ORIGINAL ARTICLE

An X chromosome-wide association analysis identifies variants in *GPR174* as a risk factor for Graves' disease

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ABSTRACT

Background Graves' disease is a female preponderant autoimmune illness and the contribution of the X chromosome to its risk has long been appreciated. However, no X-linked susceptibility loci have been indentified from recent genome-wide association studies (GWAS).

Methods We re-examined the X chromosome data from our recent GWAS for Graves' disease by including males that were previously excluded from the X chromosome analyses. The data were analysed using logistic regression analysis including sex as a covariate, and an additive method assuming X chromosome inactivation, implemented in snpMatrix.

Results A cluster of single nucleotide polymorphism (SNPs) at Xq21.1 was found showing association with genome-wide significance, among which rs3827440 was a non-synonymous SNP of *GPR174* ($P_{logistic\ regression}=9.52\times10^{-8}$; $P_{snpMatrix}=4.60\times10^{-9}$; OR=1.76, 95% CI 1.45 to 2.13). The association was reproduced in an independent sample collection set including 4564 Graves' disease cases and 3968 sex matched controls (combined $P_{logistic\ regression}=5.53\times10^{-21}$; combined $P_{snpMatrix}=4.26\times10^{-22}$; OR=1.69, 95% CI 1.53 to 1.86). Notably, *GPR174* was widely expressed in immune related tissues and rs3827440 genotypes were associated with distinct mRNA levels (p=0.002). *GPR174* did not show sex biased gene expression in our expression analysis. Resequencing study suggested the contribution of some rare variants in the *GPR174* gene region to disease risk with a collapsing p value of 1.16×10^{-3}.

Conclusions The finding of an X-linked risk locus for Graves' disease expands our understanding of the role of the X chromosome in disease susceptibility.

INTRODUCTION

Consistent with many other autoimmune illnesses, Graves' disease exhibits a pronounced gender bias, with a female to male ratio of about 5:1 in the Chinese population, similar to that in Caucasian populations.[1 2] The X chromosome is partly responsible for the hyperresponsiveness of the female immune system. It is reasonable to presume that some genes located on the X chromosome may play an important role in the susceptibility of Graves' disease. However, recent genome-wide association studies (GWAS) did not reveal any loci on the X chromosome to be associated with this disorder.[3 4] Reviewing the published GWAS for complex diseases showed that the focus of GWAS and subsequent meta-analyses has been on the autosomes, whereas X chromosomal data have usually been collected but not analysed.[5–8] One reason for the neglect of the X chromosome in GWAS results could be the lack of a consensus analytical approach taking into account the specific features of X chromosomal data.

The special features of the X chromosome make tests for association less straightforward than those for autosomal chromosomes in mixed sex population studies.[6–9] A female has two X chromosomes, while a male has only one. It should be noted that the pseudo-autosomal region (PAR) of the X chromosome has an homologous region on the Y chromosome, where loci are inherited like autosomal loci. Additionally, one X chromosome in females undergoes the X chromosome inactivation (XCI) process to maintain equal expression between sexes.[10] The traditional approach is to stratify by sex, and several ways have been proposed for combining evidence across strata.[9] Recently, Clayton proposed an additive test in which modelling was performed in the context that one of the female X chromosomes is inactivated.[5] However, all these methods did not appear to have gained widespread use in GWAS analysis.[8]

In our previous study, 1536 Graves' disease cases and 1516 sex matched controls of the Chinese Han population were genotyped using Illumina Human 660-Quad BeadChips including 14 141 chromosome X single nucleotide polymorphisms (SNPs).[4] In total, 10 925 X chromosome SNPs in 1468 cases and 1490 controls matched criteria for quality control. The X chromosome data were investigated using the Cochran–Armitage test for trend ignoring males entirely, but no significant association at the X chromosome was found. In the current work, we expand our recent GWAS of Graves' disease to include males when studying the X chromosome and follow-up our results using a larger independent case–control sample.

METHODS

Samples and clinical characteristics

As described in our previously published data,[4] 1536 Graves' disease cases and 1516 sex matched

controls were recruited for stage 1 of the GWAS. In the current work, an additional 4564 Graves' disease cases and 3968 sex matched controls were recruited for the replication study. A subset of samples from Shandong Province including 2608 cases and 2328 sex matched controls were sequenced for the *GPR174* gene region. All individuals were of Chinese Han descent and provided informed consent with protocols approved by the local institutional review board. Diagnosis of Graves' disease was based on documented clinical and biochemical evidence of hyperthyroidism, diffuse goitre, and the presence of at least one of the following: positive thyroid stimulating hormone (TSH) receptor antibody tests, diffusely increased I-131 (iodine-131) uptake in the thyroid gland, or exophthalmos. All individuals classified as having Graves' disease were interviewed and examined by experienced clinicians.

Genotyping and quality control

DNA samples from 1536 Graves' disease cases and 1516 controls were genotyped using Illumina (San Diego, California, USA) Human660-Quad BeadChips at the Chinese National Human Genome Center in Shanghai, China.[4] After quality filtering of samples as described previously,[4] 1468 Graves' disease cases and 1490 controls were used in the current analysis. The estimated genomic inflation factor was 1.02, indicating that overall population structure had negligible impact on the case–control association results. Therefore, we did not correct for population stratification in the association analysis. While examining the data of 14 141 X chromosome SNPs assayed in this study, we first eliminated the results of 'heterozygous' genotypes in males likely due to genotyping errors. Sequentially, we discarded five markers with Hardy–Weinberg equilibrium p value $<10^{-6}$ in female controls, 870 with high missing call rates (>2%), and 2341 with minor allele frequency <1%, leaving 10 925 SNPs for subsequent analysis. The quality control procedure was performed with PLINK.[11]

Replication samples were genotyped for rs3827440 with TaqMan SNP Genotyping Assays (C_25954273_10) using the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, California, USA). The data were analysed using the ABI Prisms SDS V.2.1 software package.

Statistical analysis

After quality control, we used the genotypes of 10 925 SNPs in 1468 cases and 1490 controls for association analyses using two methods. The logistic regression analyses were performed in PLINK entering sex as a covariate.[11] To perform a joint analysis for rs3827440 across the GWAS and replication stages, we used logistic regression analysis adjusted for gender and study stages to compute the p value. The one degree of freedom test described by Clayton was performed in snpMatrix.[5] Under this test, the hemizygous males were treated as equivalent to the corresponding homozygous females at non-PAR X chromosome loci. The heterozygous females were modelled as half of the risk as the hemizygous males or homozygous females carrying the risk genotype. The p values calculated by snpMatrix for two stages were combined using Fisher's test. In females, the ORs of SNPs were estimated as the ORs of the homozygotes. In males, the ORs of SNPs were estimated as the per allele ORs. In the mixed sex samples, the hemizygous males were treated as equivalent to the homozygous females and the ORs were estimated from the comparison between the combined homozygous females and corresponding hemizygous males. A Mantel–Haenszel common OR was calculated across the two sample sets.

