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The PTPN22 gene is associated with juvenile and adult UK Caucasian idiopathic inflammatory myopathy independent of the HLA 8.1 haplotype

H. Chinoy[1,2], **H. Platt**[1], **J.A. Lamb**[1], **Z. Betteridge**[3], **H. Gunawardena**[3], **N. Fertig**[4], **H. Varsani**[5], **J. Davidson**[6], **C.V. Oddis**[4], **N.J. McHugh**[3], **L.R. Wedderburn**[5], **W.E.R. Ollier**[1], and **R.G. Cooper**[2]

[1]Centre for Integrated Genomic Medical Research, University of Manchester, Manchester, UK

[2]The University of Manchester Rheumatic Diseases Centre, Hope Hospital, Salford, UK

[3]Rheumatology Dept, Royal National Hospital for Rheumatic Diseases, Bath, UK

[4]Division of Rheumatology & Clinical Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

[5]Rheumatology Unit, Institute of Child Health, UCL, London, UK

[6]Rheumatology Dept, Rheumatology Dept, Royal Hospital for Sick Children, Glasgow, UK

Abstract

Objective—To examine single nucleotide polymorphisms (SNPs) from the protein tyrosine phosphatase N22 (PTPN22) gene as part of a large UK adult and juvenile idiopathic inflammatory myopathy (IIM) case-control association study, and to study the relationship of the PTPN22 gene with the HLA region.

Methods—A cross-sectional, case-control study of PTPN22 SNPs, comparing cases of polymyositis (PM, n=114), dermatomyositis (DM, n=102), myositis associated with another connective tissue disorder (myositis/CTD-overlap, n=64) and juvenile DM (JDM, n=101) with 748 control subjects. 17 PTPN22 SNPs were genotyped using the Sequenom MassArray iPLEX platform. Serotyping for myositis specific/associated antibodies (MSA/MAA) was performed by radio-immunoprecipitation.

Results—A significant association was noted between the R620W variant (rs2476601) and IIM, and in the clinical subgroup PM (corrected p [p_{corr}]=0.0007 for both). A weaker association was noted for JDM (p_{corr}=0.019). No significant associations were noted after stratification by serological subgroup. The R620W variant association was independent of alleles forming the HLA 8.1 haplotype. No other PTPN22 SNPs were associated with IIM. The PTPN22 haplotype containing the R620W T allele was the only haplotype significantly associated with IIM.

Address for reprints and correspondence: Dr Robert G Cooper, The University of Manchester Rheumatic Diseases Centre, Hope Hospital, Salford, M6 8HD, Tel/fax: +44-(0)161-206-4367, robert.g.cooper@manchester.ac.uk.

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Conclusion—The R620W variant is a significant risk factor in IIM and is independent of the HLA 8.1 haplotype. Unlike the HLA region, risk is not increased in individuals possessing MSA/MAA. This data is further evidence that the PTPN22 gene confers autoimmune susceptibility.

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Introduction

The idiopathic inflammatory myopathies (IIM) are a heterogeneous group of rare autoimmune diseases characterised by acquired proximal muscle weakness, characteristic inflammatory cell infiltrates in muscle biopsies and the presence of circulating myositis-specific/associated autoantibodies (MSA/MAA). The most common myositis subgroups are polymyositis (PM), dermatomyositis (DM) and myositis overlapping with another connective tissue disorder (myositis/CTD-overlap). IIM may also present in children, the most common subgroup being juvenile DM (JDM). The aetiopathogenesis of IIM is likely to be multifactorial, i.e. arising due to both genetic and environmental factors and their interactions (1).

It is well recognised that a major genetic contribution conferring susceptibility in a variety of autoimmune diseases arises from the major histocompatibility complex (MHC). However, recent genetic research has demonstrated that genes outside of the MHC may also be important for conferring susceptibility to autoimmunity. For instance, large-scale genetic association studies have confirmed a missense single nucleotide polymorphism (SNP) in the gene coding for protein tyrosine phosphatase N22 (PTPN22), on chromosome 1. The SNP in question is a C to T change at position 1858, which causes an amino acid substitution at residue 620 from arginine (R) to tryptophan (W) (R620W) in the LYP (lymphocyte phosphatase) protein. This SNP represents a disease susceptibility gene in several autoimmune diseases (2), especially where HLA markers and disease-specific autoantibodies are associated.

