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► **To cite this version:**

Matthew J Simmonds, Oliver Brand, Jeffrey Barrett, Paul Roger Newby, Jayne A Franklyn, et al.. Association of Fc receptor like 5 (FCRL5) with Graves' disease is secondary to the effect of FCRL3. Clinical Endocrinology, 2010, 73 (5), pp.654. 10.1111/j.1365-2265.2010.03843.x . hal-00553273

HAL Id: hal-00553273

<https://hal.science/hal-00553273>

Submitted on 7 Jan 2011

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Association of *Fc receptor like 5 (FCRL5)* with Graves' disease is secondary to the effect of *FCRL3*

Journal:	<i>Clinical Endocrinology</i>
Manuscript ID:	CEN-2010-000315.R2
Manuscript Type/Office:	1 Original Article - UK/Europe
Date Submitted by the Author:	14-Jun-2010
Complete List of Authors:	<p>Simmonds, Matthew; University of Oxford, Oxford Centre for Diabetes, Endocrinology and Metabolism</p> <p>Brand, Oliver; University of Oxford, Oxford Centre for Diabetes, Endocrinology and Metabolism</p> <p>Barrett, Jeffrey; Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus</p> <p>Newby, Paul; University of Birmingham, Medical and Dental Sciences</p> <p>Franklyn, Jayne; University of Birmingham, Medical and Dental Sciences</p> <p>Gough, Stephen; University of Oxford, Oxford Centre for Diabetes, Endocrinology and Metabolism</p>
Key Words:	Genome Wide Screening, Graves' disease, Linkage Disequilibrium, FCRL3, FCRL5

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6 ***FCRL3***
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38 **Running Title:** Role of *FCRL5* in Graves' disease

39
40 **Key words:** Linkage disequilibrium, *FCRL3*, *FCRL5*, Graves' disease, genome wide screening
41

42 **Word Count:** 2,352
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44

45 **Acknowledgements:** The authors have no relevant financial or other conflicting interests to
46
47 declare.
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Abstract (Words = 235 [250 maximum])

Objective: The Fc receptor like 3 (FCRL3) molecule, involved in controlling B cell signalling, may contribute to the autoimmune disease process. Recently a genome wide screen detected association of neighbouring gene *FCRL5* with Graves' disease (GD). To determine whether *FCRL5* represents a further independent B cell signaling GD susceptibility loci we screened 12 tag SNPs, capturing all known common variation within *FCRL5*, in 5192 UK Caucasian GD index cases and controls.

Design: A case control association study investigating twelve tag SNPs within *FCRL5* which captured the majority of known common variation within this gene region.

Patients: A dataset comprising 2504 UK Caucasian GD patients and 2688 geographically matched controls taken from the 1958 British Birth cohort.

Measurements: We used the chi-squared test and haplotype analysis to investigate association between the tag SNPs and GD before performing regression analysis to determine if association at *FCRL5* was independent of the known *FCRL3* association.

Results: Three of the *FCRL5* tag SNPs, rs6667109, rs3811035 and rs6692977 showed association with GD (P=0.015-0.001, OR=1.15-1.16). Logistic regression performed on all *FCRL5* and, previously screened, *FCRL3* tag SNPs revealed that association with *FCRL5* was secondary to linkage disequilibrium with the *FCRL3*, rs11264798 and rs10489678 SNPs.

Conclusions: *FCRL5* does not appear to be exerting an independent effect on the development of GD in the UK. Fine mapping of the entire *FCRL* region is required to determine the exact location of the etiological variant/s present.

ORIGINAL ARTICLE

Introduction

The Fc receptor like (FCRL) gene family, which encode products that play a key role in controlling B cell signalling^{1, 2}, has been shown to be associated with several autoimmune diseases (AIDs). Originally, single nucleotide polymorphism (SNP) screening of chromosome 1q23 in a Japanese rheumatoid arthritis (RA) dataset narrowed down association to within two linkage disequilibrium (LD) blocks, which encoded five *FCRL* family members, *FCRL1-FCRL5* and lymphocyte antigen CD5-like (*CD5L*)³. Association was further refined to four SNPs within *FCRL3* with functional studies revealing that the rs7528684 SNP affected NF-κB binding to *FCRL3*, thereby causing variation in *FCRL3* expression on B cells, with the potential to impact on B cell regulation³. Further functional work has confirmed an inhibitory role for FCRL3 in B cell signalling, with the disease risk genotype causing variation in inhibitory signalling, which in turn could lead to a breakdown in B cell tolerance and autoreactive B cell activity⁴. Association of the rs7528684 SNP was also replicated in Japanese Graves' disease (GD) ($P=1.7 \times 10^{-5}$, OR=1.79) and Systemic lupus erythematosus (SLE) ($P=0.0017$) subjects³.

Replication of *FCRL3* within many AIDs including RA, SLE and type 1 diabetes (T1D) has been inconsistent^{1, 5}. However in GD we replicated association of rs7528684 (and the other three SNPs which we later found to be in complete linkage disequilibrium (LD) with rs7528684) in a UK Caucasian GD cohort of 983 GD patients and 733 controls, although the effect was smaller than that seen in the Japanese GD dataset ($P=0.024$, OR=1.17)^{3, 6}. Association of additional *FCRL3* SNPs was also detected in a smaller independent GD dataset consisting of 625 GD patients and 490 controls (OR=1.50 and 1.25, respectively)⁷ and within a 15,000 non-

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synonymous SNP genome wide screen performed by the Wellcome Trust Case Control Consortium (WTCCC) within 900 GD cases and 1500 controls⁸. Seven tag SNPs employed to screen the whole *FCRL3* region for association with GD in a Caucasian collection of 2280 GD and 2616 controls⁸, revealed association with disease of rs3761959 ($P=9.4 \times 10^{-3}$, OR=1.16) (which tags previously associated SNPs rs7522061 and rs7528684 with $r^2 > 0.80$) and another more strongly associated SNP rs11264798 ($P=1.6 \times 10^{-5}$, OR=1.22)⁸.

