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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, attt5–8, 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 attt5–8 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 most 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 tetranucleotide repeat (attt₆) 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 amplified effect. 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, attt₆, 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, MEADOWS 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 operationalized 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’CCATGTCGTTAACAGACTTCC3’, reverse primer 5’GAGTAGTTCATCTTTAC3’, both from Promega). 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 105bp product for the attt₆ allele.

Labelled PCR products were combined with a 400bp 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’TCCAAGAGCTGAGGCCGGGCGA-3’ and reverse primer 5’ACAGAATTCCCCCTTGTTACACTGCCA-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 AluII (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 sample 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 attt₆ 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 participants was 52.20 ± 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 ± 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 attt₆ 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 attt, attt, or attt. 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 attt 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 attt 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 attt 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 \( n = 179, \chi^2 = 2.95, df = 1, P = .086 \).

Gender was not related to the age of onset of dementia \( t = -1.19, df = 84, P = .24 \). The presence of an APOE-e4 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-e4 allele were associated with attt 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 attt allele in intron 7 of APP and a substantially earlier age of onset of dementia. People with attt alleles developed dementia 7 years earlier. The next step is to determine how the acceleration of dementia in DS is brought about in those people with 3 attt alleles and to understand the implications for AD in the wider population, so that the related novel therapeutic opportunities can be fully utilized.

The tetranucleotide repeat region is located 1200 bp into the 2598 bp sequence of intron 7 and is 193 bp 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.

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].

A further possibility is that the tetranucleotide repeat attt is a marker for a functional polymorphism which is altering the expression, activity, or regulation of APP, which in turn alters the development of pathology and onset of dementia. Further examination of other SNPs in APP and how they are linked to attt is required to understand fully the mechanism involved.

Levels of exon 7-containing isoforms of APP are found to be increased in AD brain [10] and associated with increased deposits of Aβ [24]. The protease inhibitor activity of the KPI domain has been shown to act on an extracellular trypsin-like serine protease which degrades secreted APP [25] and therefore, isoform variation may play an important role in regulating the activity of this protease. KPI-containing APP may also bind to low-density lipoprotein receptor-related protein (LRP), which is involved in the clearance of Aβ from the brain into peripheral tissues, and this has been shown to reduce Aβ clearance [26]. Receptors and other proteins involved in this clearance process may prove to be useful drug targets, and early intervention in this pathway may provide a method of preventing increased deposition of Aβ in the brain.

The role of this polymorphism, or other associated polymorphisms, in regulation of APP splicing is not known, and examining this will play an important role in determining how dementia risk is affected. It will also be important to
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 over the age of 80. The incidence of AD in this age group is approximately 25%, which is similar to that for people with DS in their 40s, and those over the age of 80 also have increased brain Aβ 1–40 and 1–42 levels [28]. A large study in such a population would allow us to analyse the effects of this attt5–8 in the general population at risk of developing AD.

In this study we have taken a unique opportunity to examine AD-type dementia in a population in which a high proportion of people will develop dementia with an early age of onset. This has allowed us to examine more closely the association of this polymorphism to the development and progression of dementia. The amplification of the effects of APP polymorphisms in DS allows us to detect differences which may be more subtle in the wider population.

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 attt5–8 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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