After excluding SNPs which did not pass the quality control filters, we imputed untyped and/or missing SNPs separately in cases and controls using the software IMPUTE2[12] and 1000 Genomes Phase I integrated variant set (March 2012) as reference. SNPTEST v2 was used to test for association with disease for genotyped and imputed SNPs (probability >0.9) under a logistic regression model with sex as a covariate. The effects of rare variants were assessed using collapsing methods.[13] Because the observed variants were rare and, consequently, the females homozygous for the rare allele were extremely rare, the homozygous and heterozygous females and the hemizygous males were collapsed together, and a 2×2 table was constructed. We tested the difference between the proportions of individuals with rare variants in cases and controls using Fisher's exact test.[13] All statistical analyses were performed with R (V.2.13) and SPSS software (V.17.0 for Windows) unless specified.

Tissue/cell gene expression patterns

We examined the expression of *GPR174* in 16 different human tissues. cDNA samples of 15 tissues were from the Human Immune System Multiple Tissue cDNA (MTC) Panel and Human MTC Panel I (Clontech, Palo Alto, California, USA). In addition, Human Thyroid Total RNA (Clontech) was reverse transcribed using reverse transcription PCR (RT-PCR) reagents with random hexamers (Promega, Fitchburg, Wisconsin, USA) in accordance with the instructions of the manufacturer. The control cDNA contained in Clontech human MTC Panels were used as the positive control. Quantitative RT-PCR was performed using SYBR Green (TaKaRa, Otsu, Japan) in each 20 µl reaction containing 2 µl cDNA template on an ABI PRISM 7900 Sequence Detector (Applied Biosystems) with SDS V.2.1 software. *GAPDH* was used as an endogenous control. Primer sequences used are shown in online supplementary table S3. PCR products were visualised on a 3% agarose gel to confirm correct band sizes (see online supplementary figure S1). Each reaction was performed in duplicate, with final calculations resulting from means of duplicate wells. Normalisation for cDNA quantity was performed with *GAPDH* for each template and final abundance figures were adjusted to yield an arbitrary value of one for levels of gene specific expression in leucocytes using the ΔΔcq method.[14]

Quantification of allelic variation in gene expression

A total of 185 individuals including 141 females and 44 males were recruited for gene expression analysis. We drew 3 ml of peripheral blood from individuals participating in the study under fasting conditions. Genomic DNA was isolated from whole blood by the FlexiGene DNA Kit (Qiagen, Hilden, Germany). The genotypes of rs3827440 were determined by directed sequencing using Applied Biosystems 3730 platform. Sixty-four females with rs3827440 CC genotype and 43 females with TT, as well as 20 males with rs3827440 C allele and 19 males with T allele, were included in allele specific expression analysis. The females heterozygous for rs3827440 were excluded from allele specific analysis to avoid the influence of skewed XCI.

The RNA extraction was carried out using the QIAamp RNA Blood Mini Kit (Qiagen). Total RNA were reverse transcribed using RT-PCR reagents with random hexamers (Promega, Fitchburg, Wisconsin, USA) in accordance with instruction of the manufacturer. Quantitative RT-PCR was performed using SYBR Green (TaKaRa, Otsu, Japan) on an ABI PRISM 7900 Sequence Detector (Applied Biosystems) with SDS V.2.1 software. Each reaction was performed in triplicate, with final

calculations based on the means of triplicate wells. *GAPDH* was used as an endogenous control. Primer sequences used are shown in online supplementary table S3. The ΔΔCq method was used to determine the expression levels of *GPR174* for each sample.[14] Mean threshold cycle (Cq) was calculated for each sample from three replicates and then used to calculate relative expression level (ΔCq), which is the difference between *GPR174* Cq and *GAPDH* Cq. A median ΔCq value in the samples was used as a calibrator and the ΔΔCq was calculated using ΔCq of each sample minus the calibrator. The relative quantity of each sample was calculated using the relative quantification (RQ) formula ($RQ=2^{-\Delta\Delta Cq}$). Distribution of relative gene expression levels was compared among males and females with different genotypes of rs3827440 by unpaired two-tailed Student t tests, respectively. In the combined samples, the difference of expression levels with genotypes was tested using an analysis of variance model adjusted for gender.

Resequencing

The GPR174 gene region was resequenced using the Applied Biosystems 3730 platform (see online supplementary table S4 for primers). We analysed traces using Phred, Phrap and Consed[15 16] and identified variants with Polyphred.[17] The variants identified were confirmed by sequencing the amplicons in both forward and reverse directions.

RESULTS

A cluster of SNPs in strong linkage disequilibrium (LD) showed significant association in our GWAS samples when analysed using either of the two methods (figure 1A and online supplementary table S1). The associated SNPs were located near or within the G protein-coupled receptor 174 (*GPR174*) gene on Xq21.1 (figure 1B). The most significant association signal was observed at rs5912838 ($P_{logistic\ regression}=4.60\times10^{-8}$; $P_{snpMatrix}=1.36\times10^{-9}$; OR=1.80, 95% CI 1.48 to 2.18; see online supplementary table S1), which lies about 155 kb distal to *GPR174*. No significant signals were observed in the previously reported two Graves' disease linkage regions, namely Xq21.33-q22[18 19] and Xp11[20 21]; *GPR174* locates midway between these two regions. Association analysis of imputed genotype data did not provide superior additional associated SNP in this region (see online supplementary table S2). Among the GWAS hits, a non-synonymous SNP rs3827440 ($r^2=0.93$ to rs5912838) within *GPR174* was an obvious functional variant of interest, though not presenting the top p value ($P_{logistic\ regression}=9.52\times10^{-8}$; $P_{snpMatrix}=4.60\times10^{-9}$; OR=1.76, 95% CI 1.45 to 2.13; figure 1B and table 1). Subsequently, rs3827440 was genotyped in an additional sample of 4564 Graves' disease cases and 3968 sex matched controls. The association of rs3827440 to Graves' disease was confirmed in the replication collection set and reached genome-wide significance in the combined analyses (combined $P_{logistic\ regression}=5.53\times10^{-21}$; combined $P_{snpMatrix}=4.26\times10^{-22}$; OR=1.69, 95% CI 1.53 to 1.86; table 1). The OR of rs3827440 across the two sample sets is 1.69 (95% CI 1.53 to 1.86), which is only lower than that of the SNP rs4947296 in the *HLA* gene region (OR=1.77, 95% CI 1.65 to 1.91),[4] establishing *GPR174* as an important locus with regard to the genetic susceptibility to Graves' disease in the Chinese population.

Since the expression profiles of most G protein coupled receptors (GPCRs) are unique, a highly selective tissue expression pattern may provide a clue with respect to receptor function.[22] Therefore, we investigated the expression level of *GPR174* in multiple human tissues/cells. *GPR174* is widely expressed and has especially high expression levels in immune related organs and cells, including spleen, lymph nodes, thymus, tonsil, leucocytes, and bone marrow (figure 2A). Of note, although over 90% of GPCRs are expressed in the brain,[22] no expression of *GPR174* was observed in this organ. Moderate expression of *GPR174*, however, was detected in the thyroid tissue.