In adult and juvenile IIM, many characteristic MSA/MAA are strongly associated with the HLA region, and notably with components of the 8.1 common ancestral haplotype (8.1 haplotype) (HLA-B*08/DRB1*03/DQB1*02/DQA1*05) (3-7). We have therefore examined SNPs from the PTPN22 gene as part of a large, ongoing UK adult and juvenile IIM case-control association study, and investigated the relationship of the PTPN22 gene with genetic markers from the HLA region.

Patients and Methods

Subjects

DNA was available from 381 UK Caucasian IIM cases. Adult IIM patients (n=280), aged 18 years of age or older at disease onset, were recruited through the UK Adult Onset Myositis Immunogenetic Collaboration, (AOMIC) (7). JDM patients (n=101) were recruited via the UK JDM National Registry and Repository (8,9). Patients with PM, DM or JDM had probable or definite myositis, based on the Bohan and Peter criteria (10,11). For patients with myositis/CTD-overlap, use of these criteria is problematic, as myositis is often diagnosed less rigorously in the context of another CTD (likely reflecting the lack of expertise of electromyography [EMG] and muscle histology in UK non-teaching centres).

Thus, 15 of the 64 (23%) myositis/CTD-overlap patients were included if they fulfilled all of the following: a) met published criteria for their primary CTD (12-16) or mixed CTD (MCTD) (17); b) possessed at least two of four Bohan and Peter criteria (proximal muscle weakness, elevated muscle enzymes, characteristic myopathic EMG changes, diagnostic muscle biopsy); (c) possessed at least one MSA/MAA. The remaining 49 myositis/CTD-overlap patients all fulfilled criteria for their primary disease/MCTD and probable/definite myositis according to Bohan and Peter. A standardised clinical data collection form, detailing demographics and individual clinical details, was used. For adult patients, the collaborating physicians at each AOMIC study site confirmed/excluded the presence of interstitial lung disease (ILD) by pulmonary function tests and thoracic imaging, and cancer-associated myositis (CAM) by relevant investigations. CAM was defined as cancer occurring in patients with probable/definite PM/DM, within three years of myositis diagnosis (as per modified Bohan and Peter classification (18)).

Controls

Seven hundred and forty eight UK Caucasian control subjects were recruited from blood donors and general practitioner registers as described (7). Collection of data and blood from patients and controls was undertaken under the regulation of the local research ethics committees and informed consent was obtained according to the Declaration of Helsinki.

Autoantibody typing

Serum was obtained from patients for determination of MSAs: anti-synthetases: -Jo-1, -PL-7, -PL-12, -EJ, -OJ, -KS; anti-Mi-2, anti-SRP, anti-155/140 and MAAs: anti-PM-Scl, anti-Ku, anti-U1-RNP, anti-U3-RNP using radio-immunoprecipitation, as previously described in adult (7,19) and juvenile IIM (9). The presence of the newly described anti-small ubiquitin-like modifier 1 activating enzyme [SAE] was also determined, as recently described (20).

Genotyping

DNA samples were extracted from a peripheral blood sample obtained from both cases and controls using a standard phenol-chloroform method. SNPs were genotyped using the Sequenom MassArray iPLEX platform, as per the manufacturer's instructions (http://www.sequenom.com/seq-genotyping.html). Cases were typed for the HLA-DRB1, -DQB1 and -B loci at broad specificity, using a commercially available polymerase chain reaction-sequence-specific oligonucleotide probe typing system (Dynal Biotech GmbH, Hamburg, Germany). Data for the HLA-DQA1 locus were derived from DRB1 and DQB1 results, as previously outlined (9). The HLA class II typing has been described previously (7).

PTPN22 SNPs

Seventeen SNPs within the PTPN22 gene were selected: 9 haplotype tagging (ht) SNPs were selected for genotyping using the Hapmap CEU population, (release 20, NCBI B35 assembly) by pairwise tagging, r^2 cut-off ≥0.8 and a minimum allelic frequency (MAF) of 10%. Eight other SNPs were selected on the basis of having a putative functional role. Of these SNPs, 10 were previously reported in an RA study (2). Three SNPs were removed

from further analysis where the assay success rate was <90% (rs1217412, rs1775759, rs3789604). For analysis, sample success cut off was set at 80%.