The WTCCC non-synonymous SNP screen also reported association of three SNPs within *FCRL5* ($P=1.3 \times 10^{-4}$ - 2.8×10^{-4}) with GD, located within the same region as *FCRL3* (approximately 125kb away)⁸. However, the typing of rs6667109, which tagged all three associated *FCRL5* SNPs, in our GD dataset of 2280 cases and 2616 controls showed only a trend towards association with GD ($P=0.077$)⁸. The aim of this study, therefore, was to use tag SNPs to screen all common variation within *FCRL5* in our UK Caucasian GD population to determine if other tag SNPs within *FCRL5* are more strongly associated with GD and whether association is independent of the previously detected *FCRL3* associations.

Materials and Methods

Subjects

A dataset consisting of 2504 unrelated white Caucasian patients with GD from the UK National Autoimmune Thyroid Collection was screened. Patients were recruited from specialist clinics in Birmingham, Bournemouth, Cambridge, Cardiff, Exeter, Leeds, Sheffield and Newcastle in the UK. Clinical definition of GD was as previously described⁹. A total of 2688 geographically matched white Caucasian control subjects were also obtained from the 1958 British Birth cohort. All patients gave informed written consent and the project was approved by the local ethics committee.

Tag SNP selection and genotyping

Genotyping data for the 39.14 kb *FCRL5* region (Phase 2, NCBI Build 36, Caucasian (CEU) population) was downloaded from the International Haplotype Mapping Project website (<http://www.hapmap.org>). Our analysis of Hapmap genotyping data identified 40 SNPs with minor allele frequencies of $\geq 5\%$ in the CEU population present within this region. The Tagger pairwise function within Hapmap was used to assign tag SNPs. Twelve tag SNPs: rs6667109, rs12036228, rs6692977, rs6427383, rs3811033, rs3811035, rs3811036, rs3843307, rs17416676, rs3900770, rs3845586 and rs1332714, were chosen to capture the majority of the common variation within this gene and the surrounding area with a minimum r^2 of 0.80 (for information on the SNPs which they tag see Supplementary Table 1). The rs6667109 SNP was previously typed as part of the WTCCC genome screen⁸, in which we used a smaller subsection of our current case control dataset⁸. We have subsequently increased our dataset by an additional 224

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3 GD cases and 72 controls. As all of our current dataset is being used for this study we wanted to
4 bring the number of GD samples and controls investigated for rs6667109 in line with those
5 investigated for all the other *FCRL5* tag SNPs, so screened rs6667109 in the additional 224 GD
6 cases and 72 controls not previously typed.
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14 Assays for all of the above SNPs were purchased from Applied Biosystems, Warrington, UK and
15 genotyped on an ABI7900HT using Taqman[®] genotyping technologies (Applied Biosystems,
16 UK).
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20 21 22 23 *Statistical and haplotype analyses* 24

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27 All SNPs were in Hardy Weinberg equilibrium in both the cases and controls ($P=0.92-0.05$).
28 When investigating prospective power in our dataset we had >80% power to exclude an
29 $OR_{\geq 1.13-1.24}$ at $\alpha = 0.05$ for our selected tag SNPs (see Supplementary Table 1 for the power
30 associated with each individual tag SNP). Allelic and genotypic analysis of case-control data
31 was performed using the χ^2 -test within the MINITAB statistical package (MINITAB Release
32 15.1.2, © 1972-2007, Minitab Inc., State College, PA, USA). Odds ratios (OR) with 95%
33 confidence intervals (CI) were calculated by the method of Woolf with Haldane's modification
34 for small numbers, where appropriate¹⁰. Linkage disequilibrium (LD) between tag SNPs was
35 analyzed using the pairwise LD measure D' and r^2 and haplotype blocks constructed using the
36 solid spine haplotype algorithm within the computer program Haploview Version 3.2
37 (<http://www.broad.mit.edu/mpg/haploview>). Logistic regression and imputation analysis was
38 performed using the PLINK software package¹¹.
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Results

FCRL5 genotyping results

Out of the twelve tag SNPs screened, the rs6667109 SNP, which when previously typed in a smaller sub-section of our case control dataset only showed a trend towards association with GD⁸, was weakly associated with GD in our larger cohort of 2504 GD index cases and 2688 controls (P=0.010, OR=1.16 [95% CI=1.04-1.30]) (see Table 1). Two additional SNPs, rs3811035 (P=0.001, OR=1.15 [95% CI=1.06-1.25]) and rs6692977 (P=0.015, OR=1.11 [95% CI=1.02-1.21]), were also associated with GD in our larger dataset. A minor difference in allele frequency between index cases and controls was also observed with SNPs rs3843307 (P=0.037) and rs3845586 (P=0.019). None of the remaining eight tag SNPs showed any association with GD (P=0.988-0.063) see Table 1.

