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The deleted in colorectal carcinoma (DCC) gene 201 R → G polymorphism: no evidence for genetic association with autoimmune disease

Richard J Hall¹, Marilyn E Merriman¹, Rachel A Green¹, Valerie H Markham¹, Deborah J Smyth², Joanne M Heward³, Claire E Jennings⁴, Antony W Braithwaite⁵, Tim Cundy⁶, Brian A Darlow⁷, Peter J Gow⁸, Andrew A Harrison⁹, John Highton¹⁰, Penny J Hunt¹¹, Patrick Manning¹², Violetta Pokorny¹³, Russell S Scott¹⁴, Barry J Taylor¹⁵, Jinny A Willis¹⁴, Sue Yeoman¹⁶, Lachy McLean¹³, Stephen CL Gough³, Simon H Pearce⁴ and Tony R Merriman^{*,1}

¹Department of Biochemistry, University of Otago, Dunedin, New Zealand; ²Cambridge Institute for Medical Research, Addenbrooke's Hospital, UK; ³Department of Medicine, Birmingham Heartlands Hospital, UK; ⁴Institute of Human Genetics, University of Newcastle, UK; ⁵Department of Pathology, University of Otago, Dunedin, New Zealand; ⁶Department of Medicine, University of Auckland, New Zealand; ⁷Department of Paediatrics, Christchurch Hospital, New Zealand; ⁸Department of Rheumatology, Middlemore Hospital, Auckland, New Zealand; ⁹Wellington School of Medicine and Health Sciences, New Zealand; ¹⁰Department of Medicine, University of Otago, Dunedin, New Zealand; ¹¹Department of Endocrinology, Christchurch Hospital, New Zealand; ¹²Department of Endocrinology, Dunedin Hospital, Dunedin, New Zealand; ¹³Department of Molecular Medicine, University of Auckland, New Zealand; ¹⁴Lipid and Diabetes Research Group, Christchurch Hospital, New Zealand; ¹⁵Department of Paediatrics, Dunedin Hospital, Dunedin, New Zealand; ¹⁶Department of Rheumatology, Auckland Hospital, New Zealand

The product of the deleted in colorectal carcinoma (DCC) gene has a role in apoptosis and is a positional candidate for *IDDM6*, the putative chromosome 18q12–q23 autoimmune disease locus. We hypothesised that a nonconservative substitution (DCC 201 R → G; nucleotide (nt) 601 C → G), located in an extracellular immunoglobulin-like domain of DCC, is an aetiological determinant of autoimmunity. We tested this hypothesis by genetically testing the nt 601 C → G polymorphism for association with three autoimmune phenotypes in a large population-based case–control study. There was no evidence for association of DCC nt 601 C → G with autoimmune disease in cohorts comprising 2253 subjects with rheumatoid arthritis, type I diabetes and Graves' disease, and 2225 control subjects, from New Zealand and the United Kingdom. Furthermore, using the transmission disequilibrium test, there was no significant evidence for biased transmission of the nt 601 C → G polymorphism to probands within a 382 family type I diabetes affected sibpair cohort from the United Kingdom. Thus, the DCC 201 R → G polymorphism does not appreciably influence risk of developing the autoimmune diseases tested.

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*Correspondence: Dr T Merriman, Department of Biochemistry, University of Otago, Dunedin, New Zealand. Tel: +64 3 4795 798; Fax: +64 3 4797 866; E-mail: tony.merriman@stonebow.otago.ac.nz
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Introduction

Genetic predisposition to autoimmune disease is universally associated with human leucocyte antigen class II alleles within the major histocompatibility complex (MHC) on chromosome 6p21.¹ However, genome-wide

linkage scanning has implicated numerous other non-MHC regions in autoimmune disease susceptibility.^{2–10} One of these loci, the putative locus *IDDM6* on chromosome 18q12–q23, has been linked to type I diabetes (T1D), rheumatoid arthritis (RA), Graves' disease (GD) and systemic lupus erythematosus.^{5–12} Meta-analysis provides evidence that the orthologous region in rodent (on distal chromosome 18 in mouse and rat) contains an autoimmune susceptibility locus¹¹ with congenic mapping, proving that rat has a distal chromosome 18 diabetes locus (*Iddm3*)¹³ and that mouse has a chromosome 18 diabetes locus (*Idd21*).¹⁴ Thus, there is considerable evidence to support linkage of chromosome 18q12–q23 and the rodent orthologous region to autoimmune phenotypes.