SNP rs3827440 is a nucleotide transition (519C>T) in the single exon of *GPR174* that causes the amino acid substitution P162S. Two SNPs, rs3810711 and rs3810712, both being located in the 5′ untranslated region (UTR) of *GPR174*, were in perfect LD with rs3827440 in our re-sequencing data. Real-time RT-PCR analysis revealed a significant correlation between expression levels of *GPR174* in freshly isolated peripheral blood cells (PBCs) and the genotypes of rs3827440. Both female homozygous carriers and male carriers of the risk allele T were associated with a higher level of *GPR174* expression (p=0.009 and p=0.029, respectively). When the combined homozygous and hemizygous T allele carriers were compared with the combined C allele carriers, the difference in expression levels gave a p value of 0.002 (figure 2B). These results suggest that rs3827440 and/or one or more variants in strong LD with rs382440 (eg, rs3810711 and rs3810712 in the 5′UTR) could influence *GPR174* expression, thereby leading to the association with Graves' disease. Of note, the P162S substitution in GPR174 maps to the second extracellular loop region (figure 2C), which is required for ligand recognition and receptor activation,[23] and may therefore alter these activities.

Since genes with sex biased expression are enriched on the sex chromosomes,[24] we also investigated whether *GPR174* has sex biased expression. Although the mRNA level of *GPR174* was slightly higher in the PBCs of females compared to those of males, no significant differences were observed (p=0.54 for TT females vs T males; p=0.09 for CC females vs C males; p=0.08 for combined females vs combined males, respectively).

Recent resequencing studies on complex diseases revealed common alleles of modest effect and rarer alleles with more considerable impact coexisting in the same disease genes.[25] To investigate whether there are rare variants in *GPR174* associated with risk to Graves' disease, we sequenced the exon region and the 5′ as well as 3′ UTRs of *GPR174* in 2608 Graves' disease cases and 2328 controls. We identified 22 novel variants in addition to the three common ones (rs3810711 and rs3810712 in 5′ UTR, and rs3827440, table 2 and figure 2C). All of the 22 variants were very rare (minor allele frequency <0.5%), and none of them was previously listed in the dbSNP database. Of the 16 coding variants, 10 were non-synonymous variants. Although nine rare variant carriers were found among the 1272 female controls, no rare variants were observed in 1,056 male controls. Using a collapsing method,[13] we tested the difference between the proportions of individuals with rare variants in cases and controls. The result suggested an enrichment of rare variants in cases with a p value of 1.16×10^{-3}, but the evidence is not robust.

DISCUSSION

The X chromosome spans about 155 million base pairs and contains more than 1000 genes; however, the X chromosome data have received surprisingly little attention in the wave of GWAS.[5 7 8] Although several models for the X chromosome association analysis have been proposed and assessed, neither the traditional stratification analysis nor the newly developed methods appear to have gained widespread use in GWAS analysis.[7–9] Moreover, the calculation of the ORs for

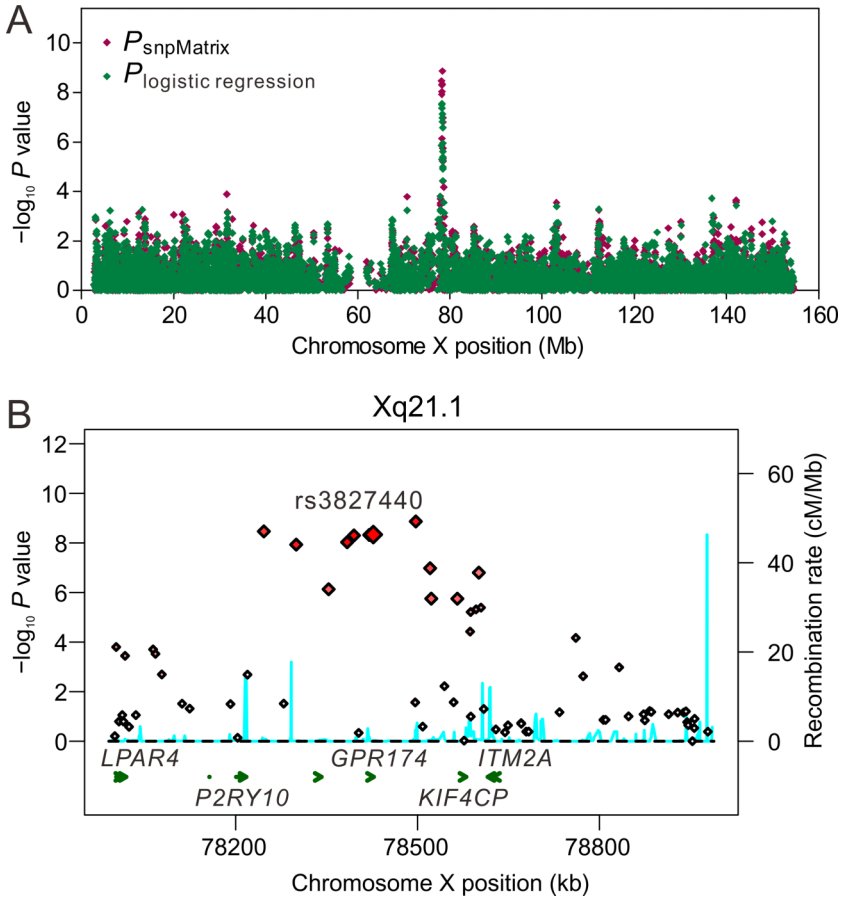

Figure 1 X chromosome-wide association results and regional plot of association results at Xq21.1. (A) X chromosome-wide association results calculated by using two methods. Values of $-\log_{10}$ p are plotted against chromosome positions. Purple and green dots represent p values calculated using Clayton's method by snpMatrix and logistic regression analysis by PLINK, respectively. (B) Association results of single nucleotide polymorphisms (SNPs) in genome-wide association study samples at Xq21.1. p Values were calculated using Clayton's methods. The colour of each genotyped SNP spot reflects its r^2 to rs3827440 (large red diamond), changing from red to white. Genetic recombination rates, estimated by using the HapMap CHB (Han Chinese in Beijing, China) and JPT (Japanese in Tokyo, Japan) samples, are shown in cyan. Physical positions are based on NCBI (National Center for Biotechnology Information) build 37 of the human genome.

X chromosome SNPs in sex mixed case–control studies was often missed in the literature.[5 9] It is worth noting that in case–control studies, the OR is a commonly used statistic for measure of association and risk assessment. In the current study, we used the logistic regression method entering sex as a covariate and the additive method developed by Clayton[6] to reanalyse the X chromosome data from our GWAS for Graves' disease; the two methods gave consistent results, both showing that *GPR174* was associated with disease susceptibility.