Haplotype analysis between FCRL5 and FCRL3

Although tag SNPs are selected to take into account known LD within a gene region, tag SNPs information is only based on reference data within Hapmap generated from screening 90 CEPH individuals which provides us with a generalized overview of the LD within the region. The additional data obtained from typing larger datasets allows us to carefully dissect local haplotype structures which may not be evident in the smaller reference dataset from which tag SNPs are chosen. As *FCRL3* is within 125kb of *FCRL5* and as seven *FCRL3* tag SNPs (representing all known common variation within this gene region) were also screened in our GD dataset⁸, we performed a haplotype analysis of *FCRL3* and *FCRL5* tag SNPs (see Figure 1).

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3 No haplotype blocks were detected between *FCRL3* and *FCRL5*. Four LD blocks were detected
4 within *FCRL5*. Association of the rs3811035 allele T-rs6692977 allele C in haplotype block 2
5 showed the strongest association with GD ($P=9.00 \times 10^{-4}$, OR=1.15 [95% CI=1.06-1.24]). Other
6 smaller associations were also observed in block 2 ($P=0.008$), block 1 ($P=0.042-0.017$) and in
7 block 4 which was comprised of the previously associated rs6667109 SNP and the rs17416676
8 and rs6667109 SNPs ($P=0.022-0.001$). One haplotype block (block 5) was detected within
9 *FCRL3* spanning the previously associated rs11264798 and rs3761959 SNPs and previously non-
10 associated rs112644794, rs6691569 and rs10489678 SNPs. This block produced one common
11 haplotype block strongly associated with GD ($P=6.87 \times 10^{-5}$) and several rarer protective
12 haplotypes with minor allele frequencies $\leq 5.1\%$ ($P=0.030-0.002$) see Figure 1.
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29 *Logistic Regression Analysis*

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32 Logistic regression analysis was performed to determine if association present within *FCRL5*
33 was independent of previously detected associations within neighbouring *FCRL3*. Logistic
34 regression analysis was performed using the PLINK software package¹¹. When we first entered
35 all of the *FCRL3* and *FCRL5* tag SNPs into a regression model the *FCRL3* rs11264798 SNP was
36 shown to be the most associated SNP within the model ($P=1.84 \times 10^{-7}$). We found that the
37 rs11264798 SNP improved all other *FCRL3* and *FCRL5* SNP associations ($P=0.004-7.95 \times 10^{-8}$).
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39 When conditioning for the effects of the rs11264798 SNP, all of the other SNPs except
40 rs10489678 ($P=0.001$), which previously showed no association with GD, were no longer
41 associated with disease ($P=0.984-0.082$). When conditioning on both rs11264798 and
42 rs10489678 no other SNP within the model remained associated with GD ($P=0.872-0.057$),
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3 suggesting that association within *FCRL5* was the result of LD with the *FCRL3* rs11264798 and
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5 rs10489678 SNPs.
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10 Interestingly when screened in our case control study the rs10489678 SNP was not associated
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12 with GD (see Table 1). There was little evidence for historical recombination between
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14 rs10489678 and rs11264798 ($D' = 0.94$), but the $r^2 = 0.19$ suggested these SNPs were at least
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16 partially independent (see Figure 1). Haplotype analysis revealed that rs10489678 formed part
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18 of a haplotype block within *FCRL3* containing rs11264798 together with rs11264794, rs6691569
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20 and rs3761959 (see Figure 1 block 5). The most strongly associated haplotype within *FCRL3*
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22 contains the rs11264798 disease associated allele G and the rs10489678 allele C ($P=6.87 \times 10^{-5}$)
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24 present within 22.3% of the cases and 18.8% of the controls. However when analysing the only
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26 haplotype to contain the rs11264798 allele G and the rs10489678 allele T, this haplotype which
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28 was present in 18.8% of cases and 17.5% of controls showed no association with GD ($P=0.1218$)
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30 (see Figure 1). Furthermore, several other less common haplotypes with the rs11264798 allele
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32 G-rs10489678 allele C were not associated with GD. This suggests that the causal variant could
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34 lie exclusively on the most common rs11264798 allele G- rs10489678 allele C haplotype.
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Discussion

Genome wide screening of 15,000 non-synonymous SNPs in a UK Caucasian GD cohort not only confirmed association of the previously detected GD susceptibility loci *FCRL3* but also detected association of neighbouring *FCRL5* with GD⁸. In our present study we performed tag SNP screening of *FCRL5* in an enlarged dataset, confirming association of this gene with GD. Logistic regression analysis performed on *FCRL5*, and previously typed *FCRL3* tag SNPs, revealed that any associations within *FCRL5* were the result of LD with *FCRL3* rs11264798 and rs10489678 tag SNPs. Although association at *FCRL5* was shown to be secondary to association at *FCRL3*, we are unable to say that *FCRL3* contains the etiological variant represented by these associations. Whilst it may be tempting to embark upon a functional analysis of rs11264798 (the most strongly associated *FCRL3* tag SNP), haplotype analysis suggests that this may not be the etiological variant, as the high risk G allele is only associated when present with the rs10489678 C allele (not the rs10489678 T allele), suggesting that the etiological variant could be elsewhere within this region.

