

# T cell receptor $\beta$ chain polymorphisms are associated with cystic fibrosis

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**SUMMARY** The *BgIII* polymorphism near the constant region of the T cell receptor  $\beta$  chain (TCR  $c\beta$ ) has been investigated in normal controls, patients with cystic fibrosis (CF), and CF carriers. A significant increase was found in the frequency of the 10.0:9.2 kb heterozygous genotype in the CF carrier group (71%) as compared with normal controls (44%) ( $p=0.005$ ). Patients with CF also showed an increased frequency of the heterozygous genotype but this was not significant. These results represent a previously unreported disease association and suggest that there may be some form of epistatic interaction between the CF gene and the TCR  $\beta$  genes such that the double heterozygote is immunologically advantaged.

The T cell receptor (TCR) is the membrane bound recognition molecule on the surface of T cells which is specific for a combination of foreign antigen and a molecule of the major histocompatibility complex (MHC).<sup>1</sup> The TCR is composed of four distinct polypeptides ( $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$ ), which form two different heterodimers ( $\alpha:\beta$  and  $\gamma:\delta$ ) that are encoded by genes very similar to immunoglobulin genes in their modes of rearrangement and organisation. The  $\beta$  chain is encoded by genes constructed from variable (V), diversity (D), joining (J), and constant (C) genetic elements which undergo rearrangement during T cell differentiation.<sup>1</sup> Restriction fragment length polymorphism (RFLP) analysis has been used to detect polymorphisms in the  $\beta$  chain.<sup>2</sup> One such polymorphism has been found at a *BgIII* site, 5' to the second constant region gene segment. The heterozygous genotype for this polymorphism has been shown to be increased in autoimmune disorders, for example, insulin dependent diabetes<sup>3,4</sup> and Graves's disease.<sup>5</sup> We are at present studying this disease association in patients with organ and non-organ specific autoimmune disease. This association is not the result of linkage disequilibrium as the allele frequencies are similar in all groups studied. During the investigations we used as one of the control groups patients with cystic fibrosis (CF) and CF carriers. An increased frequency of the heterozygous genotype for the *BgIII* polymorphism has been observed in these subjects.

## Methods

### BLOOD SAMPLES

We studied 68 subjects who are carriers for CF (parents of living affected CF children), 47 patients affected with CF (from 43 families), and 57 normal subjects (samples obtained from the DNA bank, Department of Medical Genetics, Queen's University of Belfast). Each subject had 20 ml of peripheral blood collected into 5 ml of 5% EDTA.

### DNA ANALYSIS

DNA was extracted from white cell pellets (stored at  $-20^{\circ}\text{C}$  for less than one month) by the method of Jeanpierre.<sup>6</sup> The DNA samples were digested with *BgIII* (2 units/ $\mu\text{g}$  DNA) overnight at  $37^{\circ}\text{C}$  and the restriction fragments electrophoresed through a 20 cm 0.7% agarose gel at 2.5 V/cm until the 0.5 kb size marker was at the bottom of the gel. The DNA was transferred to a nylon membrane (Hybond N, Amersham) by the method of Southern<sup>7</sup> and then covalently bound by UV irradiation (302 nm for five minutes). Membranes were placed in a Hybad blot processing system and treated for a minimum of four hours at room temperature with prehybridisation mixture (50% formamide, 0.1% Denhardt's,  $5\times$  salt solution [0.75 mol/l NaCl, 0.05 mol/l  $\text{NaH}_2\text{PO}_4$ , 5 mmol/l EDTA], 1% sodium dodecyl sulphate [SDS], 5% dextran sulphate, 200  $\mu\text{g}/\mu\text{l}$  sheared salmon sperm DNA). This was then replaced with hybridisation solution (prehybridisation solution without salmon sperm DNA) containing 50 ng  $^{32}\text{P}$  oligolabelled probe DNA which was labelled using

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the Multiprime system (Amersham). The membranes were then washed in 2× salt solution at room temperature followed by a brief wash in 2× salt solution, 0.5% SDS at 65°C. The hybridised membranes were then placed in Cronex cassettes with intensifying screens and Fuji RX medical x ray film at -70°C. Films were developed after one to four days.