GPR174 consists of one exon encoding a protein of 333 amino acids, which belongs to the GPCR superfamily and is grouped into GPCR 1 (or rhodopsin-like) family. These integral membrane proteins are characterised by the presence of seven α-helical transmembrane domains and play important roles in cell signal transduction. To date, more than 50% of the effective drug targets are GPCRs.[26 27] Very recently, lysophosphatidylserine (LysoPS) was found as a ligand for GPR174.[28 29] LysoPS is secreted by the immune system in vivo, and acts a lipid mediator that regulates immune system processes.[29] LysoPS interacting with GPR174 stimulates an increase of intracellular cyclic adenosine monophosphate (cAMP) in a dose dependent manner.[29] cAMP-elevating or cAMP-mimicking agents could inhibit production of the T-helper 1 (Th1) cytokines, whereas production of the Th2 cytokines remains unchanged or even enhanced.[30] It is often considered that Graves' disease is a Th2 disorder. Therefore, the elevated level of cAMP might drive the Th2 polarisation and be involved in the pathogenesis of Graves' disease. This assumption corresponded with our result that the

Table 1 Association results for rs3827440 using two methods

Stage	Sex	No. of cases (%)			No. of controls (%)			p Value		OR (95% CI)*
		TT/T	CC/C	TC	TT/T	CC/C	TC	Logistic regression	snpMatrix	
GWAS	Female	444 (39.8)	163 (14.6)	508 (45.6)	367 (32.6)	219 (19.4)	541 (48.0)			1.63 (1.27 to 2.08)
	Male	232 (68.0)	109 (32.0)		186 (52.0)	172 (48.0)				1.97 (1.45 to 2.68)
	Combined	676 (46.4)	272 (18.7)	508 (34.9)	553 (37.2)	391 (26.3)	541 (36.4)	9.52×10^{-8}	4.60×10^{-9}	1.76 (1.45 to 2.13)
Replication	Female	1298 (38.5)	471 (14.0)	1606 (47.6)	957 (33.2)	584 (20.2)	1344 (46.6)			1.68 (1.45 to 1.95)
	Male	606 (68.1)	284 (31.9)		526 (57.0)	396 (43.0)				1.60 (1.33 to 1.95)
	Combined	1904 (44.6)	755 (17.7)	1606 (37.7)	1483 (39.0)	980 (25.7)	1344 (35.3)	7.76×10^{-15}	1.71×10^{-15}	1.67 (1.48 to 1.87)
Meta-analysis								5.53×10^{-21}	4.26×10^{-22}	1.69 (1.53 to 1.86)

*In females, the ORs were estimated as the ORs of TT genotypes. In males, the ORs were estimated as the per allele ORs. In the mixed sex samples, the ORs were estimated as the ORs of the combined TT and T genotypes.
GWAS, genome-wide association studies.

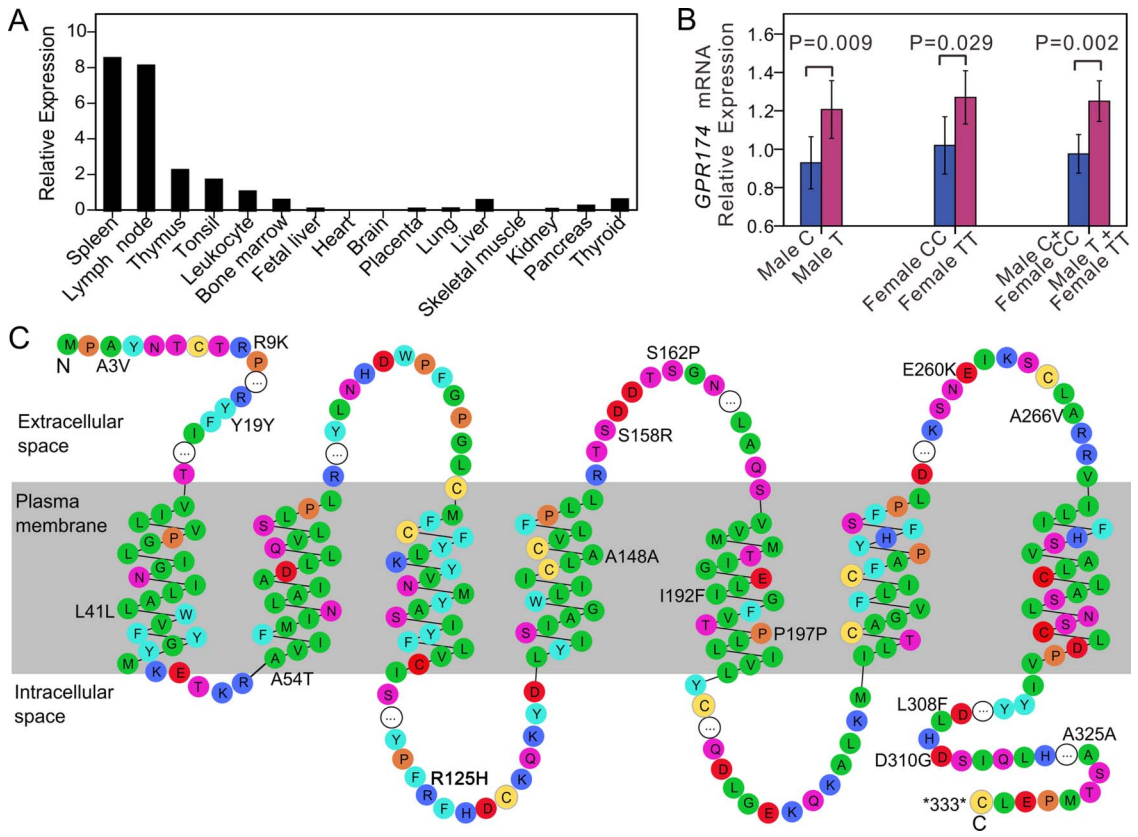

Figure 2 Expression analysis of *GPR174* and distribution of the coding variants in GPR174. (A) Expression profiles of *GPR174* in various human tissues by real-time reverse transcriptase PCR (RT-PCR). We performed real-time quantitative RT-PCR reactions in duplicate and plotted the means. Normalisation for cDNA quantity was performed by comparison with *GAPDH* controls and plotted as arbitrary relative expression units, where the leucocytes' RNA expression level is equal to 1. (B) Relative expression levels of *GPR174* against the distinct genotypes of rs3827440 were measured in peripheral blood cells (PBCs) from 39 males (C, n=20; T, n=19) and 107 females (CC, n=64; TT, n=43). Error bars,±SD. (C) Domain structure of GPR174 protein and the distribution of the coding variants. The structures are based on UniProtKB entry Q9BXC1 and the figure was prepared using RbDe. Amino acid residues are coloured according to residue types (red: acidic; blue: basic; purple: neutral hydrophilic; green: aliphatic; cyan: aromatic; orange: imino; yellow: thiol containing). The white circles represent contiguous stretches of amino acid residues which are omitted from this diagram.

susceptible T allele of rs3827440 was associated with a higher level of *GPR174* expression, since the higher level of *GPR174* expression might elevate the intracellular cAMP concentration. Our RT-PCR analysis showed *GPR174* was widely expressed in immune related tissues and moderately expressed in thyroid. This expression pattern suggested a possible involvement of *GPR174* in immune processes and a potential link of this gene to the thyroid structure/function, which could be crucial in the aetiology of Graves' disease. Graves' disease shares genetic susceptibility factors with other autoimmune diseases such as type 1 diabetes, multiple sclerosis, and rheumatoid arthritis.[4] It is therefore reasonable to address the possibility of an association of *GPR174* with these autoimmune diseases in the future.