When trying to determine the Crohn's disease etiological variant represented by associated markers present within a 1.25-Mb gene desert on chromosome 5, LD was investigated between these markers and a series of SNPs identified by Dixon *et al* that affected gene expression¹². Markers within this 1.25Mb gene desert, which contains no known genes or CpG islands which are a common feature of many gene promoters, were shown to be associated with neighbouring SNPs known to alter Prostaglandin E Receptor 4 (*PTGER4*) gene expression, thereby suggesting neighbouring *PTGER4* as a major potential susceptibility locus for Crohn's disease within this

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3 region of linkage¹³. As Dixon *et al*¹² identified SNPs which altered gene expression throughout
4 the genome, we investigated whether known gene expression altering SNPs in neighbouring
5 genes could also explain association of rs11264798 or rs10489678 with GD. Although eight
6 functional gene expression altering SNPs were located 200 kb either side of rs11264798 or
7 rs10489678, additional analysis revealed that there was no LD between any of these eight SNPs
8 and either rs11264798 or rs10489678, suggesting that this approach could not provide further
9 insights into the location of the etiological variants within the *FCRL* region for GD.
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22 As there are numerous other members of the *FCRL* family encoded within this region, all of
23 which play important roles in B cell signalling^{1, 2}, detailed mapping of the entire region is
24 essential to enable the etiological variant/s to be localized before determining how variation in B
25 cell signalling could be aiding autoimmune onset. To try to determine if other stronger
26 associations than rs11264798 and rs10489678 could exist elsewhere within the *FCRL* region, in
27 particular within *FCRL4* which is located between *FCRL3* and *FCRL5*, we performed imputation
28 analysis within the PLINK software package¹¹ to statistically predict additional SNP genotyping
29 results within the surrounding region. Although a further ninety six SNPs were imputed within
30 the region, including eleven SNPs located within *FCRL4*, the *FCRL3* rs11264798 SNP remained
31 the most associated SNP within this region (see Supplementary Figure 1 for further details).
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48 Interestingly association of the *FCRL3* rs7528684 SNP, identified by regression in the Oriental
49 population to be the most associated SNP within this region, was not replicated in many
50 Caucasian AIDs datasets, apart from GD^{1, 5}. Although we detected association of rs3761959,
51 that tagged rs7528684, with GD, logistic regression showed that association of rs3761759 was
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3 secondary to the *FCRL3* rs11264798 and rs10489678 SNPs. This suggests that rs3761759, and
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5 by proxy rs7528684, is not the likely *FCRL* etiological variant in our Caucasian GD population.
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8 Although studies on the rs7528684 SNP in many other AIDs has ruled out association of *FCRL3*
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10 with these diseases, once the *FCRL* etiological variant/s have been established for Caucasian GD
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12 patients these can then be screened in additional Caucasian AID datasets to confirm whether or
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14 not this gene region is associated with other AIDs within the Caucasian population.
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20 This study highlights the well described effects of LD between genes and the problems that this
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22 poses when attempting to identify primary aetiological disease causing DNA variants¹⁴. Further
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24 fine mapping within the entire *FCRL* region is needed to confirm the imputation results and to
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26 enable localisation of the GD etiological DNA variant/s present before embarking upon
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28 functional studies to determine how variation in B cell signalling molecules could be
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30 contributing to GD.
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Acknowledgements

We would like to thank patients, nurses and doctors for recruiting into the AITD National Collection. We also wish to acknowledge The Society for Endocrinology for awarding MJS a small project grant and the Wellcome Trust for funding this project. We acknowledge use of DNA from the British 1958 Birth Cohort Collection, funded by the Medical Research Council grant G0000934 and the Wellcome Trust grant 068545/Z/02.

Conflicts of interest

None of the authors have any conflicts of interest to declare in connection with this manuscript.

References

- 1 Davis, R.S. (2007) Fc receptor-like molecules. *Annu Rev Immunol* **25**, 525-560.
- 2 Ehrhardt, G.R., Leu, C.M., Zhang, S., Aksu, G., Jackson, T., Haga, C., Hsu, J.T., Schreeder, D.M., Davis, R.S. & Cooper, M.D. (2007) Fc receptor-like proteins (FCRL): immunomodulators of B cell function. *Adv Exp Med Biol* **596**, 155-162.
- 3 Kochi, Y., Yamada, R., Suzuki, A., Harley, J.B., Shirasawa, S., Sawada, T., Bae, S.C., Tokuhiko, S., Chang, X., Sekine, A., Takahashi, A., Tsunoda, T., Ohnishi, Y., Kaufman, K.M., Kang, C.P., Kang, C., Otsubo, S., Yumura, W., Mimori, A., Koike, T., Nakamura, Y., Sasazuki, T. & Yamamoto, K. (2005) A functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. *Nat Genet* **37**, 478-485.
- 4 Kochi, Y., Myouzen, K., Yamada, R., Suzuki, A., Kurosaki, T., Nakamura, Y. & Yamamoto, K. (2009) FCRL3, an autoimmune susceptibility gene, has inhibitory potential on B-cell receptor-mediated signaling. *J Immunol* **183**, 5502-5510.
- 5 Chistiakov, D.A. & Chistiakov, A.P. (2007) Is FCRL3 a new general autoimmunity gene? *Hum Immunol* **68**, 375-383.
- 6 Simmonds, M.J., Heward, J.M., Carr-Smith, J., Foxall, H., Franklyn, J.A. & Gough, S.C. (2006) Contribution of single nucleotide polymorphisms within FCRL3 and MAP3K7IP2 to the pathogenesis of Graves' disease. *J Clin Endocrinol Metab* **91**, 1056-1061.
- 7 Owen, C.J., Kelly, H., Eden, J.A., Merriman, M.E., Pearce, S.H. & Merriman, T.R. (2007) Analysis of the Fc receptor-like-3 (FCRL3) locus in Caucasians with autoimmune disorders suggests a complex pattern of disease association. *J Clin Endocrinol Metab* **92**, 1106-1111.