Statistical analyses

Genotype frequencies for each PTPN22 SNP were tested for Hardy-Weinberg equilibrium (HWE) in each group. Allele and genotype frequencies of these PTPN22 SNPs were compared between myositis cases and controls, using Fisher's exact test. Where significant, data were expressed as odds ratios (OR) with exact 95% confidence intervals (CI). Pointwise p values were corrected using permutation testing (10,000), as implemented in the PLINK program (21). Linkage disequilibrium (LD) was calculated using both *D'* and pairwise r^2 values, and PTPN22 haplotypes were estimated and constructed using the Expectation/Maximisation (EM) algorithm, using HelixTree (version 3.1.2, Golden Helix, Inc., Bozeman, MT, USA). The analyses were also repeated after stratification for myositis serology and the presence of CAM/ILD. Unless otherwise stated, the statistical package Stata (release 9.2, Stata Corp., College Station, TX) was used to perform statistical analysis. A power calculation was applied to the SNP with the lowest MAF (rs2476601), using allelic data deposited by Dr Anne Barton, arc Epidemiology Unit, UK, and published on-line from the British 1958 Birth Cohort DNA Collection (22). For 80% power to detect an effect size of 1.65 at a 95% significance level (based on data from an RA discovery study (2)), 390 cases and 390 controls were required. Significant allelic results were also verified using the 1958 Birth Cohort data.

Results

Clinical details

The 381 cases recruited for the study included: 114 PM, 102 DM, 64 myositis/CTD-overlap and 101 JDM patients. The myositis/CTD-overlap patients had the following primary diagnoses: 41 systemic sclerosis (SSc), 9 MCTD, 6 Sjögren's syndrome, 7 SLE and 1 RA. The median age at myositis onset was 49 (+/− 14.1) years for adult onset IIM and 6 (+/− 3.6) years for JDM. The overall female percentage was 73%.

Genotype and allele associations

All of the analysed PTPN22 SNPs conformed to HWE in both cases and controls. **Error! Reference source not found.** summarises the genotype and allele frequencies for the tested PTPN22 SNPs. The minor T allele (allele 2) for the rs2476601 SNP (R620W) was a significant risk factor in combined cases *vs.* controls (see Table 2). This result remained present when the 1958 Birth Control cohort was used as an additional control dataset (Table 2). A significant genotype association for R620W was also present, using both recessive (TT *vs.* CT + CC) and dominant (CT + TT *vs.* CC) models of inheritance (see Table 3). The genotype data were also analysed by heterozygosity or homozygosity of the T allele. The association was significant for TT homozygotes (TT *vs.* CC); a weaker association was noted for TC heterozygotes (TC *vs.* CC). No genotype or allele associations were observed for the other tested SNPs.

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Clinical and MSA/MAA associations

To examine the R620W association in more detail, data were stratified by clinical subgroups and by MSA/MAA. The clinical subgroup and significant MSA/MAA associations are summarised in Table 2. Significant R620W allele associations were observed in the PM subgroup compared to both sets of controls. A weaker association was observed in the JDM group, and possible associations (not significant for corrected probabilities) in the anti-Jo-1 and anti-155/140 antibody positive subgroups. No significant associations were detected in any of the other clinical or MSA/MAA subgroups, or in those patients with no demonstrable antibodies. An additional association was noted in cases with ILD (uncorrected p=0.007, OR 2.2, 1.2-3.9), but no association was noted with CAM. No association or interactive effect was noted with gender or age at myositis onset.

Interaction with the HLA 8.1 haplotype

Alleles forming part of the 8.1 haplotype are known to confer risk of disease in adult and juvenile IIM (3-7). It was thus of interest to investigate the relationship between the HLA region and the PTPN22 gene in the IIMs. The R620W SNP association was examined with each of HLA-B*08, -DRB1*03, -DQA1*05, -DQB1*02, in a multivariate logistic regression model. Both the R620W and 8.1 haplotype associations remained significant (where the R620W association was significant in univariate analysis). However, no multiplicative interaction was noted with any of the tested 8.1 alleles. As anti-Jo-1 and -PM-Scl antibodies are known to be strongly associated with the 8.1 haplotype (7), data were also stratified for the absence of these antibodies. The R620W association remained significant in anti-Jo-1 and -PM-Scl negative cases *vs.* controls (uncorrected p=0.0007, OR=1.7, 1.3-2.4).

