi², Kristopher Harrup¹, Johnathan Frank³, Stephen O’Rahilly², Stefan M. Pulst¹,4*

¹Department of Neurology, University of Utah, Salt Lake City, Utah, United States of America, ²Metabolic Research Laboratories, Institute of Metabolic Science, Addenbrooke’s Hospital, University of Cambridge, Cambridge, United Kingdom, ³David Geffen Medical School, University of California Los Angeles, Los Angeles, California, United States of America, ⁴Program in Neurosciences and Brain Institute, University of Utah, Salt Lake City, Utah, United States of America

Abstract

Background: Expansion of a CAG repeat in the coding region of exon 1 in the ATXN2 gene located in human chromosome 12q24.1 causes the neurodegenerative disease spinocerebellar ataxia type 2 (SCA2). In contrast to other polyglutamine (polyQ) disorders, the SCA2 repeat is not highly polymorphic in central European (CEU) controls with Q22 representing 90% of alleles, and Q23 contributing between 5–7% of alleles. Recently, the ATXN2 CAG repeat has been identified as a target of adaptive selection in the CEU population. Mouse lines deficient for atxn2 develop marked hyperphagia and obesity raising the possibility that loss-of-function mutations in the ATXN2 gene may be related to energy balance in humans. Some linkage studies of obesity related phenotypes such as antipsychotic induced weight gain have reported significant lod scores on chromosome 12q24. We tested the hypothesis that rare loss-of-function ATXN2 variants cause obesity analogous to rare mutations in the leptin, leptin receptor and MC4R genes.

Methodology/Principal Findings: We sequenced the coding region of ATXN2 including intron-exon boundaries in 92 severely obese children with a body mass index (BMI) >3.2 standard deviations above age- and gender-adjusted means. We confirmed five previously identified single nucleotide polymorphisms (SNPs) and three new SNPs resulting in two synonymous substitutions and one intronic polymorphism. Alleles encoding >Q22 were overrepresented in our sample of obese children and contributed 15% of alleles in children identified by their parents as white. SNP rs695872 closely flanking the CAG repeat showed a greatly increased frequency of C/C homozygotes and G/C heterozygotes compared with reported frequencies in the CEU population.

Conclusions/Significance: Although we did not identify variants leading to novel amino acid substitutions, nonsense or frameshift mutations, this study warrants further examination of variation in the ATXN2 gene in obesity and related phenotypes in a larger case-control study with emphasis on rs695872 and CAG repeat structure.

Introduction

The ataxin-2 protein is a 1312 amino acid protein of unknown function encoded by 25 coding exons of the SCA2/ATXN2 gene [1,2]. The protein contains a polyglutamine domain, which is encoded by a CAG/CAA repeat in exon 1 of the gene. Expansion of the CAG repeat and the consequent elongation of the polyQ domain to ≥32 repeats causes the neurodegenerative disease spinocerebellar ataxia type 2 (SCA2). ATXN2 is widely expressed in neuronal and non-neuronal tissues and is highly conserved in the mouse at the nucleotide and amino acid level [3,4]. The region containing the ATXN2 gene on 12q24.1 has undergone significant selection in Central Europeans (CEU) [5]. The selected allele is associated with a specific twice CAG repeat that is interrupted twice by a CAA codon.

The function of ATXN2 has remained largely unknown. It is a cytoplasmic protein with Golgi association [6] which may play a role in RNA splicing via its interaction with A2BP1/FOX-1 [7,8]. An association with polyribosomes has been observed in the fly [9]. Ralser and colleagues identified interactions between ataxin-2 and endophilin proteins in plastin-associated cellular pathways [10]. ATXN2 also interacts with the DEAD/H-box RNA helicase DDX6, components of P-bodies and stress granules[11].