- 1
2
3 8 WTCCC & TACS (2007) Association study of 14,500 nsSNPs in four common diseases
4 identifies variants involved in autoimmunity. *Nature Genetics* **39**, 1329-1337.
5
6
7
8 9 Manji, N., Carr-Smith, J.D., Boelaert, K., Allahabadia, A., Armitage, M., Chatterjee,
9 V.K., Lazarus, J.H., Pearce, S.H., Vaidya, B., Gough, S.C. & Franklyn, J.A. (2006) Influences of
10 age, gender, smoking, and family history on autoimmune thyroid disease phenotype. *J Clin*
11 *Endocrinol Metab* **91**, 4873-4880.
12
13
14
15
16
17 10 Mathews, J.D. (1984) Statistical aspects of immunogenetic association with disease. In
18 *Detection of immune-associated genetic markers of human disease* eds. M. J. Simons & B. D.
19 Tait). Churchill Livingstone, London, pp. 106-136.
20
21
22
23
24 11 Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A., Bender, D., Maller,
25 J., Sklar, P., de Bakker, P.I., Daly, M.J. & Sham, P.C. (2007) PLINK: a tool set for whole-
26 genome association and population-based linkage analyses. *Am J Hum Genet* **81**, 559-575.
27
28
29
30
31 12 Dixon, A.L., Liang, L., Moffatt, M.F., Chen, W., Heath, S., Wong, K.C.C., Taylor, J.,
32 Burnett, E., Gut, I., Farrall, M., Lathrop, G.M., Abecasis, G.R. & Cookson, W.O.C. (2007) A
33 genome-wide association study of global gene expression. *Nature Genetics* **39**, 1202-1207.
34
35
36
37
38 13 Libioulle, C., Louis, E., Hansoul, S., Sandor, C., Farnir, F., Franchimont, D., Vermeire,
39 S., Dewit, O., de Vos, M., Dixon, A., Demarche, B., Gut, I., Heath, S., Foglio, M., Liang, L.M.,
40 Laukens, D., Mni, M., Zelenika, D., Van Gossum, A., Rutgeerts, P., Belaiche, J., Lathrop, M. &
41 Georges, M. (2007) Novel Crohn disease locus identified by genome-wide association maps to a
42 gene desert on 5p13.1 and modulates expression of PTGER4. *Plos Genetics* **3**, -.
43
44
45
46
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48 14 McCarthy, M.I. & Hirschhorn, J.N. (2008) Genome-wide association studies: potential
49 next steps on a genetic journey. *Hum Mol Genet* **17**, R156-165.
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Table 1: Allelic and genotypic association of FCRL5 tag SNPs within a large UK Caucasian Graves' disease cohort

