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Screening exons 16 and 17 of the amyloid precursor protein gene in sporadic early-onset Alzheimer's disease

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ABSTRACT

Early-onset Alzheimer's disease (EOAD) can be familial (FAD) or sporadic EOAD (sEOAD); both have a disease onset ≤65 years of age. A total of 451 sEOAD samples were screened for known causative mutations in exons 16 and 17 of the amyloid precursor protein (*APP*) gene. Four samples were shown to be heterozygous for 1 of 3 known causative mutations: p.A713T, p.V717I, and p.V717G; this highlights the importance of screening EOAD patients for causative mutations. Additionally, we document an intronic 6 base pair (bp) deletion located 83 bp downstream of exon 17 (rs367709245, IVS17 83-88delAAGTAT), which has a nonsignificantly increased minor allele frequency in our sEOAD cohort (0.006) compared to LOAD (0.002) and controls (0.002). To assess the effect of the 6-bp deletion on splicing, COS-7 and BE(2)-C cells were transfected with a minigene vector encompassing exon 17. There was no change in splicing of exon 17 from constructs containing either wild type or deletion inserts. Sequencing of cDNA generated from cerebellum and temporal cortex of a patient harboring the deletion found no evidence of transcripts with exon 17 removed.

1. Introduction

Alzheimer's disease (AD) was estimated to cause 500,000 deaths in the U.S. in 2010 (Alzheimer's Association, 2014), and this number is steadily increasing with the aging population. Sporadic early-onset Alzheimer's disease (sEOAD) patients have a disease onset ≤65 years of age and do not harbor a known causative mutation; the remaining sporadic cases are classified as late-onset Alzheimer's disease (LOAD). Both sporadic forms have a complex etiology, with LOAD estimated to be 70% heritable. Patients with a causative mutation are classified as Familial Alzheimer's disease (FAD) or autosomal dominant Alzheimer's disease. Since the 1990s, many causative mutations have been identified, all located in 1 of 3 genes; amyloid precursor protein (*APP*), presenilin 1 (*PSEN1*), and presenilin 2 (*PSEN2*) (Goate et al., 1991; Levy-lahad et al., 1995; Sherrington et al., 1995). Studies have found that mutations in *PSEN1* and *APP* account for most FAD cases, approximately 55% and 13%, respectively (Janssen et al., 2003). The remainder are caused by mutations in *PSEN2* and duplications in *APP*. The median age of onset for all these variants is between 45 and 55 years of age, with onset presenting as early as the mid 20s or as late as the mid 70s (Ryman et al., 2014). Given their similar clinical presentation, it is highly likely that FAD patients have been misclassified as sEOAD (or vice versa) if a proband's family history has not been documented, or genetic screening of the proband has not been conducted.

Screening sEOAD patients for causative mutations would help prevent introduction of FAD into sEOAD cohorts and thereby increase the power to detect true genetic loci associated with sEOAD. Causative mutations in *APP*, *PSEN1*, and *PSEN2* are documented in the AD&FTD mutation database (Cruts et al., 2012). Unlike *PSEN1* and *PSEN2*, causative *APP* mutations are clustered into just 2 exons (16 and 17), both of which are less than 500 base pairs (bp) and therefore amenable to screening via Sanger sequencing.

The ARUK Consortium members are listed in Appendix.

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In this article, we report on screening of 451 sEOAD samples from the ARUK DNA Resource for causative mutations in exons 16 and 17 of *APP* using a sequencing reaction for each exon. Additionally, there is a documented 6-bp deletion (rs367709245; IVS17 83-88delAAGTAT) located downstream of exon 17, and the design of this PCR product also permitted genotyping of this variant. As rs367709245 occurs near an intron and/or exon boundary, we have also investigated its possible effect on splicing and generation of potential alternative isoforms of *APP*.

2. Materials and methods

All methods were conducted according to the manufacturer's instructions unless otherwise stated. sEOAD samples were screened for causative mutations by Sanger sequencing. In addition, the sequencing of exon 17 permitted genotyping of rs367709245 (*APP* 6-bp intronic deletion, 83 bp downstream of exon 17). Further genotyping of rs367709245 in LOAD and controls was achieved via a custom designed KASP genotyping assay.

2.1. Samples

The 451 sEOAD samples had an age of disease onset (AAO) ≤65 years of age (Table 1), the 584 LOAD samples had an AAO >65 years of age (Table 2), and the 528 controls had an age at death >65 years of age (Table 3). Where AAO was not documented, it was derived assuming 8 years disease duration from age at death (Brookmeyer et al., 2002), or age at sampling was used with the understanding it would approximate to disease onset.

All case samples were diagnosed as either definite (postmortem confirmed) or probable AD according to National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association, and the Consortium to Establish a Registry for Alzheimer's Disease guidelines. All samples used in this study were received with informed consent and were approved by the local Ethics Committee.

DNA was extracted from blood or brain tissue using a standard phenol chloroform extraction method. DNA quality and quantity was assessed via gel electrophoresis and NanoDrop 3300 spectrometer, respectively.