ATXN2 deficiency in the rodent did not result in a neurodegenerative phenotype, but led to marked obesity [12]. These results have recently found independent confirmation [13]. Genetic linkage studies of human obesity-related traits have also implicated the ATXN2 region on human chromosome 12q24. Li and coworkers identified linkage for body mass index (BMI) and

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* E-mail: stefan.pulst@hsc.utah.edu
total fat percentage to D12S2070, a marker that is 4MB telomeric to the ATXN2 gene[14]. This region has also been implicated in studies of obesity associated with antipsychotic medication. Highest lod scores were obtained with the marker D12S79, which is 4MB telomeric of ATXN2[15]. Parent-of-origin analysis of obesity traits implicated the region around D12S2070 as well with higher lod scores obtained for maternal transmissions[16]. Other groups, however, identified linkage signals further distal to the ATXN2 locus in 12q24.3[17,18]. To elucidate a potential role for ATXN2 in human obesity we examined the coding region of ATXN2 in 92 patients with severe obesity of early onset recruited to the UK Genetics of Obesity Study.

Results

Based on our finding of hyperphagia and obesity in mice hemizygozously or homozygously deficient for ATXN2 we tested the hypothesis that loss-of-function mutations in ATXN2 are responsible for a subset of human obesity. We sequenced the 25 coding exons of the human ATXN2 gene in 92 severely obese children enrolled in the Genetics of Obesity Study (GOOS) with a mean body mass index 3.2 standard deviations above the mean. Using primers located in the introns flanking the respective exon (Table S1) we searched for coding sequence variation as well as for variants leading to an alteration in splicing.

Table 1 lists the location and nature of eight DNA sequence variants separately for our entire cohort (n = 92) and those children with two self-identified white parents (n = 77) as well as SNP frequencies reported in the Hap-Map CEU population (n = 55). Overall, no variants were detected that changed the amino acid sequence of ataxin-2 other than one previously reported single nucleotide polymorphism (SNP). This SNP (rs695871) had the same genotype frequencies as reported for the CEU reference population (Table 1). We also identified two rare synonymous changes in exons 1 and 23 that had not previously been reported. These basepair changes are likely rare normal variants and are not expected to induce cryptic splice sites.

Two additional SNPs had been previously reported in European and Asian populations; rs695872 is located in exon 1 just upstream of the CAG repeat and results in a synonymous change, rs2073950 is located in intron 22. For both SNPs, genotype frequencies in the samples of obese white children from the U.K. were significantly different from those reported in the Hap-Map CEU sample (Table 1; rs695872, chi-square 36, DF 2, nominal p < 0.0000001; rs2073950, chi-square 10.2, DF 2, nominal p < 0.007). Both SNPs have significantly different allele frequencies in white and Asian populations.

Table 1. ATXN2 Sequence Variants found in the Genetics of Obesity Study (GOOS) Cohort.

| Location | SNP ID | Type | Flanking Sequence | Sequence Variation Site | Amino acid change | Genotype Frequency | Genotype Frequency | Frequency |
|----------|--------|------|------------------|------------------------|------------------|--------------------|--------------------|-----------|
| Exon 1   |        | Coding | GCCCGGG/GCTGGCGG | c.81G>C                | p. =             | C/C = 0.00         | C/C = 0.00         | NA        |
|          |        |       |                  |                        |                  | C/G = 0.02         | G/G = 0.98         |           |
| Exon 1   | rs695871 | Coding | GTCGTC/GTCTTTC  | c.319C>G               | p.Leu107Val      | C/C = 0.07         | C/C = 0.08         | C/C = 0.07 |
|          |        |       |                  |                        |                  | C/G = 0.35         | G/G = 0.60         | G/G = 0.51 |
| Exon 1   | rs695872** | Coding | CGCCCGC/TGGCTCC | c.390C>T               | p. =             | C/C = 0.08*        | C/C = 0.08         | C/C = 0.00 |
|          |        |       |                  |                        |                  | C/G = 0.36*        | G/G = 0.60         | G/G = 0.51 |
| Intron 15|        | Non-coding | TAGACCA/CTCCTGT | c.2882+24C>A          |                  | C/C = 0.96         | C/C = 0.96         | NA        |
|          |        |       |                  |                        |                  | A/A = 0.04         | A/A = 0.00         |           |
| Intron 21| rs12301585 | Non-coding | TGGAGGT/TTGAGG | c.3371-46T>G           |                  | G/G = 0.00         | G/G = 0.00         | G/G = 0.00 |
|          |        |       |                  |                        |                  | G/T = 0.02         | G/T = 0.00         | G/T = 0.00 |
| Intron 21| rs2301622 | Non-coding | TTGTAG/CTGAG   | c.3371-40G>C           |                  | C/C = 0.08         | C/C = 0.07         | C/C = 0.03 |
|          |        |       |                  |                        |                  | C/G = 0.40         | C/G = 0.39         | C/G = 0.41 |
| Intron 22| rs2073950 | Non-coding | TTCTCTG/ATCGTT | c.3517-12G>A           |                  | A/A = 0.03         | A/A = 0.04         | A/A = 0.03 |
|          |        |       |                  |                        |                  | A/G = 0.21*        | A/G = 0.21         | A/G = 0.42 |
| Exon 23  |        | Coding | GCCCGGG/GTTATAC | c.3744G>A              | p. =             | A/A = 0.00         | A/A = 0.00         | NA        |
|          |        |       |                  |                        |                  | A/G = 0.04         | G/G = 0.96         |           |