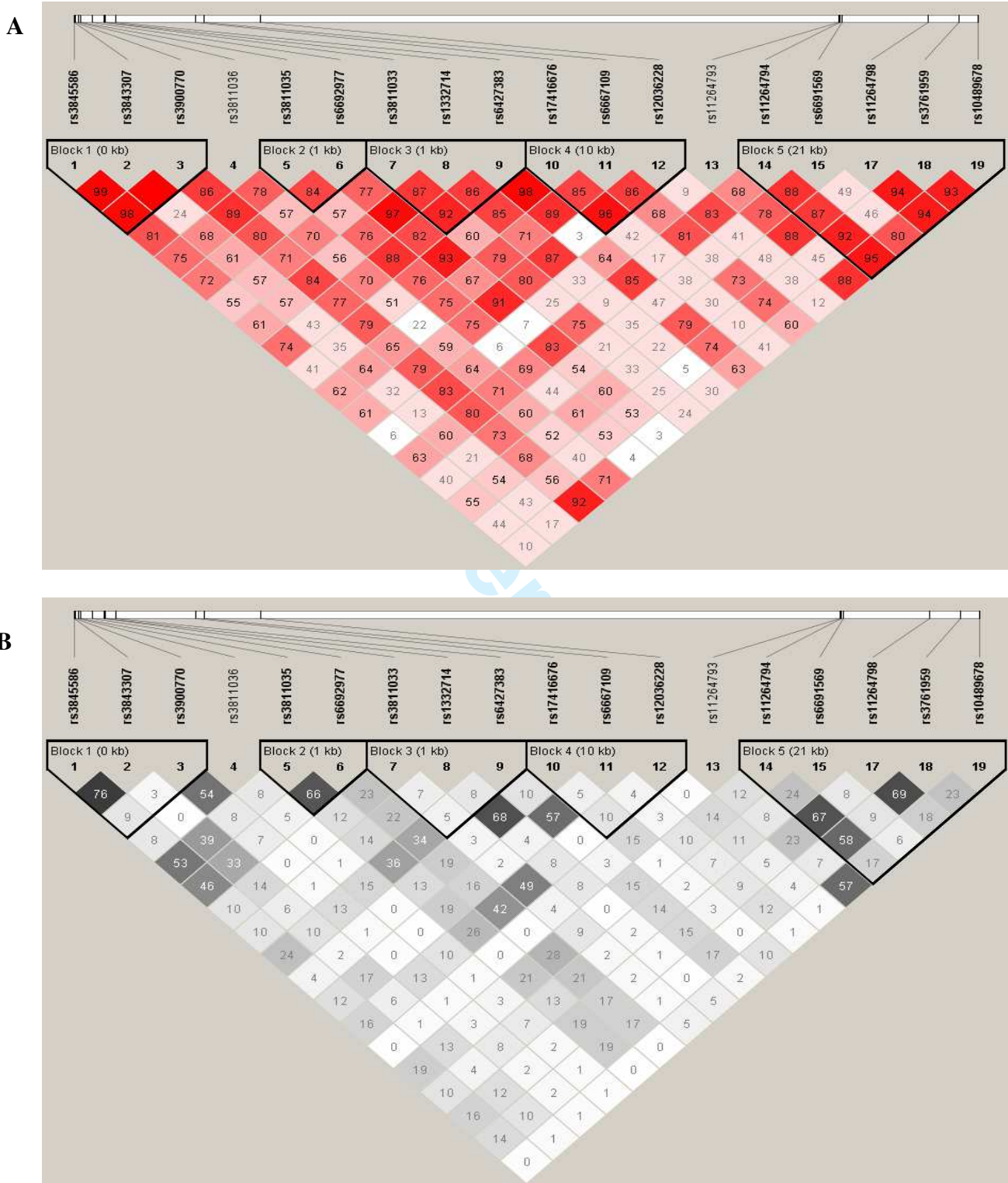
<i>FCRL5</i>	Genotype / Allele	Graves' disease (%)		Control subjects (%)		Chi-squared	P	OR	95% CI
rs3845586	A	2842	(60.4)	3125	(62.7)	5.461	0.019	1.10	1.02-1.20
	G	1866	(39.6)	1861	(37.3)				
	AA	853	(36.2)	973	(39.0)	5.519	0.063		
	AG	1136	(48.3)	1179	(47.3)				
GG	365	(15.5)	341	(13.7)					
rs3843307	G	3079	(66.1)	3355	(68.1)	4.372	0.037	1.09	1.01-1.119
	A	1581	(33.9)	1573	(31.9)				
	GG	1004	(43.1)	1131	(45.9)	4.491	0.106		
	GA	1071	(46.0)	1093	(44.4)				
AA	255	(10.9)	240	(9.7)					
rs3900770	A	4490	(94.2)	4738	(94.2)	0.037	0.847	0.98	0.83-1.17
	G	274	(5.8)	294	(5.8)				
	AA	2115	(88.8)	2232	(88.7)	0.380	0.827		
	AG	260	(10.9)	274	(10.9)				
GG	7	(0.3)	10	(0.4)					
rs3811036	T	4390	(92.5)	4505	(92.1)	0.569	0.451	0.94	0.81-1.10
	G	356	(7.5)	387	(7.9)				
	TT	2030	(85.5)	2078	(85.0)	1.112	0.574		
	TG	330	(13.9)	349	(14.2)				
GG	13	(0.6)	19	(0.8)					
rs3811035	A	2733	(61.4)	3242	(64.7)	11.009	0.001	1.15	1.06-1.25
	G	1719	(38.6)	1770	(35.3)				
	AA	830	(37.3)	1030	(41.1)	11.610	0.003		
	AG	1073	(48.2)	1182	(47.2)				
GG	323	(14.5)	294	(11.7)					
rs6692977	C	2883	(63.8)	3257	(66.3)	5.886	0.015	1.11	1.02-1.21
	T	1629	(36.1)	1657	(33.7)				
	CC	930	(41.2)	1076	(43.8)	6.485	0.039		
	CT	1023	(45.3)	1105	(45.0)				
TT	303	(13.4)	276	(11.2)					
rs3811033	G	3718	(81.6)	4130	(82.8)	2.055	0.152	1.08	0.97-1.20
	A	836	(18.4)	860	(17.2)				
	GG	1524	(66.9)	1702	(68.2)	3.824	0.148		
	GA	670	(29.4)	726	(29.1)				
AA	83	(3.7)	67	(2.7)					
rs1332714	T	3132	(70.3)	3431	(70.1)	0.026	0.872	0.99	0.91-1.08
	G	1324	(29.7)	1461	(29.9)				
	TT	1117	(50.1)	1221	(49.9)	0.025	0.988		
	TG	898	(40.3)	989	(40.4)				
GG	213	(9.6)	236	(9.7)					
rs6427383	A	3539	(77.8)	3968	(79.1)	2.486	0.115	0.99	0.91-1.08
	T	1011	(22.2)	1048	(20.9)				
	AA	1382	(60.7)	1575	(62.8)	2.452	0.294		
	AT	775	(34.1)	818	(32.6)				
TT	118	(5.2)	115	(4.6)					
rs17416676	G	3128	(70.6)	3475	(71.2)	0.370	0.543	1.03	0.94-1.12
	A	1304	(29.4)	1409	(28.8)				
	GG	1107	(50.0)	1256	(51.4)	1.589	0.452		
	AG	914	(41.2)	963	(39.5)				
AA	195	(8.8)	223	(9.1)					
rs6667109*	T	3445	(82.5)	3912	(84.6)	6.601	0.010	1.16	1.04-1.30
	A	729	(17.5)	714	(15.4)				
	TT	1428	(68.4)	1660	(71.8)	6.465	0.039		
	TA	589	(28.2)	592	(25.6)				
AA	70	(3.4)	61	(2.6)					
rs12036228	C	3639	(77.7)	3981	(78.9)	1.858	0.173	1.07	0.97-1.18
	T	1043	(22.3)	1067	(21.1)				
	CC	1420	(60.7)	1572	(62.3)	1.930	0.381		
	CT	799	(34.1)	837	(33.2)				
TT	122	(5.2)	115	(4.5)					