XCI skewing or other X chromosome associated abnormalities might contribute to disturbances in self reaction and ultimately to autoimmunity, which might enhance the susceptibility of the female sex to autoimmune disease including Graves' disease. Several hypotheses have been proposed to explain the mechanisms.[31] The loss of mosaicism hypothesis postulated that autoreactive T cells may fail to be tolerated by self-antigens encoded by one of the two X chromosomes, and these autoreactive T cells may stimulate B cells and induce autoimmunity in the periphery.[32] A high prevalence of skewed XCI in females with Graves' disease supports this hypothesis.[33–35] The haploinsufficiency hypothesis states that haploinsufficiency for X-linked genes results in some autoimmune disorders.[31 36] The observation that women with Turner's syndrome (loss of one X chromosome; X0) have an increased risk of developing autoimmune thyroid disease including Graves' disease corroborated this hypothesis.[37] Other evidence supporting this hypothesis is the higher rate of circulating cells with X chromosome monosomy that were found in females with Graves' disease and other autoimmune disorders.[36] Further study is required to clarify whether *GPR174* is involved in the X chromosome-specific abnormalities or whether *GPR174* plays a role in the pathogenesis of Graves' disease via the above mechanisms.

Although skewing of XCI or X chromosome associated abnormalities might play a role in female preponderance, it is unlikely that variation in a single gene would be responsible for the higher susceptibility of females to Graves' disease. If this was the case, we would expect a higher frequency of autoimmune diseases in males. The risks in males and females derived from genotype distribution of rs3827440 (table 1) support minimal female:male difference in risk. Specifically, using the larger replication cohort, we obtained relative risks (RRs) of 1.60 for the T allele in males, and RRs of 1.68 and 1.48 for the TT and TC genotypes in females, respectively. These RRs gave an attributable risk of 26% in males and 31% in females, attributable to this polymorphism—that is, nearly no difference. In fact, using both the original and replication data

Table 2 Rare variants in GPR174 indentified in 2608 cases and 2328 controls from resequencing

Location	Nucleotide	Amino acid	Number of variant carriers			
			Female cases	Male cases	Female controls	Male controls
5′UTR	C-20T	/	7†	1	1	0
5′UTR	T-17C	/	1	0	0	0
Exon	C8T	Ala3Val	1	0	0	0
Exon	G26A	Arg9Lys	1	0	0	0
Exon	C57T	Tyr19Tyr	1	0	0	0
Exon	C121T	Leu41Leu	1	0	0	0
Exon	G160A	Ala54Thr	1	0	0	0
Exon	G374A	Arg125His	0	1	0	0
Exon	C444T	Ala148Ala	5†	2	1	0
Exon	T474A	Ser158Arg	0	0	1	0
Exon	A574T	Ile192Phe	1	0	0	0
Exon	G591A	Pro197Pro	1	1	0	0
Exon	G778A	Glu260Lys	0	0	1	0
Exon	C797T	Ala266Val	0	0	1	0
Exon	G924T	Leu308Phe	0	1	0	0
Exon	A929G	Asp310Gly	1	0	0	0
Exon	A975G	Ala325Ala	1	0	2	0
Exon	A1001G	*333*	1†	0	0	0
3′UTR	C1082A	/	1	0	0	0
3′UTR	G1132A	/	1	0	0	0
3′UTR	T1184C	/	0	0	1	0
3′UTR	C1194T	/	0	1	1	0

Female cases, n=1830; male cases, n=778; female controls, n=1272; male controls, n=1056.
*Represents a stop codon.
†The number includes one female carrying a homozygous rare variant.
UTR, untranslated region.

together provides even less difference. Therefore, the genotype distributions of rs3827440 explain little of the difference in Graves' disease prevalence between males and females in the Chinese population.

In conclusion, the identification of *GPR174* as a risk factor for Graves' disease resulted from an extended analysis of the X chromosome data. Exploring the X chromosome data in GWAS studies more carefully and making full use of the sample would help to reveal the X-linked loci with susceptibility to complex disease. This study has not only identified a new X chromosome risk locus for Graves' disease but also suggests the X chromosome targets for study in other autoimmune diseases.

Useful websites

- snpMatrix, http://bioc.ism.ac.jp/2.8/bioc/html/snpMatrix.html
- R statistical environment V.2.13.2, http://www.r-project.org/
- PLINK v1.07, http://pngu.mgh.harvard.edu/~purcell/plink/
- IMPUTE2, http://mathgen.stats.ox.ac.uk/impute/impute_v2.html
- SNPTEST v2, https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html.

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Table S1 Genotypes and association results for SNPs at Xq21.1 in the initial genome-wide scan