Linkage disequilibrium

The tested PTPN22 SNPs were assessed for LD with each other in the control group. Strong pairwise LD was noted between SNPs using the *D*‘ measure. Using the more stringent measure of pairwise r^2, rs1217388 showed the greatest LD with rs2476601 (pairwise r^2 =0.24). Little LD was noted between the tested PTPN22 SNPs and the tested HLA alleles (*D*‘<=0.27).

PTPN22 haplotypes

Using the selected htSNPs, only the haplotype containing the R620W T allele was increased in cases compared to controls. To enable comparison of haplotype results with previously published data, haplotypes were constructed from htSNPs previously identified in RA (2). Thus, five haplotypes were identified in the control population at a frequency >5% (Table 4). The most frequent haplotype corresponded to the haplotype most frequently observed in the Carlton study (2). Only one haplotype (C-A-G-A-**T**-T-C-T) carried the R620W T allele. This haplotype was significantly associated with all IIM combined cases *vs.* controls. When stratified by disease subtypes, associations for this haplotype were also seen in PM and JDM. Other associations were also observed in serological subtypes (Mi-2, C-G-A-G-C-C-T-T 11%, p=0.01; Jo-1, C-A-G-A-C-T-C-T 6%, p=0.007; 155/140, C-A-G-A-**T**-T-C-T 19%, p=0.009), although low numbers post-stratification are likely to affect the validity of these results. Haplotype frequencies were re-analysed in the absence of the risk T allele (forming

the R620W variant). Other than the C allele haplotype associations already described, no further associations were observed.

Discussion

We describe an association of the R620W variant of the PTPN22 gene in adult and juvenile IIM, representing the first description of a major IIM disease susceptibility gene outside of the HLA region. No significant associations are present for the remainder of the tested PTPN22 SNPs. The R620W association appears to be strongest in individuals homozygous for the T allele. The R620W association is also observed after stratification by clinical disease subgroups; the association being present for PM and JDM, but not the DM or CTD/overlap groups. It is interesting that even after stratification into traditional IIM clinical subgroups, the association within PM remains strong. The major PTPN22 haplotype association is that containing the R602W variant, in keeping with published RA data (2,23).

Until now, the major IIM disease susceptibility gene has been thought to reside in the HLA region, as part of the 8.1 haplotype (6,7,9). We demonstrate that the R620W IIM association is independent of the 8.1 haplotype (albeit with a weaker strength of association). After stratification by anti-synthetase or -PM-Scl antibodies, the association with the 8.1 haplotype becomes stronger (7,24). In contrast, the R620W association becomes far weaker, or loses significance altogether after stratification by MSA/MAAs. PM and JDM, the IIM subgroups associated with strongest risk for the R620W variant, have the lowest overall MSA/MAA frequencies when compared to the DM or myositis/CTD-overlap subgroups (7,19). A weak association is noted between the anti-155/140 antibody and the R620W variant; notably this antibody is associated with an HLA allele outside of the 8.1 haplotype (HLA-DQA1*0301) (25). The R620W association is also observed for IIM cases with ILD, although this may represent a general association with the IIMs. No effect is noted for gender or age at disease onset, in contrast to findings in RA (26,27).

Association with the R620W SNP has already been described in other autoimmune diseases, including RA, systemic lupus erythematosus, type 1 diabetes mellitus and autoimmune thyroid disease, with effect sizes similar to those seen in the present IIM study (28). Generally, PTPN22 associations have been found in diseases where autoantibodies potentially play a prominent role, and not in others where antibodies are generally not detectable (e.g. psoriasis, psoriatic arthritis, multiple sclerosis) (23). In RA, the R620W variant also shows no interaction with the HLA shared epitope in one study (29), although a pooled study has suggested a gene-gene interaction (30). A strong interaction has also been demonstrated between the R620W variant and anti-cyclic citrullinated antibodies in the development of RA (26,27). A further study failed to demonstrate association in rheumatoid factor (RF) negative patients (31). However, a further RA study demonstrated association of the R620W variant in both ANA positive and negative juvenile idiopathic arthritis and RF positive and negative RA patients (23). The lack of PTPN22 association in anti-synthetase and anti-PM-Scl antibody positive IIM cases may reflect the strong relationship that these antibodies have with the 8.1 haplotype. The R620W variant and other PTPN22 SNP variants may exert a smaller effect in other IIM antibody systems or in cases lacking detectable antibodies, but the current study lacked the power to detect such associations. Thus in the

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IIMs, the R620W variant appears to generate susceptibility for disease regardless of MSA/MAA status.