*The genotype frequencies are statistically different than those reported for the Hap-Map CEU population.

**Frequencies for Hap-Map CEU not available for this SNP, CEPH frequencies given.

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We also determined the size and structure of the CAG/CAA repeat as the CAG repeat is known to be interrupted by CAA codons. This repeat is relatively invariant in the CEU population, but highly variable in Africans. The most common allele worldwide has 22 repeats with a composition of (CAG)22CAA(CAG)8 and has undergone adaptive selection in the CEU population [5]. We confirmed the known repeat configurations in our sample and detected a total of 15 different CAG/CAA repeat alleles (Table 2, Table S2). Five of the alleles (CAG)10CAA(CAG)11; (CAG)9CAA(CAG)5CAA(CAG)7; (CAG)22CAA(CAG)11; (CAG)13CAA(CAG)9; (CAG)12CAA(CAG)5 have not been previously identified in over 900 alleles worldwide. Overall, we detected a higher frequency of alleles with 23 or more repeats in our white cohort (14.9%) than reported for the CEU (9.1%, n = 110) or the Polish (7.7%, n = 234) populations. Given the large numbers of comparisons necessary, we did not attempt a statistical analysis of CAG repeat allele frequencies and repeat alleles. A larger number of obese individuals need to be examined to determine whether particular repeat alleles are associated with the development of obesity.

Discussion

The ATXN2 gene was initially identified as the gene mutated in SCA2, a human neurodegenerative disease. The only mutations identified so far consist of expansion of a CAG repeat in exon 1, which encodes a polyglutamine domain leading to aggregation of the protein in neurons [3,19,20]. Knock-out of the gene in mice [12,13] and the presence of the (CAG)8CAA(CAG)4CAA(CAG)8 configuration appears to have been selected in Northern Europeans. Although CAA interruptions do not change the amino acid sequence, they lead to branched structures at the DNA and RNA level [22]. We therefore analyzed not only the repeat length, but also the repeat structure.

Recent studies suggested that evolutionary selection acts upon the ATXN2 CAG repeat or closely linked SNPs[5]. An allele with the (CAG)8CAA(CAG)4CAA(CAG)8 configuration appears to have been selected in Northern Europeans. Although CAA interruptions do not change the amino acid sequence, they lead to branched structures at the DNA and RNA level in vitro [22].

