

TNF α -308 (but not IL1 α -889, IL1 β +3953 or IL12 β 3'UTR) Is Associated with AIDS Dementia Complex

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Abstract

Background: AIDS dementia complex (ADC) affects only approximately 20% of advanced HIV disease patients without antiviral therapy. Both viral and host factors affect the development of ADC. Candidate host factors associated with increased cytokine synthesis may predispose patients to ADC, given that the best marker of ADC severity is the degree of microglial activation, which in turn is related to excess cytokines. We hypothesized that polymorphisms in genes encoding the cytokines TNF α , IL-1 α , IL-1 β , and IL-12 β could be associated with ADC. We examined the relationship between ADC and ApoE4, as there have been conflicting results from previous studies.

Methods: Genomic DNA was isolated from cryopreserved peripheral blood mononuclear cells from 56 HIV + ADC patients (mean age 36 years), 112 to 203 patients with HIV disease without ADC and 60 to 204 patients without HIV disease. PCR RFLP assays were used to determine the alleles carried at TNF α -308, IL1 α -889, IL1 β +3953, IL12 β -3'UTR and ApoE. Alleles carried at BAT1(intron 10) in the central MIC were included as a marker of a conserved MIC haplotype associated with multiple immunopathological diseases [HLA-A1,B8,BAT1(intron 10)*2, TNF α -308*2, DR3,DRQ2]. Fisher's exact test was used to evaluate differences in allelic carriage.

Results: Carriage of TNF α -308*(2,2) with BAT1(intron10)*(2,x) was significantly more common in the ADC patients than in either control group (p=0.005 for HIV-positive controls and p=0.024 for HIV-negative controls). Carriage of the other polymorphisms was similar in the ADC patients and control groups.

Conclusions: Two previous studies (one clinically based, the other based on pathology) reported contradictory findings in relation to TNF α -308 and ADC. The present study is the largest thus far, so we suggest that the association is pathogenetically important. Indeed the association is of the same magnitude as that of the MCP-1 polymorphism. The lack of association with ApoE4 status may be related to the younger age of the ADC patients in our study. Presently the genetic risk signature for ADC appears to be TNF α -308*(2,2) with BAT1(intron10)*(2,x), MCP1*2578G and ApoE4 (4,4), the latter being important in older patients. Other yet-to-be-determined genetic risk factors may be important. The elucidation of the genetic signature will allow the identification of patients who will require more intensive monitoring and perhaps the earlier introduction of highly active antiretroviral therapy.

Introduction

AIDS dementia complex (ADC) affects only ~20% of patients with advanced untreated HIV disease

- The limited incidence of ADC may reflect both viral and host factors.
- Candidate host factors include cytokine genes and genes affecting tissue vulnerability.

Evidence of a role for cytokines:

- * The best marker of ADC severity is the degree of microglial activation, which is likely to reflect cytokine levels.
- * Elevated levels of cytokines have been found in the cerebrospinal fluid (CSF) and brains of ADC patients and correlates with ADC severity.

Cytokines implicated in ADC pathogenesis:

- TNF- α .
- IL-1
- interferon- γ (elevated in ADC)
- IL-12 (induces interferon- γ)

Several polymorphisms within the genes encoding TNF- α , IL-1 α , IL-1 β and IL-12 affect the production of the respective cytokines.

Here we included a polymorphism in intron 10 of the BAT1 gene, adjacent to TNF α , as it is a specific marker of a conserved disease-associated haplotype (HLA-A1,B8,DR3).

Candidate host factors associated with increased tissue vulnerability:
APOE4 (increases vulnerability to oxidative stress).

Hypothesis

Alleles carried at TNF α -308, IL1 α -889, IL1 β +3953, IL12 β 3'UTR and ApoE4 are associated with the presence of ADC.

Patient Selection

ADC Patients

- 58 ADC stage \geq 1 patients aged 19 to 64 years (mean 36 years).
- Clinical, neuropsychological, MRI and CSF evaluation confirm ADC.

Control Groups:

- 112-203 patients with HIV disease without ADC.
- 60-204 donors without HIV disease.

Methods

Isolation of DNA from Human peripheral blood mononuclear cells (PBMCs)

- PBMC were isolated by Ficoll-Paque centrifugation, resuspended in 90% FCS/10% DMSO and stored in liquid nitrogen.
- To isolate DNA, cells were washed with PBS, re-suspended in PCR lysis buffer (50mM KCl, 150mM Tris-HCl, 2.5mM MgCl₂, 0.1mg/ml gelatin, 0.45% Nonidet-P40 (NP40) and 0.45% Tween 20 containing 0.6 μ l 10mg/ml Proteinase K (in H₂O) (all from Sigma, Australia) per 100 μ l of solution and incubated at 50-60°C for 1 hour. The proteinase was then inactivated at 95°C for 10 minutes and the solution was used as template in PCR reactions.