A previous small US case-control study examined the R620W variant in the IIMs, but no significant associations were observed (32). However, the control group frequency of the T allele was higher than that seen in the present study (US Caucasian IIM 10%, US controls 12% *vs.* UK Caucasian IIM 14%, UK controls 8%). As the frequency of T homozygotes is low in Caucasians, a small sample size may overestimate the relative C allele frequency. It is therefore possible that an association does exist in US Caucasian IIM patients, but the cited study was insufficiently powered to detect this.

The amino acid at residue 620 is located in a proline-rich motif of the LYP protein. R620 (arginine) enables binding of LYP to the Src homology domain of C-terminal Src kinase (Csk) (28,33). The LYP-Csk complex inhibits the T cell receptor (TCR) signaling pathway, but W620 (tryptophan) disrupts the complex rendering LYP*W620 unable to bind Csk (33). Initially, LYP*W620 was thought to be a loss-of-function variant, due to a reduced ability to down-regulate T cell activation thus leading to increased autoimmune reactivity. Recently, data has suggested that W620 is a gain-of-function mutation, able to dephosphorylate signaling proteins more efficiently than LYP*R620, leading to increased inhibition of T and B lymphocytes, thymic hyporesponsiveness and increased circulating auto-reactive T cells (34,35). Furthermore, LYP*W620/ LYP*W620 homozygote T cells suppress TCR signaling more than LYP*R620/ LYP*W620 heterozygotes (34). The findings of the current study are in keeping with this hypothesis of a R620W gene-dosage effect, whereby a greater risk of IIM occurs in individuals homozygous for the T allele.

Due to the rarity of adult and juvenile IIMs, it is challenging to recruit sufficient case numbers to perform genetic association studies examining SNPs with a modest effect size. The current study was not powered to detect associations after disease stratification (although a strong association was still observed within the PM subgroup). This may also explain why no significant associations were observed for other PTPN22 SNPs which may have a more modest effect size, and why no interaction was noted with the 8.1 haplotype. Although to date, there are no replication data from other IIM cohorts to support our findings, the demonstrated IIM association is consistent with findings from other autoimmune diseases.

The findings from the current study demonstrate that the R620W variant is a significant risk factor in IIM and is independent of the 8.1 haplotype. Genetic risk for the IIMs thus resides not only within HLA, but possibly within multiple genetic regions, all of which may contribute to disease susceptibility. The polygenic nature of susceptibility risk is already becoming apparent in RA and other common autoimmune diseases, where novel associations with modest effect sizes are coming to light, for instance as a result of the recent Wellcome Trust case control whole genome scan (36). To further our understanding of IIM genetics, large scale collaborative genetic studies now appear necessary to identify further susceptibility genes.