### Table 2. Allele Frequency in the GOOS Cohort and 9 other Populations.

| Repeat Structure | Repeat size | Mixed Pop n = 184a | White Only* n = 154b | CEU n = 110b | French n = 17c | Spanish n = 11d | Polish n=234e | Indian n=215f | CHB n = 86g | JPT n = 129h | YRI n = 106b |
|------------------|-------------|-------------------|---------------------|-------------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|
| (CAG)12CAA(CAG)8 | 21          | 0.006 (1)         | 0.004 (1)           | 0.005 (1)   |              |              |              |              |              |              |              |
| (CAG)8CAA(CAG)4CAA(CAG)7 | 21          | 0.005 (1)         | 0.006 (1)           |              |              |              |              |              |              |              |              |
| (CAG)22          | 22          | 0.005 (1)         | 0.006 (1)           |              |              |              |              |              |              |              |              |
| (CAG)8CAA(CAG)13 | 22          | 0.005 (1)         | 0.006 (1)           |              |              |              |              |              |              |              |              |
| (CAG)10CAA(CAG)11| 22          | 0.005 (1)         | 0.006 (1)           |              |              |              |              |              |              |              |              |
| (CAG)13CAA(CAG)8 | 22          | 0.14 (26)         | 0.136 (21)          | 0.173 (19)  | 0.118 (2)   | 0.145 (34)  | 0.205 (44)  | 0.442 (38)  | 0.217 (28)  | 0.132 (14)  |              |
| (CAG)8CAA(CAG)4CAA(CAG)8 | 22          | 0.696 (128)       | 0.688 (106)         | 0.727 (80)  | 0.529 (9)   | 0.455 (128) | 0.688 (106) | 0.727 (80)  | 0.529 (9)   | 0.455 (128) |              |
| (CAG)8CAA(CAG)5CAA(CAG)7 | 22          | 0.05 (1)          | 0.006 (1)           |              |              |              |              |              |              |              |              |
| (CAG)9CAA(CAG)4CAA(CAG)8 | 23          | 0.005 (1)         | 0.006 (1)           |              |              |              |              |              |              |              |              |
| (CAG)13CAA(CAG)9 | 23          | 0.043 (8)         | 0.052 (8)           | 0.009 (1)   | 0.021 (5)   | 0.014 (3)   |              |              |              |              |              |
| (CAG)14CAA(CAG)8 | 23          | 0.049 (9)         | 0.058 (9)           | 0.064 (7)   | 0.030 (7)   | 0.014 (3)   |              |              |              |              |              |
| (CAG)8CAA(CAG)14 | 23          | 0.05 (1)          | 0.006 (1)           | 0.059 (1)   | 0.028 (3)   |              |              |              |              |              |              |
| (CAG)8CAA(CAG)5CAA(CAG)8 | 23          | 0.016 (3)         | 0.059 (1)           | 0.377 (40)  |              |              |              |              |              |              |              |
| (CAG)25          | 25          | 0.011 (2)         | 0.013 (2)           |              |              |              |              |              |              |              |              |
| (CAG)13CAA(CAG)11| 25          | 0.005 (1)         | 0.006 (1)           |              |              |              |              |              |              |              |              |
| (CAG)13CAA(CAG)13 | 27          | 0.005 (1)         | 0.006 (1)           |              |              |              |              |              |              |              |              |

*Pulst et al (2009), Gibbs et al (2005), Imbert et al (1996), Pujana et al (1999), Krzyzosiak et al (2004), Choudhry et al, Mizuhima et al (1999), Sanpei et al (1996).

**This number is contained within the n = 184 of the Mixed population.

**Repeats ≈27 CAG repeats not found within our cohort.

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structure of the repeat with respect to the location and number of CAA interruptions (Table 2, Table S8). As reported for virtually all populations, the allele with 22 repeats and two CAA interruptions made up the vast majority of alleles. We did not find unusually expanded repeat lengths in the obese children, but 5 repeat structures that had not been previously seen in over 900 alleles worldwide. Four of these had ≥23 repeats. Overall repeats ≥23 were more common in white obese children than previously reported for CEU and Polish populations. It is important to note that an effect of the ATXN2 repeat structure on obesity-related traits would be difficult to detect using adjacent SNPs in genome-wide association studies due to the higher mutation frequency of truncotillide repeats in human populations.

Several limitations of our study need to be recognized. A sample size of 92 severely obese children may not be sufficient to detect rare variants. Our sequencing strategy would not have detected regions that likely control expression of the rare variants. Our sequencing strategy would not have detected size of 92 severely obese children may not be sufficient to detect that an effect of the reported for CEU and Polish populations. It is important to note that an effect of the ATXN2 repeat structure on obesity-related traits would be difficult to detect using adjacent SNPs in genome-wide association studies due to the higher mutation frequency of truncotillide repeats in human populations.