Polymorphism Screening

- PCR RFLP assays were performed to determine the alleles carried at TNF α -308, IL12 β -3'UTR, IL12 β -promoter, IL1 α -889 and IL1 β +3953.
- Allele 1 at TNF α -308 (C) yielded 87 and 20 bp products and allele 2 (A) gave a 107 bp band.
- Allele 1 (A) at IL12 β -3'UTR (position 16974 of IL12 β sequence; Genbank AY008847) yielded a 300 bp band and allele 2 (C) yielded bands of 166 and 134 bp.
- The IL12 β -promoter polymorphism was typed using primers:
5'-TCAGACACATTAACCTTGGCA-3' and 5'-TAATGTGGTCATTGGCAGGT-3'.
Allele 1 was designated as the 4 bp larger allele; the smaller product was designated as allele 2.
- IL1 α -889 allele 1 (C) yielded 83 and 16 bp products while allele 2 (T) yielded a single band of 99 bp.
- IL1 β +3954 allele 1 (C) yielded 97 and 85 bp bands, whilst allele 2 (T) yielded a single band of 182 bp.
- Alleles of BAT1 intron 10 were determined by PCR-RFLP based on Nco1 digestion.
- APOE restriction isotyping used oligonucleotides to amplify APOE gene sequences encoding amino acid positions 112 and 158. The amplification products were digested with HhaI and subjected to electrophoresis on polyacrylamide gels. HhaI cleaves at GCGC encoding 112arg (E4) and 158arg (E3, E4) but does not cut at GTGC encoding 112eys (E2, E3) and 158eys (E2).

Statistical Analysis

- Statistical analyses were based on Fisher's exact test as some cells contained < 5 values.
- p \leq 0.05 was accepted as a significant difference.
- No correction was made for multiple analyses as this was an exploratory study.

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Carriage of the TNF α -308 2/2 genotype was more common in patients with ADC (8.9%) compared with HIV positive and negative controls (0.9 and 3.9%). These differences were statistically significant (p=0.005 and 0.024, respectively).

The excess ADC patients had the genotype TNF α -308(2,2) BAT1intron 10 (2,x)

Carriage of all other polymorphisms was similar in ADC patients and controls.

Results

Polymorphism	1/1	1/2	2/2
TNFα-308			
ADC (n=56)	33 (59%)	18 (32%)	5 (8.9%)
HIV Positive Controls (n=112)	71 (63%)	39 (35%)	1 (0.9%)
HIV Negative Controls (n=204)	137 (67%)	59 (29%)	8 (3.9%)
BAT1 Intron 10			
ADC (n=56)	40 (71%)	16 (29%)	0 (0%)
HIV Positive Controls (n=203)	146 (72%)	56 (27%)	1 (0.5%)
HIV Negative Controls (n=204)	156 (76%)	46 (22%)	2 (1%)
IL1α-889			
ADC (n=57)	33 (58%)	19 (33%)	5 (8.8%)
HIV Positive Controls (n=171)	96 (56%)	61 (36%)	14 (8%)
HIV Negative Controls (n=60)	33 (55%)	24 (40%)	3 (5%)
IL1β+3953			
ADC (n=57)	39 (67%)	15 (28%)	2 (3.5%)
HIV Positive Controls (n=185)	111 (60%)	71 (38%)	3 (1.6%)
HIV Negative Controls (n=60)	36 (60%)	23 (38%)	1 (2%)
IL12β 3'UTR			
ADC (n=58)	34 (60%)	20 (33%)	3 (7%)
HIV Positive Controls (n=204)	120 (59%)	72 (35%)	12 (6%)
HIV Negative Controls (n=96)	68 (71%)	24 (25%)	4 (4%)
APOE			
ADC (n=58)	0.12	0.77	0.11
HIV Negative Controls (n=624)	0.072	0.77	0.16

Conclusions

Our results extend the findings of published studies:

- Quasney *et al* (1998) described an association between an increase in the frequency of TNF α -308 2/2 genotype and ADC diagnosed by autopsy (n = 16). The effect was clearer in African Americans than in Caucasians. This increase was not seen however by Diaz-Arrastia *et al*, (2004) (n=53). When our data was combined with these two studies, there was an increase in the frequency of TNF α -308 1/2 and TNF α -308 2/2 between the ADC group and the HIV positive control group (p = 0.0093, CI = 1.11 - 1.94, OR = 1.773).
- Corder *et al* (1998) described an association between the APOE4 genotype and ADC. This was not evident in other studies (Dunlop *et al*, 1997; Diaz-Arrastia *et al*, 2004). Valcour *et al* (2004) suggested genetic effects may be more apparent in older patients (mean age = 55, in our study mean age = 36).

Presently the genetic risk signature for ADC appears to be TNF α -308*(2,2) with BAT1(intron10)*(2,x), MCP1*2578G and ApoE4 (4,4), the latter being important in older patients.

Elucidation of the genetic signature will allow the identification of patients who will require more intensive monitoring and perhaps the earlier introduction of highly active antiretroviral therapy.

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