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Table 1

PTPN22 SNP allele and genotype frequencies in combined cases versus controls

SNP	rs number	n		% minor allele frequency		% genotype frequency (11, 12, 22)	
		Cases	Controls	Cases	Controls	Cases	Controls
1	**rs1217385**	360	286	0.44	0.42	0.33, 0.46, 0.21	0.33, 0.49, 0.18
2	**rs1235005**	365	289	0.44	0.42	0.33, 0.46, 0.21	0.34, 0.48, 0.18
3	**rs2488457**	362	287	0.23	0.18	0.60, 0.34, 0.06	0.65, 0.33, 0.02
4	**rs17510162**	364	289	0.18	0.20	0.67, 0.29, 0.04	0.64, 0.32, 0.04
5	**rs1217414**	364	708	0.27	0.25	0.55, 0.37, 0.08	0.56, 0.39, 0.05
6	**rs2488458**	361	680	0.27	0.25	0.55, 0.36, 0.09	0.56, 0.39, 0.05
7	**rs1217418**	364	289	0.46	0.43	0.30, 0.48, 0.22	0.32, 0.49, 0.19
8	**rs12760457**	361	678	0.28	0.31	0.51, 0.42, 0.07	0.47, 0.44, 0.09
9	**rs12730735**	350	645	0.28	0.31	0.52, 0.41, 0.07	0.47, 0.43, 0.10
10	**rs2476601** [1]	**363**	**735**	**0.14**	**0.08**	**0.75, 0.22, 0.03**	**0.84, 0.15, 0.01**
11	**rs1310182**	361	587	0.45	0.45	0.31, 0.47, 0.22	0.31, 0.49, 0.20
12	**rs1217389**	356	279	0.27	0.23	0.55, 0.37, 0.08	0.58, 0.38, 0.04
13	**rs1217388**	357	693	0.26	0.25	0.55, 0.37, 0.08	0.55, 0.40, 0.05
14	**rs3811021**	344	678	0.19	0.19	0.65, 0.31, 0.04	0.65, 0.32, 0.03

Key: genotype frequencies, 1 refers to major allele; 2 refers to minor allele.

[1] For associations, see Tables 2 & 3.

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Table 2

rs2476601 allele associations in IIM subgroups stratified by clinical and antibody IIM subgroups

	n	Case frequency				
		C	T	OR, 95% CI	p	p_{corr}
Controls	735	91.8	8.2			
Combined cases	363	86.4	13.6	1.8, 1.3-2.4	0.00007	0.0009
PM	110	83.6	16.4	2.2, 1.4-3.3	0.0001	0.003
DM	98	90.3	9.7	1.2, 0.7-2.0	NS	NS
CTD/overlap	63	88.9	11.1	1.4, 0.7-2.5	NS	NS
JDM	91	84.1	15.9	2.1, 1.3-3.3	0.0006	0.009
Jo-1	50	86.0	14.0	1.8, 0.9-3.3	0.05	NS
155/140	32	82.8	17.2	2.3, 1.1-4.6	0.01	NS

Note: only significant antibody associations are shown. NS=not significant.

Using 1958 Birth Control Cohort control data, Combined cases: p=0.0004, OR 1.5, 1.2-2.0; PM: p=0.0006, OR 1.9, 1.3-2.8; JDM, p=0.003, OR 1.8, 1.2-2.8.

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Table 3

rs2476601 genotype associations in overall IIM cases *vs.* controls

Genotype test	p	OR, 95% CI
TT *vs.* CT + CC	0.005	4.1, 1.3-15.5
CT + TT *vs.* CC	0.0005	1.7, 1.3-2.4
TT *vs.* CC	0.003	4.5, 1.4-17.0
TC *vs.* CC	0.004	1.6, 1.1-2.2

Key: OR=odds ratio, CI confidence interval. P values are uncorrected.

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Table 4
PTPN22 haplotype frequencies for IIM subgroups and controls

SNP number								Control	Combined cases	PM	DM	CTD-overlap	JDM
5	**6**	**8**	**9**	**10**	**11**	**13**	**14**	**2n=1124**	**2n=622**	**2n=216**	**2n=192**	**2n=122**	**2n=114**
C	G	A	G	C	C	T	T	0.31	0.27	0.26	0.30	0.29	0.20[1]
T	G	G	A	C	C	T	T	0.24	0.28	0.29	0.29	0.25	0.29
C	G	G	A	C	T	T	C	0.19	0.19	0.16	0.20	0.20	0.21
C	A	G	A	C	T	C	T	0.16	0.12	0.11	0.10	0.15	0.15
C	**A**	**G**	**A**	**T**	**T**	**C**	**T**	**0.08**	**0.13**[2]	**0.17**[3]	**0.10**	**0.10**	**0.15**[4]

Key: Haplotype tagging SNPs listed above refer to Carlton SNPs 1, 2, 18, 21, 22, 27, 32, 36 (2).

[1] JDM *vs.* controls, p=0.01, OR 0.5, 0.3-0.9.

[2] All *vs.* controls, p=0.0007, OR 1.7, 1.2-2.4;

[3] PM *vs.* controls, p=0.00003, OR 2.4, 1.5-3.6;

[4] JDM *vs.* controls, p=0.01, OR 2.1, 1.1-3.6.