Materials and Methods

Cohort

The Genetics of Obesity Study (GOOS) is a large cohort of patients with severe early onset obesity [23]. Inclusion criteria for the GOOS cohort are severe obesity defined as a BMI standard deviation score (S.D.S) >5.2, and onset of obesity before 10 years of age. Ninety-two patients in whom the known monogenic causes of obesity had been excluded by sequencing of the genes encoding LEP, LEPR, POMC and MC4R were selected for this study. 77 patients in this study were U.K. white, 6 were Indonesian, 2 Afro-Caribbean and the remainder 7 of mixed background. Mean BMI was ≥5.2 standard deviations above the mean (corrected for age and gender), age of onset of obesity was 4 years.

Ethics Statement

All subjects gave their written informed consent and the study was approved by the Cambridge Local Regional Ethics Committee, U.K. The study was conducted according to the principles of the Helsinki Declaration.

Sequence Analysis

PCR reactions were done under the following conditions: 50 ng (5 μL) of genomic DNA, 80 ng of each primer (Table S1), 10 mM dNTP mix (Roche), 1X (2 μL) of 10X Buffer (Qiagen), 1 unit (0.2 μL) of Hot Taq (Qiagen), 2 μL of Q solution (Qiagen) and ddH2O up to 20 μL, utilizing step down PCR in which the initial denaturation of 95°C for 15 s was followed by 10 cycles consisting of: 95°C 1′, annealing of 5°C higher than optimal temperature listed on primer table for 30″, 72°C 1′30″ followed by 30 cycles of 95°C 1′, optimal annealing temperature as listed per each exon for 30″, 72°C 1′ 30″ followed by an extension of 72°C for 5′. Due to the large size of exon 1, it was amplified at 95°C for 15′, 35 cycles of 95°C 1′ 30″, 65°C 30 sec, 72°C 2 min with a final extension of 72°C for 7 min. PCR products were checked on agarose gels, and then sequenced as detailed below. Each fragment containing mutations was PCR amplified and sequenced a second time to confirm that the identified mutations were not due to PCR artifact.

DNA sequencing was performed using the ABI BigDye Terminator v3.1 cycle sequencing kit and the following protocol: 10 ng (2 μL) of purified PCR amplicon, 3 μL sequencing reaction pre-mix, 2 μL 5X sequencing buffer, 80 ng (2 μL) of primer and 11 μL of ddH2O. The reaction mix was run in a PCR thermocycler (Bio-Rad MyCycler v 1.065) and cycled as follows: 96°C for 3 min followed by 25 cycles consisting of 96°C for 10 s, 50 for 5 s and 60°C for 4 min. Sequencing products were purified using ABI Centri-Sep spin columns. Resuspended samples were electrophoresed on an ABI 377 DNA sequencer. All sequences were analyzed using BioEdit biological sequence alignment editor (v5.0.9.1, Tom Hall, Isis Pharmaceuticals).

Sequencing of the CAG repeat: Primer labeling was set up with 25pmol of the SCA2-A (5′GGGCCCCCTCACCATGTCG3′) oligonucleotide primer end-labeled with γ-32P ATP (3,000 Ci/nmol, 10 mCi/ml, 50 pmol total), 5 units (5 μL) of T4 Polynucleotide Kinase (Epicentre), 5 μL of 10X Kinase Buffer and ddH2O up to 50 μL. This reaction mix was incubated at 37°C for 30 min then heat inactivated at 70°C for 5 min.

Sequencing took place with the same cycling conditions described above for exon 1. As template, we used 50–100fmol of exon 1 amplicon. The sequencing protocol was followed as described in the SequiTherm EXCEL II DNA sequencing kit. Reaction products were resolved in a standard 6% denaturing polyacrylamide gel, 8 M urea.

Supporting Information

Table S1 SCA2 Primer Table and predicted amplicon sizes

Table S2 Comprehensive SCA2 CAG Repeat Structure and Frequency in 9 Populations

Author Contributions

Conceived and designed the experiments: KPF SMP. Performed the experiments: KPF KH JF. Analyzed the data: KPF JF SMP. Contributed reagents/materials/analysis tools: KPF SF SMP. Wrote the paper: KPF SMP.

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