Research Article
An Intron 7 Polymorphism in APP Affects the Age of Onset of Dementia in Down Syndrome

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People with Down syndrome (DS) develop Alzheimer’s disease (AD) with an early age of onset. A tetranucleotide repeat, att5, in intron 7 of the amyloid precursor protein has been associated with the age of onset of AD in DS in a preliminary study. The authors examine the impact of this polymorphism in a larger cohort of individuals with DS. Adults with DS were genotyped for att5 and APOE. The results were analysed with respect to the age of onset of dementia. The presence of three copies of the six-repeat allele resulted in onset of dementia seven years earlier than in the presence of other genotypes. Further study is essential to elucidate the mechanism by which this polymorphism functions, with an exciting opportunity to identify novel treatment targets relevant for people with DS and AD.

1. Introduction

Down syndrome (DS), defined cytogenetically by trisomy 21, is the most common chromosomal disorder associated with learning disability, occurring in approximately 1/1000 live births [1]. The prevalence of Alzheimer’s disease (AD) in people with DS increases significantly with age. However, the nature of the early clinical presentation, course, and incidence rates of dementia are uncertain. Despite the nearly universal occurrence of AD pathology by age 40 [2, 3], there is wide variation in age of onset of clinical dementia. Most studies have indicated that the average age at onset of dementia is between 50 and 55 years of age, with a range from 38–70 years [4]. Many studies have now confirmed that age-related cognitive decline and dementia affecting people with DS occurs 30–40 years earlier than in the general population. Age-specific rates of dementia begin to increase in the patient’s 30s from 1-2% to 40% in the 50s [5], hence dementia is becoming an increasingly important issue in people with DS as life expectancy continues to increase.

The neuropathological manifestations of AD in DS have been at least in part attributed to triplication and overexpression of the gene for amyloid precursor protein (APP) located on chromosome 21 [6]. In fact, an additional copy of APP can cause early onset AD with cerebral amyloid angiopathy, even when only small regions of chromosome 21 including only the APP gene are triplicated [7–10]. Triplication of chromosome 21 leads to an increase in expression levels of its genes. APP is expressed at levels which are four- to five-fold higher in DS than in the general population [11]. This is not only due to triplication of the gene, but is also caused by regulators of APP expression, for example ETS2, present on chromosome 21, which have increased expression levels [12]. Processing of APP can result in the production of beta-amyloid (Aβ) which is deposited extracellularly as a core disease feature in the brains of people with AD.

Along with overexpression, other APP changes are seen in AD, which include variations in the proportion of APP splice variants. Three of these isoforms are APP695, APP751, and APP770. APP695 is predominantly expressed in neurons
whilst APP751 and APP770 are ubiquitously expressed. A
kunitz protease inhibitor domain (KPI) encoded by exon 7 is
present in both APP751 and APP770, but not in APP695. The
ratio of APP751:APP695 mRNA is found to be increased in
the brains of people with AD [13, 14], and there appears to
be a relationship between this ratio and the density of plaques
in the hippocampus and entorhinal cortex [15]. In fact, mice
expressing human APP751, but not APP695, develop an AD-
like pathology, which closely resembles that found in the
brains of young people with DS [16]. This pathology not
only involves Aβ, but also abnormal tau isoforms. Therefore,
it would seem that not only overexpression of APP in DS, but
also variation in isoforms, may have a role in determining the
onset of AD.

Intron 7 of APP has been sequenced previously to determine if polymorphic sites which may have the potential
to regulate exon 7 splicing were present [17]. The tetra-
nucleotide repeat (attt5–8) was identified and the attt6 allele
was found to be the most common, present in 96% of the
genotyped population. When this region was genotyped in a
preliminary study of 105 people with DS, there was found to
be an association between the number of attt6 alleles
and the age of onset of dementia [18]. Specifically, those
individuals carrying three copies of the attt6 allele developed
dementia with an earlier age of onset than those with any
other combination of the repeats.

In DS polymorphisms on chromosome 21 may have an
effected. As most people with DS develop AD with an
early age of onset, these amplified genetic effects may allow
us to more clearly detect the associated phenotypes, such
as accelerated onset of dementia or severity. Such genetic
effects may be too subtle to detect in the general population,
but the mechanistic information provided will be valuable
in drug design for all those at risk of developing AD.

Following the preliminary study [18] we examine whether
the tetranucleotide repeat, attt5–8, in intron 7 impacts upon
age of onset of dementia in a larger cohort of individuals with
DS.