In humans, a study comprising 3380 families provided evidence for association with autoimmunity of a haplotype of two microsatellite markers within the *IDDM6* positional candidate, the deleted in colorectal carcinoma (DCC) gene ($P = 5 \times 10^{-6}$).¹¹ The DCC gene, a member of the immunoglobulin superfamily, encodes a receptor dependent on the ligand netrin-1. In the absence of netrin-1, DCC induces cell death through interaction with a membrane-associated adenosine A2b receptor.^{15–17} Cell death occurs via a cascade involving activation of caspase-3 by caspase-9, independent of the mitochondrial and caspase-8/death receptor pathways.¹⁸ It is possible that DCC has uncharacterised role(s) in the regulation of central and/or peripheral tolerance to self-antigens or in abnormal apoptosis of cells targeted in autoimmunity.

The DCC gene has an exon 3 polymorphism (601 C→G) that results in a codon 201 R→G nonconservative substitution within the second of the four DCC extracellular Ig-like domains.^{19,20} We hypothesised that this substitution plays a role in the aetiology of autoimmune disease. There are strong *a priori* reasons to support the formulation of this hypothesis; evidence supporting linkage of the DCC region to human and rodent autoimmune phenotypes,^{5–12} association of the DCC gene with autoimmunity in humans,¹¹ and the involvement of DCC in regulating apoptotic events. The hypothesis was tested by performing a population- and family-based study testing the genetic association of the DCC nucleotide (nt) 601 C→G polymorphism with several autoimmune phenotypes (RA, T1D and GD).

Materials and methods

Subjects

Type I diabetes cases ($n = 354$) were collected from the Auckland, Canterbury and Otago regions of New Zealand (NZ). The average and median ages at onset were 12.3 (range <1–40 years) and 11 years, respectively. All 671 RA cases were drawn from the Auckland, Bay of Plenty, Wellington, Otago and Southland regions of NZ, and satisfied the 1987 American College of Rheumatology

criteria for disease.²¹ GD was defined by the presence of hyperthyroidism and diffuse goitre, supported by the presence of either thyroid antibodies (peroxidase and/or thyroglobulin) or thyroid-associated orbitopathy. The NZ GD cases ($n = 180$) were drawn from the Canterbury and Otago regions. All 1246 NZ control subjects were healthy, over 17 years of age, and were drawn from the Otago region. The Birmingham GD cases ($n = 731$, recruited from Birmingham, Bournemouth and Exeter, UK) and control subjects ($n = 668$, recruited within Birmingham) have been described previously.²² The Newcastle GD cases ($n = 317$) were recruited from Northern England and the Lothian region of Scotland. Healthy control subjects ($n = 311$) were recruited within Newcastle (UK). The 382 T1D UK affected sibpair families are described elsewhere.⁴ All case and control subjects and families were of white ethnicity. All subjects gave informed consent and the study was approved by the appropriate regional Ethics Committee.

Genotyping

The DCC nt 601 C→G polymorphism was genotyped by a PCR-RFLP assay over the case and control subjects using primers (5'→3') ATTTGGAAGACTTATTCTTCC and CGGTAAATCCAATGTCCCTCGGTTGGAGC. The second primer has a mismatch at the 3' end (nt 603 of DCC), creating an *MspI* restriction site in the presence of 601C. The nt 601 C→G polymorphism was typed over the UK T1D families using Invader.²³

Power estimation

We hypothesised that the autoimmune disease-associated 2-10 haplotype of DCC microsatellite markers 88,21 and 55,26 is in linkage disequilibrium with an aetiological polymorphism, namely DCC nt 601 C→G, that accounts for the previously observed association.¹¹ The transmission ratio (r) of this haplotype from heterozygous parents to affected offspring (0.547 in 2484 RA and T1D families),¹¹ was used to estimate an odds ratio (OR) using the formula $r/(1-r) = 1.21$ (95% confidence interval (CI) 1.12–1.31).²⁴ *Post priori* power calculations were performed, as described,²⁵ with the frequency of the DCC nt 601G (minor) allele estimated from the appropriate healthy control cohort. A *post priori* power calculation for the TDT²⁶ was performed for the UK T1D affected sibpair family cohort using the OR estimated above and the DCC nt 601G allele frequency (0.322) observed in parents.