SNP	Position	Reference allele	Female cases	Male cases	Female controls	Male controls	P value		OR(95%CI)
			AA/Aa/aa	AA/aa	AA/Aa/aa	AA/aa	logistic regression	snpMatrix	
rs1000530	78,010,734	T	765 / 310 / 41	290/58	729 / 340 / 58	297/64	2.71E-02	4.84E-02	1.27(0.96- 1.67)
rs5912686	78,078,046	G	790 / 292 / 34	300/49	756 / 324 / 46	302/60	2.57E-02	3.17E-02	1.32(0.98- 1.78)
rs2858575	78,089,897	C	962 / 152 / 2	326/24	974 / 149 / 4	334/29	8.47E-01	7.25E-01	1.25(0.74- 2.10)
rs5959212	78,106,116	G	933 / 173 / 10	323/23	886 / 227 / 14	328/34	1.23E-03	2.06E-03	1.50(0.96- 2.36)
rs11799022	78,133,128	A	406 / 508 / 202	223/118	321 / 550 / 256	177/183	6.69E-08	3.42E-09	1.73(1.44- 2.09)
rs5912206	78,165,867	C	922 / 187 / 7	321/28	900 / 211 / 16	325/37	2.68E-02	3.04E-02	1.54(1.00- 2.37)
rs5959225	78,186,487	C	441 / 505 / 169	233/109	363 / 543 / 221	189/171	2.00E-07	1.17E-08	1.72(1.42- 2.08)
rs5912749	78,239,990	A	580 / 441 / 95	261/82	513 / 489 / 125	226/135	9.61E-06	7.38E-07	1.67(1.35- 2.07)
rs2411976	78,270,514	A	449 / 506 / 161	233/109	370 / 539 / 217	191/169	1.21E-07	9.36E-09	1.74(1.44- 2.10)
rs2411975	78,281,726	T	448 / 507 / 161	232/109	368 / 542 / 217	189/171	7.84E-08	4.99E-09	1.75(1.45- 2.12)
rs17317518	78,289,574	T	1057 / 58 / 1	338/12	1068 / 57 / 2	356/7	6.75E-01	4.61E-01	0.68(0.29- 1.59)
rs5912785	78,306,987	T	444 / 508 / 164	232/109	366 / 541 / 220	187/172	9.20E-08	4.77E-09	1.76(1.45- 2.12)
rs3827440	78,313,644	G	444 / 508 / 163	232/109	367 / 541 / 219	186/172	9.52E-08	4.60E-09	1.76(1.45- 2.13)
rs5959276	78,382,774	T	921 / 187 / 8	324/24	904 / 208 / 15	325/37	3.69E-02	2.72E-02	1.65(1.05- 2.57)
rs5912838	78,383,774	C	447 / 512 / 157	235/106	369 / 546 / 212	186/173	4.60E-08	1.36E-09	1.80(1.48- 2.18)
rs2411961	78,395,137	A	1050 / 64 / 2	339/11	1068 / 57 / 2	356/7	3.25E-01	2.57E-01	0.68(0.29- 1.58)
rs17324573	78,407,358	A	534 / 460 / 122	250/94	449 / 516 / 162	215/144	6.54E-07	1.05E-07	1.67(1.37- 2.05)
rs10521391	78,409,291	T	495 / 466 / 154	235/108	413 / 522 / 192	206/153	6.08E-06	1.77E-06	1.55(1.28- 1.88)
rs12012447	78,430,997	A	950 / 157 / 9	322/27	908 / 207 / 12	327/35	3.48E-03	6.03E-03	1.34(0.87- 2.09)
rs1736673	78,446,191	C	888 / 208 / 20	305/40	840 / 264 / 23	318/44	1.11E-02	2.68E-02	1.15(0.80- 1.65)
rs5912878	78,452,318	G	546 / 467 / 103	256/84	500 / 485 / 142	218/142	3.81E-05	1.78E-06	1.70(1.37- 2.09)
rs1736661	78,463,483	G	1041 / 72 / 3	334/14	1046 / 77 / 4	351/12	7.91E-01	9.46E-01	0.93(0.47- 1.84)
rs5959303	78,473,400	G	525 / 477 / 114	252/88	488 / 498 / 141	216/144	6.21E-04	3.78E-05	1.56(1.27- 1.92)

Table S1 continued

rs6619915	78,474,243	A	283 / 545 / 287	168/171	335 / 566 / 226	219/140	2.45E-05	6.12E-06	0.65(0.54- 0.78)
rs13441063	78,474,451	C	806 / 284 / 26	285/59	770 / 327 / 30	298/64	6.32E-02	1.01E-01	1.13(0.83- 1.53)
rs1736646	78,483,152	C	521 / 482 / 113	248/91	474 / 514 / 139	208/152	1.33E-04	4.78E-06	1.61(1.31- 1.98)
rs1751107	78,487,758	C	385 / 539 / 189	211/129	322 / 565 / 240	169/191	2.49E-06	1.58E-07	1.65(1.36- 1.99)
rs1751105	78,491,316	C	292 / 559 / 264	166/175	349 / 565 / 213	220/139	2.46E-05	4.07E-06	0.65(0.54- 0.78)
rs1040408	78,495,923	G	919 / 186 / 11	317/32	894 / 218 / 15	323/40	4.32E-02	5.00E-02	1.30(0.86- 1.95)
rs2056918	78,515,834	C	899 / 201 / 16	317/31	892 / 216 / 19	325/36	3.23E-01	3.34E-01	1.17(0.79- 1.74)
rs5912919	78,530,923	A	969 / 143 / 4	323/27	958 / 158 / 11	340/23	2.36E-01	4.37E-01	1.09(0.67- 1.79)
rs1474563	78,535,849	T	765 / 320 / 31	288/58	743 / 337 / 47	303/58	1.26E-01	2.28E-01	1.19(0.88- 1.60)
rs5959348	78,557,496	A	765 / 320 / 31	288/58	742 / 337 / 48	302/59	1.02E-01	1.86E-01	1.21(0.90- 1.63)
rs5959349	78,557,743	G	765 / 320 / 31	287/59	741 / 338 / 48	302/59	1.03E-01	1.97E-01	1.20(0.89- 1.61)
rs5912942	78,565,893	A	970 / 142 / 4	323/27	958 / 158 / 11	340/23	2.15E-01	4.10E-01	1.09(0.67- 1.79)
rs5959353	78,569,995	C	901 / 199 / 16	317/31	896 / 212 / 19	326/35	3.89E-01	4.10E-01	1.15(0.77- 1.71)
rs1120643	78,620,799	T	1006 / 106 / 4	339/11	1004 / 118 / 5	340/23	1.39E-01	6.77E-02	1.87(0.99- 3.51)
rs5958896	78,647,702	G	541 / 462 / 112	242/99	489 / 503 / 135	214/146	4.65E-04	6.78E-05	1.48(1.21- 1.82)
rs5912953	78,659,755	T	624 / 415 / 77	258/83	595 / 439 / 93	239/121	1.06E-02	2.39E-03	1.41(1.13- 1.77)
rs7059686	78,693,245	C	874 / 221 / 21	313/35	854 / 259 / 14	316/47	1.81E-01	1.38E-01	1.11(0.76- 1.60)
rs11796452	78,696,855	C	874 / 221 / 21	313/35	854 / 259 / 14	316/47	1.81E-01	1.38E-01	1.11(0.76- 1.60)
rs5959388	78,719,264	C	310 / 575 / 231	191/147	310 / 531 / 286	160/200	7.00E-03	1.05E-03	1.37(1.14- 1.65)
rs1353452	78,734,728	T	873 / 223 / 20	310/38	843 / 270 / 14	316/47	9.67E-02	9.93E-02	1.07(0.74- 1.55)
rs1496186	78,759,538	C	871 / 224 / 21	311/37	841 / 270 / 16	316/47	8.35E-02	8.19E-02	1.11(0.77- 1.60)
rs12014367	78,761,577	T	878 / 217 / 20	315/33	859 / 254 / 14	319/44	1.78E-01	1.44E-01	1.11(0.76- 1.62)
rs1496210	78,769,541	T	871 / 224 / 21	311/37	839 / 272 / 16	315/48	6.40E-02	6.06E-02	1.13(0.79- 1.63)
rs12012345	78,772,193	A	871 / 224 / 21	311/37	838 / 273 / 16	316/47	6.39E-02	6.56E-02	1.11(0.77- 1.60)
rs5959408	78,800,927	G	277 / 585 / 254	173/166	329 / 517 / 281	208/150	1.74E-01	8.15E-02	0.86(0.72- 1.03)