2. Materials and Methods

2.1. Samples and Clinical Assessments. Clinical evaluation
was completed and DNA samples were obtained from 291
adults with DS (Newcastle-181, Birmingham-78, MEAD-
OWS Clinical Trial-32), substantially extending a previous
pilot study [18]. In the previous study the difference in age of
onset between the genotypes was 13.4 years, with a standard
deviation of 7.2 years [18]. Using these values, a power
calculation shows that a study using a total of 18 participants
(9 attt6 homozygotes and 9 heterozygotes) has a 95 percent
chance to detect a difference at a two-sided 0.05 significance
level.

This study was approved by the appropriate local research
ethics committee and consent was obtained from the
participants. Detailed prospective clinical assessments were
completed which included the DAMES battery (Newcastle,
MEADOWS) and the Adaptive Behaviour Scale (ABS) [19]
(Birmingham, MEADOWS), which enabled the operational-
ized diagnosis of dementia by an expert in the field (MH, VP)
according to the International Classification of Disease [20].
Clinicians were blind to genotyping results.

2.2. Genotyping. Blood samples were collected into K2-
EDTA vacutainers (BD Diagnostics), and DNA was extracted
using the FlexiGene DNA kit (Qiagen). Genotyping of the
APP intron 7 tetranucleotide repeat was performed using a
CEQ8000 Genetic Analyser (Beckman Coulter). Briefly, the
region surrounding the repeat was amplified by PCR (Dye
4-labelled forward primer 5′GCATGCTGTTAACAGACT-
tcc3′, reverse primer 5′GAGTATCTACTCTGCTAC3′,
both from Proliago). PCR enzymes and buffers were from
Qiagen. Cycling was carried out as follows: 94°C for 2
minutes, 35 cycles of (94°C for 30 seconds, 55°C for 30
seconds, 72°C for 1 minute), 72°C for 10 minutes. This
reaction produced a 105 bp product for the attt6 allele.
Labelled PCR products were combined with a 400 bp size
standard and sample loading solution (Beckman Coulter)
and processed on the CEQ8000 genetic analyser.

APOE genotyping was carried out using PCR (forward
primer 5′TCAGAGCAGTCCAAGGCAGCTTGA-3′
and reverse primer 5′ACGAATTCTGCCGCACATGG-
tacactgca-3′, Sigma-Aldrich). AmpliTaq Gold enzyme
and buffer (Applied Biosystems) and 10% DMSO (Sigma-
Aldrich) were used, and cycling proceeded as follows: 94°C
for 5 minutes, 45 cycles of 94°C for 30 seconds, 65°C for 1
minute, 72°C for 1 minute, and 72°C for 10 minutes. The
resulting product was digested with HaeII or AffIII (New
England Biolabs). Fragments were separated on 1% standard
agarose (Invitrogen Life Technologies) gels containing 3% Metaphor agarose (Cambrex).

2.3. Statistics. Statistical analysis of clinical and genotyping
data was carried out using SPSS version 17. For the primary
analysis we compared the age of onset of dementia in people
with and without the attt6 polymorphism using independent
t-tests. The same evaluation was used to examine the
impact of APOE ε4.

3. Results

Two-hundred and ninety-one DNA samples were genotyped
for the intron 7 repeat polymorphism attt5–8 in APP and
APOE, using fragment analysis with a CEQ8000 genetic
analyser and restriction fragment length polymorphism,
respectively. Clinical data for each participant was collected
by the examining psychiatrists. The mean age of the partici-
ants was 52.20 years ± 13.93 (range from 24 to 89 years),
and 38% were female. Based upon the operationalized psychiatric
evaluations, 103 participants had dementia (35%), with a
mean age of onset of 50.46 years ± 7.85 (range 34–74 years).
A further 76 individuals were 45 or older, but did not meet
operationalized criteria for a diagnosis of dementia. Thirty
people (10.3%) were heterozygous for the attt5–8 genotype,
which is in agreement with frequencies found in previous
genotyping studies [17, 18]. Of the 103 participants with
dementia, 9 were heterozygous for the tetranucleotide repeat
polymorphism, which is an appropriately sized sample when
considering the power calculation shown in Section 2. Due to variation in the peak sizes for the fragment analysis it is not possible to tell if the heterozygotes have one or two copies of the rarer alleles, so we have chosen to group all heterozygotes together based upon the presence of alleles attt6, attt5, or attts. This may be due to variations in amplification efficiencies between the different repeat alleles.