Results

Previous reports of the frequency of the DCC nt 601 C→G variant were from Japanese subjects (601G frequency = 0.56).^{19,27,28} To determine the allele frequency in white ethnic groups, 192 NZ healthy control subjects were typed. The frequency of 601G was 0.35. The relationship between DCC nt 601 C→G and the 2-10 haplotype of

Table 1 Association analysis of the *DCC* nt 601 C→G polymorphism with autoimmune disease in NZ and UK case-control cohorts.

Phenotype	Origin	C	Cases G	Freq ^a	C	Controls ^b G	Freq	P ^c	Power (95% CI)
T1D	NZ	454	254	0.359	1614	878	0.352	0.75	0.58 (0.24–0.87)
RA	NZ	864	478	0.356				0.81	0.78 (0.36–0.97)
GD	NZ	234	126	0.350				0.93	0.36 (0.16–0.63)
T1D/RA/GD	NZ	1552	858	0.356				0.79	0.90 (0.47–0.996)
GD (Birmingham)	UK	938	524	0.358	863	473	0.354	0.81	0.68 (0.30–0.93)
GD (Newcastle)	UK	393	241	0.380	364	258	0.415	0.21	0.39 (0.17–0.66)

NZ T1D/RA/GD, NZ control; Birmingham GD, Birmingham control; Newcastle GD and Newcastle control cohorts all exhibited Hardy–Weinberg equilibrium at *DCC* nt 601 C→G. ^aFrequency of the minor allele (*DCC* 601G, *DCC* 201G) is given. ^bThe same control cohort was used in all four New Zealand comparisons. ^cThe χ^2 test (1 df) was used to compare *DCC* nt 601 C→G allele frequency between case and control cohorts.

Table 2 Transmission of *DCC* [nt 601 C→G]–[88,21]–[55,26] haplotypes from heterozygous parents to probands in UK T1D affected sibpair families

Haplotype ^a	T	NT	% T	P ^b	Freq ^c
C-2-10	117	81	59.1	0.01	0.215
C-2-11	61	45	57.5	0.12	0.103
G-7-1	32	32	50.0	1.00	0.070
C-2-12	41	25	62.1	0.05	0.060
C-7-1	19	39	32.8	0.009	0.052
G-2-10	12	20	37.5	0.16	0.034

Transmission of multilocus haplotypes from heterozygous parents to offspring in the UK affected sibpair families was assessed using TDT phase software (<http://www.hgmp.mrc.ac.uk>).³¹ ^aTransmissions are shown for the five, and eighth most common, haplotypes. ^bP calculated from the TDT statistic (χ^2 1 df).³² ^cFrequency in parents. T = transmitted, NT = not transmitted, % T = percent transmission.

T1D-associated microsatellite markers 88,21 and 55,26 (spanning exons 16–28 of *DCC*) was then investigated by genotyping the UK T1D families. The 2-10 haplotype has previously been shown to be positively transmitted to offspring with an autoimmune phenotype (in the UK T1D affected sibpair cohort transmissions to probands are 152 T, 111 NT; % T = 57.8; P = 0.01).¹¹ The C allele of *DCC* nt 601 C→G was over-represented on the 2-10 haplotype (D' , calculated as described²⁹ using parental haplotype frequencies = 0.58).

The *DCC* nt 601 C→G polymorphism was tested for association with autoimmune disease in five independent case-control cohorts from three different populations (one T1D, one RA and three GD). No association of the polymorphism was observed with disease in any of the individual case-control cohorts (Table 1). Hypothesising that the same allele of nt 601 C→G may be associated with autoimmunity *per se* the New Zealand T1D, RA and GD cohorts were combined. Again, no evidence supporting an association of the exon 3 variation with autoimmune disease was obtained (Table 1). Transmission of *DCC* nt 601 C→G from heterozygous parents to probands was also

tested in the UK affected sibpair T1D cohort. Data were 162 T, 137 NT (% T = 54.2, P = 0.15). Thus, there was no evidence to support association of *DCC* nt 601 C→G with T1D in the UK affected sibpair families. However, power for this analysis was only 42% (CI 18–71%), α = 0.05, OR = 1.21 (1.12–1.31).