Table S1 continued

rs5958916	78,815,765	A	434 / 532 / 150	218/122	456 / 486 / 185	199/159	1.99E-01	6.96E-02	1.26(1.04- 1.53)
rs2132476	78,825,632	T	434 / 531 / 151	218/122	456 / 485 / 186	199/159	2.00E-01	6.99E-02	1.26(1.04- 1.52)
rs1353456	78,829,377	C	433 / 533 / 150	218/122	455 / 487 / 185	198/160	1.90E-01	6.26E-02	1.26(1.04- 1.53)
rs5913021	78,831,415	T	666 / 384 / 65	269/72	682 / 378 / 67	251/104	4.97E-01	1.70E-01	1.25(0.98- 1.59)
rs5959428	78,833,078	G	425 / 541 / 150	217/123	457 / 505 / 165	197/163	6.15E-01	2.24E-01	1.18(0.97- 1.43)
rs7063238	78,840,039	C	810 / 285 / 21	297/50	828 / 275 / 24	307/56	8.88E-01	9.97E-01	1.10(0.79- 1.53)
rs5912329	78,843,061	G	423 / 540 / 150	206/123	457 / 502 / 166	194/163	7.02E-01	2.94E-01	1.16(0.96- 1.41)
rs5912331	78,843,683	T	667 / 384 / 65	271/72	681 / 379 / 67	253/107	4.25E-01	1.25E-01	1.28(1.00- 1.62)
rs1948538	78,865,535	A	855 / 243 / 18	300/44	868 / 237 / 22	328/35	6.19E-01	4.12E-01	0.89(0.61- 1.28)
rs2898803	78,904,750	C	854 / 244 / 18	300/44	867 / 238 / 22	328/35	6.19E-01	4.12E-01	0.89(0.61- 1.28)
rs3127143	78,948,815	T	468 / 502 / 146	220/117	492 / 476 / 159	218/142	8.05E-01	5.36E-01	1.11(0.91- 1.35)
rs4596772	78,989,702	G	854 / 244 / 18	298/47	866 / 238 / 23	328/35	5.93E-01	3.41E-01	0.86(0.60- 1.24)
rs2263534	78,990,685	T	854 / 244 / 18	298/47	865 / 239 / 23	328/35	6.22E-01	3.59E-01	0.86(0.60- 1.24)

Table S2. Association results of the imputed and typed SNPs in the region from 78Mb to 79Mb on X chromosome in initial genome-wide scan

SNP	Position	allele_A	allele_B	*p* value	Certainty	SNP Type
rs6615046	78000649	T	C	5.64E-01		Typed
rs4573413	78002855	A	C	1.61E-04		Typed
rs5912638	78007456	C	T	1.53E-01		Typed
rs5959189	78012894	T	C	8.28E-02		Typed
rs5959190	78014946	T	G	1.53E-01		Typed
rs3123295	78017518	A	G	4.14E-04		Typed
rs188727915	78018101	G	A	1.20E-01	0.999	Imputed
rs3132267	78024086	G	A	2.57E-01		Typed
rs181648847	78026553	A	G	1.70E-01	0.999	Imputed
rs144459946	78027795	A	G	5.90E-01	0.998	Imputed
rs183207722	78027869	T	A	5.80E-01	0.999	Imputed
rs144703370	78031292	C	T	6.38E-01	0.999	Imputed
rs150983838	78034163	C	T	5.00E-01	0.993	Imputed
rs5912644	78035351	C	T	8.46E-02		Typed
rs149129349	78041466	G	A	5.97E-01	0.996	Imputed
rs149855931	78050311	G	A	5.58E-01	0.998	Imputed
rs144488820	78054496	G	T	5.00E-01	0.998	Imputed
rs138005259	78056540	G	A	6.48E-01	0.998	Imputed
rs147620443	78057557	T	G	7.50E-01	0.998	Imputed
rs4263894	78063755	C	T	2.64E-04		Typed
rs5912181	78067644	A	G	3.60E-04		Typed
rs5959200	78068751	T	C	7.11E-01	0.996	Imputed
rs143866491	78075623	T	C	4.00E-01	0.998	Imputed
rs17324447	78078155	G	A	2.59E-03		Typed
rs189224654	78078836	G	C	6.41E-01	0.999	Imputed
rs72629930	78079960	T	C	5.00E-01	0.998	Imputed
rs192271545	78081799	G	A	3.20E-01	0.997	Imputed
rs181057598	78085205	C	A	4.03E-01	0.998	Imputed
rs189830243	78091038	G	A	3.93E-01	0.998	Imputed
rs185538709	78104417	T	C	9.95E-01	0.998	Imputed
rs146418470	78108351	T	A	5.83E-01	0.998	Imputed
rs12687690	78111578	G	T	3.05E-02		Typed
rs142317058	78114560	A	G	4.86E-01	0.996	Imputed
rs188799060	78116172	G	A	5.00E-01	0.997	Imputed
rs148960869	78119036	A	G	4.93E-01	0.996	Imputed
rs193041002	78120258	T	A	1.34E-01	0.999	Imputed
rs150002139	78138612	A	G	7.32E-01	0.999	Imputed
chrX:78142028:D	78142028	TAA	T	7.31E-01	0.999	Imputed
rs188797500	78150777	C	T	3.90E-01	0.998	Imputed
rs149520185	78152160	T	G	7.00E-01	0.998	Imputed
rs184828121	78164363	A	G	1.95E-01	0.998	Imputed
rs138365450	78164621	G	A	3.92E-01	0.993	Imputed

Table S2 continued

rs140963568	78170957	C	G	1.90E-01	0.997	Imputed
rs184241654	78177922	C	T	1.50E-01	0.997	Imputed
rs185627711	78182768	C	T	1.48E-01	0.999	Imputed
rs73231515	78191476	T	G	6.17E-01	0.998	Imputed
rs73231521	78213160	A	G	9.66E-01	0.999	Imputed
rs7881963	78219965	C	T	3.48E-02	0.997	Imputed
rs138941982	78220459	T	C	6.73E-01	0.999	Imputed
rs189091056	78222266	C	T	6.72E-01	0.999	Imputed
rs143329489	78224827	G	A	7.16E-02	0.998	Imputed
rs148329543	78230529	G	A	8.20E-01	0.999	Imputed
rs190657482	78231140	C	A	7.31E-01	0.999	Imputed
rs143659907	78234313	G	A	4.75E-01	0.997	Imputed
rs187331328	78238354	G	A	7.19E-01	0.999	Imputed
rs188340660	78239999	T	A	8.11E-01	0.999	Imputed
rs140070509	78250785	A	C	5.71E-01	0.998	Imputed
rs57129566	78255102	G	A	2.82E-01	0.999	Imputed
rs186812447	78255419	C	T	2.82E-01	0.999	Imputed
rs148171982	78255784	C	G	4.94E-01	0.999	Imputed
rs140760381	78258977	C	T	5.54E-01	0.998	Imputed
rs149665188	78265817	T	C	3.37E-01	0.999	Imputed
rs192353435	78266528	G	C	2.95E-01	0.999	Imputed
rs182740304	78274298	G	A	3.35E-01	0.999	Imputed
rs180734908	78275658	A	T	2.81E-01	0.999	Imputed
rs187254974	78276737	C	G	1.85E-01	1	Imputed
rs185573642	78278561	C	G	3.33E-01	0.999	Imputed
rs191651092	78283651	G	T	3.14E-01	0.999	Imputed
rs148727732	78286932	C	T	3.13E-01	0.999	Imputed
rs187444242	78288081	A	T	3.13E-01	0.999	Imputed
rs72629944	78288180	G	A	2.52E-01	0.998	Imputed
rs190881797	78296287	A	G	3.19E-01	0.999	Imputed
rs147340214	78299541	A	T	4.57E-02	0.999	Imputed
rs73231549	78303333	C	G	2.43E-01	0.999	Imputed
rs185796661	78312015	G	C	4.34E-02	0.999	Imputed
rs146655820	78312822	T	C	2.00E-01	0.996	Imputed
rs188887819	78314041	G	T	6.80E-01	0.999	Imputed
rs62606389	78316933	G	A	9.00E-01	0.992	Imputed
rs190114811	78317674	G	T	3.16E-01	0.999	Imputed
rs181435120	78318133	C	A	7.10E-01	0.998	Imputed
rs190846109	78320484	T	C	1.56E-01	0.999	Imputed
rs185296111	78327630	C	T	3.24E-01	0.999	Imputed
rs182233609	78343217	G	A	3.44E-01	0.999	Imputed
rs34758665	78353858	G	T	4.67E-01	0.999	Imputed
rs5912221	78358481	C	T	4.10E-01	0.997	Imputed
rs190915051	78359428	C	T	1.64E-01	0.999	Imputed
rs73231580	78372734	T	G	7.50E-02	0.998	Imputed