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3 All SNPs are shown within their forward oriental (5'-3'). All SNPs which showed association at
4 both the allelic and genotypic level are highlighted in bold. All P values are shown
5 uncorrected. χ^2 = Chi-squared, OR = Odds Ratio, 95% CI = 95% Confidence Intervals.
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8 *This SNP was previously typed in a subsection of our GD case control dataset as part of the
9 WTCCC genome screen⁸ and so to bring the numbers of GD samples and controls in line with
10 those investigated for all the other *FCRL5* tag SNPs we screened an additional 224 GD cases and
11 72 controls.
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Figure 1: Haplotype analysis between *FCRL5* and *FCRL3* tag SNPs



C	Haplotype block	Haplotype	Frequency	Case:Control Frequency	χ^2	P Value	OR	95% CI
	Block 1 (rs3845586-rs3843307-rs3900770)							
		AGT	0.613	0.602:0.622	4.156	0.042	1.09	1.00-1.78
		GAT	0.324	0.336:0.313	5.695	0.017	1.11	1.02-1.20
		GGC	0.058	0.058:0.058	0.040	0.841	0.98	0.83-1.16
	Block 2 (rs3811035-rs6692977)							
		TC	0.597	0.580:0.613	11.046	9.00x10⁻⁴	1.15	1.06-1.24
		CT	0.316	0.329:0.304	7.014	0.008	1.12	1.03-1.22
		CC	0.051	0.055:0.048	2.380	0.123	1.15	0.96-1.38
		TT	0.035	0.036:0.035	0.100	0.751	1.06	0.85-1.31
		CC	0.051	0.055:0.048	2.380	0.123	1.15	0.96-1.38
		TT	0.035	0.036:0.035	0.100	0.752	1.06	0.85-1.31
	Block 3 (rs3811033-rs1332714-rs6427383)							
		CAA	0.329	0.322:0.335	1.969	0.161	0.94	0.87-1.02
		CCA	0.283	0.279:0.287	0.849	0.357	0.96	0.88-1.05
		CAT	0.202	0.206:0.198	0.994	0.319	1.05	0.95-1.16
		TAA	0.167	0.170:0.165	0.519	0.471	1.04	0.94-1.16
	Block 4 (rs17416676-rs6667109-rs12036228)							
		GTC	0.341	0.324:0.356	10.822	0.001	0.87	0.80-0.95
		ATC	0.283	0.285:0.281	0.284	0.594	1.02	0.94-1.12
		GTT	0.211	0.216:0.207	1.250	0.264	1.06	0.96-1.16
		GAC	0.153	0.162:0.146	5.221	0.022	1.14	1.02-1.27
	Block 5 (rs11264794-rs6691569-rs11264798-rs3761959-rs10489678)							
		CCCGC	0.376	0.371:0.381	0.935	0.334	0.96	0.88-1.05
		ATGAC	0.205	0.223:0.188	15.848	6.87x10⁻⁵	1.50	1.35-1.67
		ACGAT	0.182	0.188:0.175	2.394	0.122	1.14	1.02-1.27
		ACGAC	0.064	0.067:0.061	1.602	0.206	1.12	0.94-1.33
		ATCGC	0.051	0.046:0.056	4.712	0.030	0.81	0.67-0.98
		ACGGC	0.038	0.038:0.037	0.016	0.900	1.01	0.81-1.27
		CCGGC	0.023	0.027:0.020	3.662	0.056	1.31	0.99-1.74
		ATGGC	0.011	0.008:0.014	5.347	0.021	0.62	0.41-0.94
		CTCGC	0.011	0.007:0.014	9.742	0.002	0.51	0.33-0.79

Linkage disequilibrium (LD) between tag SNPs was analyzed using the pairwise LD measure D' and r^2 and haplotype blocks constructed using the solid spine haplotype algorithm within the computer program Haploview Version 3.2 (<http://www.broad.mit.edu/mpg/haploview>). The solid spine haplotype method searches for a "spine" of strong LD running from one marker to another along the base of the triangle in the LD chart (this would mean that the first and last markers in a block are in strong LD with all intermediate markers but that the intermediate markers are not necessarily in LD with each other). Each diamond either represents the D' or r^2 measure of LD. A: Displays the LD parameter, D' , where the darker shades of red represent greater D' values. B: Shows the correlation coefficient of LD, r^2 , darker shades of black