Transmission of common [nt 601 C→G]–[88,21]–[55,26] haplotypes and the G-2-10 haplotype (eighth most frequent) from heterozygous parents to probands was tested in the families (Table 2). The nt 601 C→G polymorphism subdivides the 2-10 haplotype of [88,21]–[55,26] into positively associated (C-2-10, % T = 59.1, P = 0.01) and nonassociated (G-2-10, % T = 37.5, P = 0.16) haplotypes.

Discussion

We considered the *DCC* codon 201 R→G nonconservative substitution as a candidate autoimmune disease aetiological variation. There was a strong *a priori* reason for undertaking this experiment; in humans and mice the *DCC* region is strongly implicated in autoimmune disease susceptibility and *DCC* is a plausible candidate gene for autoimmune disease susceptibility by virtue of its involvement in apoptosis (see Introduction). Despite this, in the present study the nt 601 C→G variation was found not to be associated with autoimmune disease in any of the five case-control cohorts tested (Table 1), nor in the family-based T1D data set. We conclude that the *DCC* nt 601 C→G variant is not likely to have a major role in autoimmune disease susceptibility when considered as a single polymorphic locus acting independent of other polymorphisms in the *DCC* region. This conclusion is supported by the presence of the same nt 601 C→G allele on both positively and negatively associated *DCC* [nt 601 C→G]–[88,21]–[55,26] haplotypes in the UK T1D families. For example, the C allele is present on the C-2-10 (positively transmitted, % T = 59.1, P = 0.01) and C-7-1 (negatively transmitted, % T = 32.8, P = 0.009) haplotypes (Table 2).

The case-control association study was adequately powered to test our hypothesis. Reasoning that DCC nt 601 C→G accounts for the previously observed disease association at DCC¹¹, it was estimated that the New Zealand autoimmune cohort had 90% power, and a second independent data set (Birmingham GD) had 68% power to detect association with disease of a locus with effect equivalent to IDDM6 (OR=1.21) at the 5% level of significance. However, substantiation of the lack of association of DCC nt 601 C→G with autoimmune disease will require genotyping in significantly larger cohorts. A study providing 99.5% power ($\alpha=0.05$) (using the lower 95% CI OR estimate for IDDM6 (1.12)) would require 7000 cases and 7000 controls.

Even if it does not have a significant direct role in autoimmune disease susceptibility, it is conceivable that DCC 201 R→G may interact with other polymorphisms in the region to influence protein expression and/or function collectively, thus accounting for at least part of the previously observed linkage and association of DCC with autoimmune disease.^{5–12} To investigate this, the association of haplotypes of DCC with disease will need to be assessed. This will require cohort sizes of thousands of individuals to be of sufficient power to account for the reduced power concomitant with the increased degrees of freedom inherent in the analysis of multiple SNP haplotypes.

In conclusion, the DCC 201 R→G polymorphism does not significantly influence risk of developing the autoimmune diseases tested. Several strategies can be followed to investigate the role of chromosome 18q12–q23 in autoimmune disease further. In humans, we have first concentrated on DCC as an IDDM6 positional candidate. This approach should be continued, focusing in the first instance on the neighbouring centromeric SMAD2, SMAD4 and SMAD7 candidate genes. The SMAD (similar to mothers against decapentaplegic) gene products mediate transforming growth factor- β_1 (TGF- β_1) signalling from the cell membrane to the nucleus. TGF- β_1 signalling is implicated in the control of T-cell homeostasis.³⁰ However, the possibility that other polymorphisms within DCC modulate disease risk should not be neglected. Comparative mapping in rodents may be a powerful aid to IDDM6 positional cloning efforts. This would be based on the hypothesis that orthologous chromosome 18 autoimmune disease susceptibility gene(s) exist in human and rodent. It will be necessary to fine map the chromosome 18 autoimmune diabetes loci *Iddm3* (rat)¹³ and *Idd21* (mouse)¹⁴ by constructing and evaluating cumulative diabetes frequency in novel subcongenic strains.

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