

# Cytokine Genotypes Establish a Role for Inflammation in Antiretroviral Toxic Neuropathy (ATN) and Predict an Individual's ATN Risk.

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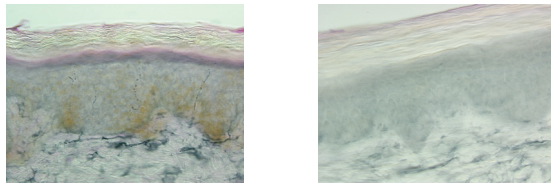
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## BACKGROUND

Sensory neuropathy is the commonest neurological problem affecting people with HIV, with a 44% prevalence in the Alfred Hospital HIV Clinic.<sup>1</sup> Distal sensory polyneuropathy (DSP) due to HIV itself and antiretroviral toxic neuropathy (ATN) associated with exposure to the nucleoside analogs (NRTIs) d4T, ddI and ddC are both recognized, and are clinically indistinguishable.<sup>2</sup> This may complicate patient management.

The histological correlates of DSP include loss of epidermal nerve fibers and increased numbers and activation of macrophages throughout the peripheral nervous system.<sup>2</sup> Epidermal nerve fiber loss is also seen in ATN (figure 1), but inflammation has not yet been well described. It is unclear why some individuals develop ATN when exposed to potentially neurotoxic NRTIs but others do not. The possible importance of inflammatory pathways in the development of ATN can also be assessed using host genotype.



**Figure 1:** Loss of epidermal nerve fibers occurs in both DSP and ATN. The micrograph on the left shows normal epidermal nerve fibers stained black. The denervated epidermis on the right is from an HIV+ patient with neuropathy

## HYPOTHESIS

1. Inflammatory pathways may be important in the pathogenesis of ATN
2. Host genotype may be a determinant of ATN risk and define the critical inflammatory pathways.

## METHODS

Established PCR-based assays<sup>3</sup> were used to analyze alleles in DNA from clinically well characterized subjects.

### Patients

HIV+ patients who had been exposed to d4T, ddI or ddC were enrolled from the Alfred and Royal Perth Hospitals, Australia.

1. No ATN: No symptoms or signs of neuropathy despite  $\geq 6$  months of d4T, ddI or ddC
2. Definite ATN: Neuropathy onset within 6 months of commencing d4T, ddI or ddC
3. Probable ATN: Neuropathy onset following d4T, ddI or ddC use. Precise timing unclear.

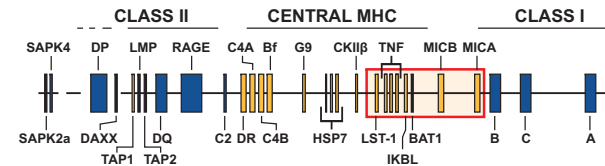
Control subjects were obtained from existing Western Australian DNA registers.

1. HIV+ controls: RPH patients not selected by ATN status
2. HIV- controls: WA bone marrow registry or the Busselton Survey

## METHODS

### Genotyping

DNA was typed by PCR-RFLP for known SNPs in genes located in the central MHC (TNFA, BAT1 and HSPA1B; figure 2) and genes encoding cytokines (IL12B, IL1A, IL1B, IL6 & IL4). A polymorphism in the BAT1 gene adjacent to TNFA was included to define carriage of a conserved pro-inflammatory haplotype [HLA-A1,B8, BAT1(intron10)\*2, TNFA-308\*2, DR3,DQ2].<sup>3</sup> Control samples carrying (1,1), (1,2) and (2,2) were included in all runs.



**Figure 2:** Location in the central MHC of some of the genes examined in this study

### Statistics

The associations of genotypes with ATN were assessed individually using 2-sided Fisher's exact tests (table 1) and jointly in a multiple case-control logistic regression with ATN as the outcome. To accommodate small numbers with the (2,2) genotypes of most alleles, genotypes were grouped as (1,1) vs (1,2 or 2,2). Exceptions were TNFA-1031 and HSP1B+1267 where there were enough patients with the rare allele.

## RESULTS

### Outcome of DNA typing

Subjects with no ATN despite prolonged ( $\geq 6$  months) exposure to d4T, ddI or ddC (n=28) and those with a definite or probable diagnosis of ATN (n=40) could be distinguished by genotype (table 1).

**Overall genotype was associated with neuropathic state (p=0.049).**

	No ATN (n=28)	Prob ATN (n=19)	Def ATN (n=21)	Def ATN vs no ATN	Def ATN vs no/prob ATN
TFNA-308*2	18	11	21	0.19	0.06
BAT182	11	11	33	0.08	0.04
TNFA-1030*2	39	42	67	0.05	0.03
TNFA-1030 (1,2)	21	26	57	0.02	0.01
HSP+1267*2	61	61	62	0.70	0.54
IL1B+3953*2	50	42	43	0.77	0.80
IL1A+4845*2	43	42	43	1.00	1.00
IL12B 3'UTR*2	50	32	19	0.04	0.10
IL6-174*2	54	58	62	0.77	0.79
IL4-589*2	32	21	48	0.38	0.17

**Table 1:** Frequencies of alleles in patients with a history of exposure to ddI, d4T, or ddC (numbers are percentages of individuals carrying each allele, p values were obtained using a 2-sided Fisher's test)

## RESULTS

### Comparison with controls

We addressed whether BAT1, IL12B and TNFA-1031 act as risk factors for ATN or may be protective against the development of this problem (table 2). Three patterns emerge:

1. Carriage of IL12B (1,1) is rare in patients with definite ATN relative to the control donors, suggesting this allele protects against ATN.
2. Carriage of TNFA-1031(1,2) is increased in patients with definite ATN, suggesting this allele marks susceptibility to ATN.
3. Carriage of [TNFA-308\*2, BAT1(intron 10)\*2] was rare in patients with no ATN compared to those with definite ATN. Neither frequency was significantly different from controls. This suggests that carriage of these alleles is a risk factor for ATN, but requires clarification in a larger population.

	HIV (n=31-246)	HIV+ (n=102-112)	No ATN (n=28)	Def ATN (n=21)
BAT1 (1,2)	20	25	11	33
IL12B (1,1)	69	60	50	19
TNFA-103 (1,2)	19	25	21	57

**Table 2(a):** Frequency of alleles (numbers shown are percentage of individuals expressing the allele) in each group in the overall model that best predicted ATN (overall p=0.001)

	HIV+vs No ATN	HIV+vs def ATN	HIV-vs no ATN	HIV-vs def ATN
BAT1 (1,2)	0.13	0.43	0.41	0.25
IL12B (1,1)	0.39	0.001	0.06	<0.0001
TNFA-103 (1,2)	0.81	0.008	1.0	0.008

**Table 2(b):** Influence of each allele in the overall model on the development of ATN compared with control populations (p values obtained using a 2-sided Fisher's test)

## CONCLUSIONS

1. These findings support a role for inflammatory pathways in the development of ATN.
2. If confirmed in a larger cohort, these findings will enable improved assessment of an individual's risk for ATN prior to commencing NRTIs, allowing more informed treatment decisions.
3. By facilitating an understanding of the cytokines involved in the development of ATN, these findings may also aid the development of rational immunotherapeutic strategies for this disabling problem.

## ACKNOWLEDGEMENTS

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## REFERENCES

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