A significant difference was evident in the age of onset of dementia between individuals with 3 attt6 alleles and those with any other allele combination ($n = 103$, independent samples $t$-test, $t = -2.65$, df = 102, $P = .009$) (a Kaplan-Meier plot is shown in Figure 1). The mean age of onset in individuals with 3 attt6 alleles was 49.85 years ± 7.81 whilst in people with a different repeat combination the mean age was 56.89 years ± 5.09. None of the individuals with less than 3 attt repeats developed dementia before 52 years.

We also examined the link between the attt5–8 polymorphism and the risk of developing dementia before the age of 45. Only individuals who had dementia or had reached the age of 45 without developing dementia were included in this analysis, as individuals under the age of 45 and without dementia have not yet reached the high-risk period for dementia development. A trend was found between the risk of developing dementia before the age of 45 and the APP polymorphism ($n = 179$, $\chi^2 = 2.95$, df = 1, $P = .066$).

Gender was not related to the age of onset of dementia ($t = -1.19$, df = 84, $P = .24$). The presence of an APOE-ε4 allele was not associated with the age of onset of dementia ($t = 0.49$, df = 80, $P = .62$). Neither gender nor the presence of an APOE-ε4 allele were associated with attt5–8 genotype ($\chi^2 = 1.22$, df = 1, $P = .27$ and $\chi^2 = 0.27$, df = 1, $P = .60$, resp.).

4. Discussion

These data highlight a striking association between 3 copies of the attt6 allele in intron 7 of APP and a substantially earlier age of onset of dementia. People with 3 attt6 alleles developed dementia 7 years earlier. The next step is to determine how the acceleration of dementia in DS is brought about in those dementia 7 years earlier. The next step is to determine how they are linked to attt6 allele.

The tetranucleotide repeat region is located 1200 bp into the 2598 bp sequence of intron 7 and is 193 bp 3’ from an Alu sequence which is key for binding of splicing factors and splicing of exons 7 and 8 from APP [7, 21] (Figure 2). The tetranucleotide repeat lies in a 524 bp region between single nucleotide polymorphisms (SNPs) rs9982544 and rs2409162, but neither of these is polymorphic in the CEU population and so cannot be used for haplotype analysis of this region for our samples. The closest SNPs which are sufficiently polymorphic in the CEU population, rs3787637 and rs8132200, enclose a region of 2313 bp extending beyond intron 7, and further analysis of how the tetranucleotide repeat is associated with these will be required before we can understand the linkage of this location.

Figure 1: Kaplan-Meier survival plot showing the effect of the tetranucleotide attt5–8 upon age of onset of dementia in 103 people with DS. Those people carrying three copies of the attt6 allele of this polymorphism develop dementia with an earlier age of onset than those with any other combination of alleles ($P = .009$).

It is unclear why the 6-repeat allele is associated with risk when neither the 5- or 7-repeat alleles are, and why risk is not associated with increasing or decreasing repeat length although similar results have been found for other genes and disease risk: CYP19 and breast cancer risk, and MIF and gastric cancer risk [22, 23].

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determine the impact of the tetranucleotide repeat upon Aβ deposition in the brain in postmortem studies. This will give an indication of how large a role this polymorphism plays in Aβ regulation.

The APOE-ε4 allele had no effect upon the age of onset of dementia in this study. Previously it has been suggested that APOE does not affect the age of onset of dementia in cases of familial Alzheimer’s disease (FAD) due to chromosome 14 mutations [27]. It may be that when APP regulation is strongly affected through triplication in DS or through particular mutations, APOE effects are masked by the presence of the more powerful APP effects. Triplication of APP in DS may have resulted in amplified effects of this polymorphism, but it may still have important potential implications for AD which have not yet been detected in the general population.

When this polymorphism was previously studied by Li et al. [17] the less common repeat sizes were only found in a small number of samples, which may result in low statistical power. Given that this polymorphism appears to take effect in a population with an increased risk of AD and increased expression of APP leading to increased Aβ levels, it would be valuable to examine the effects of this polymorphism in a second population with an increased incidence of AD and Aβ concentrations, namely those in the wider population at risk of developing AD.

Studies such as these not only provide the opportunity to discover therapies which may benefit those with DS, but also those in the older general population in which the incidence of AD is increasing. The benefits of studying the relatively small DS population must not be forgotten in the search for new therapies to tackle AD.

5. Conclusions

The attt<sub>5–8</sub> polymorphism in APP is associated with the age of onset of AD in DS, from which we conclude that it may function by accelerating the pathological process or is a marker for an, as yet unidentified, functional variant. The mechanism by which this is achieved has yet to be established, but the proteins involved may prove to be useful targets for future drug development.

Conflict of Interests

The authors report no conflict of interests.

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