2.2. Sequencing of APP

APP exon 16 (485 bp) was amplified using primers E16F 5′ CAG-GTT-TCC-CTT-ACC-CTT-TC 3′ and E16R 5′ GCG-CTC-AGC-CTA-GCC-TAT-TT 3′ (Eurogenomics). *APP* exon 17 (482 bp) was amplified using primers E17F 5′ CAA-CCA-GTT-GGG-CAG-AGA-AT 3′ and E17R 5′ CAC-GGT-AAG-TTG-CAA-TGA-ATG 3′ (Eurogenomics). Both amplicons were sequenced in the forward direction using primer E17F or E16F, those found to harbor a causative mutation were validated by sequencing in the reverse direction using reverse primer E16R or E17R.

Genomic DNA was amplified using 2 ng/μL gDNA, 1 pM forward primer, 1 pM reverse primer, 1× Buffer (BioLabs), 0.2 mM dNTPs (Thermo Scientific), 0.1 U/μL LongAmp Taq DNA polymerase (New England Biolabs). The reaction was subjected to the following conditions: initial denaturation step of 94 °C for 2 minutes, followed by 35 cycles of 94 °C for 30 seconds, 58 °C (exon 16) or 61 °C (exon 17) for 15 seconds, and 72 °C for 45 seconds, with a final extension step at 72 °C for 7 minutes. PCR products were cleaned using ExoSAP-IT (Affymetrix) and Sanger sequenced using BigDye Terminator, version 3.1 (Thermo Fisher Scientific). The reactions were cleaned using Performa DTR Gel filtration Cartridges (Edge Biosystems) and sequenced on the ABI 3130.

2.3. Exontrap minigene assay

2.3.1. TA cloning

gDNA was amplified for exon 17 using the method described in Section 2.2, but with primer E17SF 5′ CAA-ATA-GTC-GAC-CAA-CCA-GTT-GGG-CAG-AGA-AT 3′ (Eurogenomics) which has a Sall restriction site at position 7 to 12 of the primer, and E17SR 5′ GAG-CAG-TCT-AGA-CAC-GGT-AAG-TTG-CAA-TGA-ATG 3′ (Eurogenomics) which has a Xbal restriction site at position 7 to 12 of the primer. The product was 494 bp in length and contained exon 17 (147 bp) along with 167 bp upstream and 192 bp downstream intronic sequence.

Amplicon DNA ligated with the pCR 2.1-TOPO vector (Invitrogen) was transformed into One Shot TOP10 Chemically Competent *E. coli* cells (Invitrogen). Colonies harboring insert were selected and DNA extracted from liquid bacterial cultures using the QIAprep Spin Miniprep Kit (QIAGEN) and sequenced to confirm the presence and/or absence of the deletion. The APP insert for each allele was then subcloned into the Exon-trap vector pET01 (MoBiTech) using Sall and Xbal digestion followed by ligation with T4 Ligase.

2.3.2. pET01 cloning and COS-7 and BE(2)-C Cell Culture

Plasmids were sequenced to confirm successful cloning using primer pET01S 5′ GAT-CGA-TCC-GCT-TCC-TG 3′ or pET01AS 5′ GTC-ATA-GCT-GTT-TCC-TG 3′ (Eurofin Genomics). The NucleoBond Xtra Midi EF/Maxi EF kit (Macherey-Nagel) was used to extract pET01 plasmids free of endotoxins and suitable for transfection.

COS-7 and BE(2)-C cells were obtained from the European Collection of Cell Cultures. COS-7 cells were cultured in Dulbecco's modified eagle medium (Sigma) with 10% fetal bovine serum (Gibco), 2-mM L-Glutamine, 100-U/mL penicillin (Gibco), 100 μg/mL streptomycin (Gibco), and 2.5 μg/mL fungizone. BE(2)-C was cultured with 50% of eagle's minimum essential medium and 50% Ham's F12 supplement with additional components 1% of nonessential amino acids, 2-mM L-glutamine, 1-mM sodium pyruvate, 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 100 U/mL fungizone.

Table 1
Sample demographic of sporadic early-onset Alzheimer's disease

Centre	N	Mean age at onset (±SD)	Females (%)	*APOE* ε4+ (%)	*APOE* ε4ε4 (%)	*APOE* ε4 MAF	Definite	Probable
Bristol	24	53.5 (5.3)	11 (45.8)	12 (50.0)	3 (12.5)	0.31	24	0
Manchester	356	57.3 (5.4)	171 (48.0)	211 (59.3)	49 (13.8)	0.56	61	295
Nottingham	37	58.5 (6.1)	18 (48.6)	17 (45.9)	2 (5.4)	0.26	7	30
Oxford	34	55.5 (4.2)	19 (55.9)	20 (58.8)	4 (11.8)	0.35	24	10
All	451	57.0 (5.5)	219 (48.6)	260 (57.6)	58 (12.9)	0.51	116	335

Each cohort contains samples from multiple centers with each center represented one per row. The number of samples from each center (N) is given along with the mean age of onset with standard deviation (mean age at onset [±SD]), the number and percentage of female samples per center [women (%], the number and percentage of samples harboring at least 1 *APOE* ε4 allele [*APOE* ε4+ (%)], the number and percentage of samples with *APOE* ε4ε4 genotype [*APOE* ε4ε4 (%)], the minor allele frequency of the *APOE* ε4 allele (*APOE* ε4 MAF), and finally the number of samples classed as postmortem confirmed Alzheimer's disease (Definite) or probable Alzheimer's disease (Probable).