#### PROBE

The probe used was a 615 bp *Bam*HI/*Eco*RI fragment of a murine cDNA clone 86T1, which cross hybridises with the two constant genes of the human TCR β chain.<sup>8</sup>

#### Results and discussion

The genotype frequencies of the TCR β chain RFLPs in the patients with CF, CF carriers, and normal subjects are shown in the table. These show a significantly increased frequency of the heterozygous genotype of the TCR β chain *Bg*III polymorphism in CF carriers as compared with normal controls. The heterozygous genotype (fragment sizes 10.0 and 9.2 kb, figure) is present in 71% of CF carriers and in 57% of CF patients, compared with 44% of normal controls. The frequency of this genotype in our normal control group is similar to that reported by other workers in their normal subjects (between 41 and 43%).<sup>3-5</sup>

The human TCR β chain genes have been localised to the 7q32 region which is approximately 20 cM from the CF locus. The frequency of the two alleles has been shown to be similar in each group. The frequency of the 10.0 kb allele was 0.52, 0.44, and 0.46 in the controls, CF carriers, and CF affected groups and the frequency of the 9.2 kb allele was 0.48, 0.56, and 0.54. There is therefore no evidence of linkage disequilibrium between the alleles of the CF locus and the TCR β chain polymorphism. For linkage disequilibrium to occur

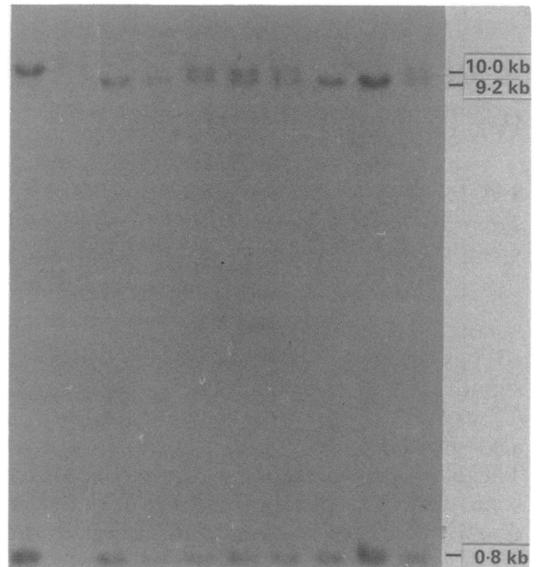


FIGURE The TCR probe in conjunction with *Bg*III detects two allelic fragments, 10.0 kb and 9.2 kb. The heterozygous and homozygous genotypes are shown.

the marker alleles have to be very close together so that the lack of recombination between them leads to certain alleles being inherited together more often than expected. If this were so, the CF gene would be preferentially inherited with one particular allele of the TCR polymorphism; however, this could not account for the increase in the heterozygous genotype found in the CF carriers or CF patients. It is therefore reasonable to assume that the results represent a disease association.

The significant shift in the Hardy-Weinberg equilibrium in the CF carriers suggests that there might be some form of epistatic interaction between the CF gene and the TCR β genes such that the double heterozygote is advantaged. Perhaps the CF gene can modulate expression or affect rearrangement of the TCR β chain genes. The polymorphism of the TCR may reflect certain rearrangements of the variable, joining, and diversity genes of the β chain or a deletion or mutation in the germline gene segments, which may correlate with variations in immune function; thus the heterozygous CF are advantaged and the homozygous CF show decreased immune function. Abnormalities in immune function have been described in patients with CF, such as decreased lymphocyte responsiveness, defective opsonic activity, raised immune complexes, and impaired neutrophil activity.<sup>9</sup> Recently, lower percentages of T helper cells and decreased function

TABLE Genotype frequency of T cell receptor β chain RFLPs in the different groups (allele 1: 10.0 kb RFLP, allele 2: 9.2 kb RFLP).

	Genotype		
	1,2	1,1	2,2
Cystic fibrosis carriers (n=68)	48 71%*	6 9%	14 20%
Cystic fibrosis patients (n=47)	27 57%†	8 17%	12 26%
Controls (n=57)	25 44%	17 30%	15 26%

\*p=0.005; †p=0.24.

of both T helper and T suppressor cells have been described in patients with CF.<sup>10</sup> Although an increased frequency of this heterozygous genotype has not been reported before in patients with CF or CF carriers it has been described in patients with autoimmune disease, especially insulin dependent diabetes.<sup>3,4</sup> It has been suggested that this heterozygous genotype is not associated with autoimmune disease per se, but with the presence of MHC antigens DR3 and 4.<sup>3,4</sup> However, there is no evidence that CF is MHC restricted.<sup>11</sup> We have no information on the HLA status of our subjects.

### Conclusion

These results represent a previously unknown disease association and may indicate some form of modulation of TCR function by the CF gene. This polymorphism may also be of use in identifying those at increased risk of carrying the CF gene, especially if combined with linkage disequilibrium data for probes closely linked to the CF gene.<sup>12</sup> Further work is necessary to determine whether altered lymphocyte function or other immune defects are present in subjects heterozygous and homozygous for the CF gene and the *Bg/II* TCR polymorphism.

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