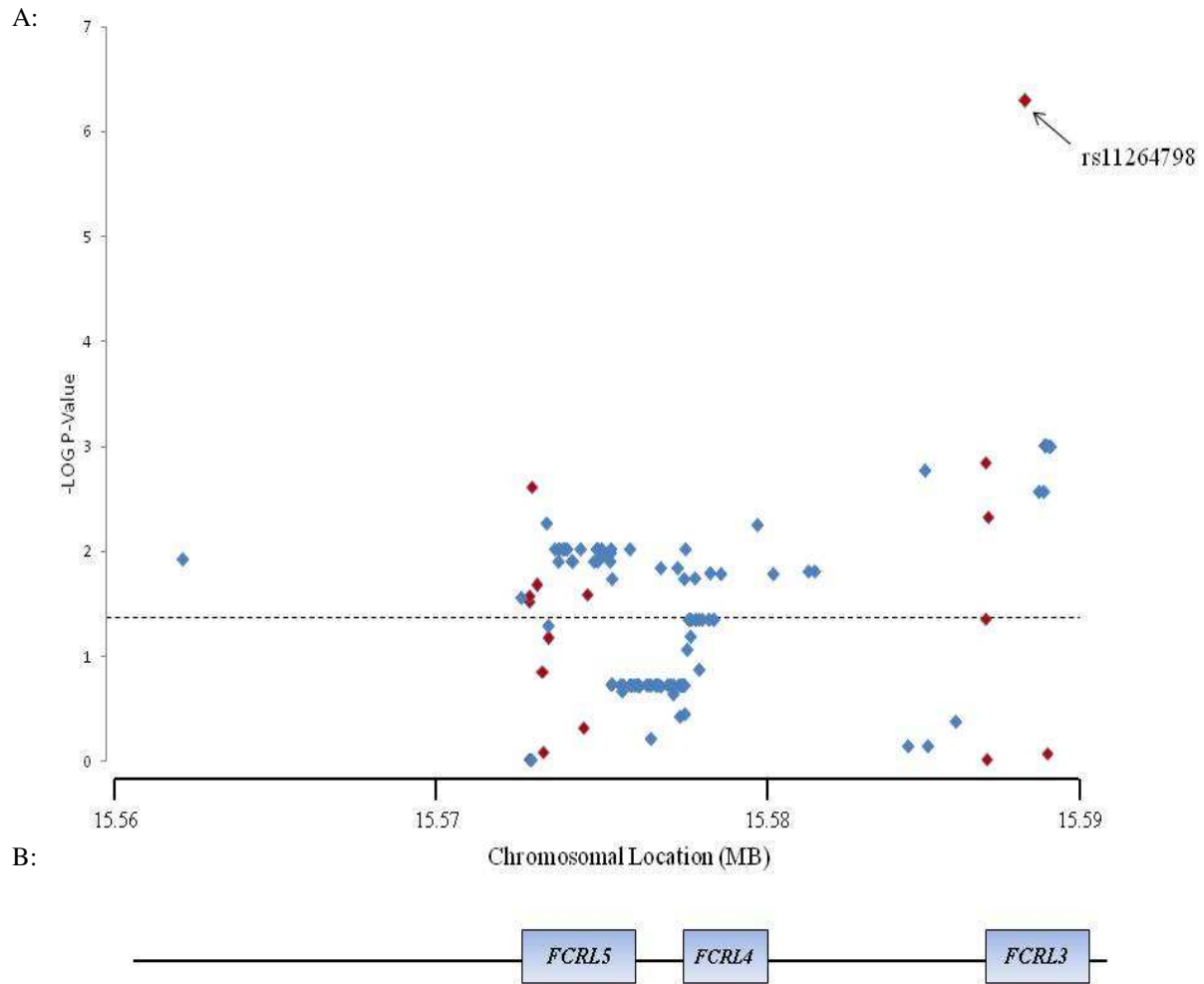
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3 represent greater r^2 values. C: Shows association of haplotypes produced by Haplotype Block 1,
4 Haplotype Block 2 and Haplotype Block 4 within *FCRL5* and Haplotype Block 5 within *FCRL3*
5 (highlighted in bold). χ^2 = Chi-squared, OR = Odds Ratio, 95% CI = 95% Confidence Intervals.
6 All P values are represented as uncorrected.
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Supplementary Table 1: Tag SNPs present in *FCRL5*, the SNPs which they capture and the level of OR that can be excluded for these SNPs

tag SNPs	SNPs Captured	Hapmap Caucasian MAF (%)	Smallest OR that can be excluded in our dataset
rs6667109	rs2317051, rs1412330, rs20122712, rs1972239, rs12745961, rs6427384, rs6686913, rs3761961, rs4971128, rs10796980, rs849836, rs2012425, rs1977588, rs6667109, rs2012199, rs7517509, rs1855121, rs10796979, rs4971127	0.108	1.19
rs12036228	rs7552348, rs7527521, rs12732692, rs7531685, rs12036228, rs11264761, rs12733916, rs1984492	0.250	1.14
rs6692977	rs6696309, rs10494316, rs6692977	0.340	1.13
rs6427383	rs6696922, rs6427383	0.186	1.15
rs3811036	rs3811036	0.092	1.21
rs3811033	rs3811033	0.170	1.16
rs3811035	rs3811035	0.325	1.13
rs3843307	rs3843307	0.275	1.13
rs17416676	rs17416676	0.267	1.14
rs3900770	rs3900770	0.067	1.24
rs3845586	rs3845586	0.345	1.13
rs1332714	rs1332714	0.267	1.14

Supplementary Figure 1: Imputation analysis of the *FCRL* region based on *FCRL3* and *FCRL5* tag SNP data and data available from Hapmap Phase II



Data from *FCRL3* and *FCRL5* tag SNPs typed in our GD cohort was merged with Hapmap Phase II genotyping data (release 23) on 90 CEPH individuals from 200kb either side of the *FCRL3* rs11264798 and rs10489678 SNPs. This data was then used to perform imputation analysis within the PLINK software package¹¹ to statistically predict additional SNP genotyping results within the surrounding region in our case control dataset. A: PLINK imputed an additional 96 SNPs (highlighted in blue) within the surrounding *FCRL* region and combined with our 19 previously typed tag SNPs (highlighted in red) the results were plotted ($-\log P$ value) against their chromosome position with the most associated SNP within the region highlighted. The dotted line represents the cut off for association and any SNPs above this line show association with GD. B: Location of *FCRL* family members relative to chromosomal location.