Table S2 continued

rs147941514	78372794	T	A	4.35E-01	0.999	Imputed
rs187093575	78384275	T	A	1.81E-01	1	Imputed
rs150073320	78387464	G	T	4.66E-01	0.999	Imputed
rs191377591	78393529	A	T	2.06E-01	0.999	Imputed
rs143137690	78400383	A	C	4.63E-01	0.999	Imputed
rs140936915	78413332	G	C	6.36E-01	0.999	Imputed
rs148416472	78416649	T	C	4.34E-01	0.999	Imputed
rs73231593	78419050	A	G	6.05E-02	0.999	Imputed
rs189033609	78420636	A	G	2.45E-01	0.999	Imputed
rs57481284	78423568	T	A	3.87E-01	0.999	Imputed
rs144830132	78425210	T	G	4.85E-01	0.999	Imputed
rs75956723	78426987	C	T	3.87E-01	0.999	Imputed
rs5958866	78436002	T	A	6.10E-01	0.998	Imputed
rs138288213	78439522	C	T	2.41E-01	0.999	Imputed
rs191687323	78439541	A	T	2.41E-01	0.999	Imputed
rs145514745	78445635	A	G	6.36E-01	0.999	Imputed
rs182770538	78448112	G	C	2.41E-01	0.999	Imputed
rs187511107	78454007	A	G	2.42E-01	0.999	Imputed
rs150381154	78456901	T	A	3.90E-01	0.999	Imputed
rs146377235	78462805	G	A	3.91E-01	0.999	Imputed
rs73233212	78468118	G	A	7.44E-02	0.999	Imputed
rs143829941	78471626	G	A	5.91E-01	0.999	Imputed
rs111995577	78471674	G	A	3.00E-01	0.998	Imputed
rs150064797	78482697	A	C	8.55E-02	0.999	Imputed
rs142095275	78488882	C	T	4.15E-01	0.998	Imputed
rs147611325	78490443	A	G	5.24E-02	0.998	Imputed
rs60379437	78490817	A	G	3.91E-01	0.999	Imputed
rs140931888	78493650	G	A	5.93E-01	0.999	Imputed
rs182983019	78495759	A	C	2.01E-01	0.994	Imputed
rs73233247	78496560	A	C	2.29E-01	0.999	Imputed
rs186370278	78507296	A	G	5.59E-01	0.995	Imputed
rs146560034	78511843	T	C	4.11E-01	0.999	Imputed
rs73233250	78515145	G	A	2.40E-01	0.999	Imputed
rs186637862	78516085	C	A	1.78E-01	0.999	Imputed
rs58706188	78517078	T	G	4.22E-01	0.999	Imputed
rs140525813	78519240	C	T	4.31E-01	0.998	Imputed
rs145336014	78523438	A	T	4.34E-01	0.999	Imputed
rs181057281	78525563	A	G	5.77E-01	0.999	Imputed
rs183798512	78525813	A	G	3.48E-01	1	Imputed
rs12012373	78536949	G	A	4.55E-01	0.999	Imputed
rs186641652	78539379	G	A	1.77E-01	0.999	Imputed
rs112855917	78543023	G	A	4.40E-01	0.998	Imputed
rs142181626	78543064	G	C	4.89E-01	0.999	Imputed
rs187291047	78552400	G	T	7.59E-01	0.998	Imputed
rs1736676	78554569	A	T	1.52E-01	0.997	Imputed

Table S2 continued

rs1736675	78554835	A	T	1.50E-01	0.997	Imputed
rs6522917	78557384	G	A	1.52E-01	0.997	Imputed
rs1751115	78560986	A	C	1.65E-01	0.997	Imputed
rs112394313	78561560	A	G	3.90E-01	0.998	Imputed
chrX:78563688:I	78563688	C	CA	1.94E-01	0.999	Imputed
rs148183187	78565266	G	A	1.55E-01	0.999	Imputed
rs146806132	78567066	C	T	2.00E-01	0.995	Imputed
rs2205808	78567988	A	G	3.80E-01	0.997	Imputed
rs190017058	78568337	A	C	1.52E-01	0.999	Imputed
rs182993003	78568372	T	C	1.47E-01	1	Imputed
rs1736665	78569128	G	A	1.00E-01	0.997	Imputed
rs112425994	78571489	G	A	4.51E-02	0.999	Imputed
rs12008051	78575514	G	A	4.17E-01	0.999	Imputed
rs1622968	78575791	T	A	1.47E-01	0.997	Imputed
rs1008567	78576095	A	G	1.00E-01	0.997	Imputed
rs184964496	78580884	G	A	4.40E-01	0.999	Imputed
rs6652327	78588457	C	T	2.06E-01	0.998	Imputed
rs57119575	78589112	A	G	2.20E-01	0.998	Imputed
rs140034494	78590075	C	T	3.06E-01	0.999	Imputed
rs181268854	78590373	G	T	3.07E-01	0.999	Imputed
rs141000792	78590374	G	T	2.26E-01	0.998	Imputed
rs182816814	78595626	T	C	9.14E-02	0.998	Imputed
rs138773248	78595830	G	C	2.25E-01	0.998	Imputed
rs146063681	78600763	C	T	2.79E-01	0.999	Imputed
rs193235884	78604057	C	T	1.08E-01	0.998	Imputed
rs150405188	78607259	C	A	6.79E-02	0.998	Imputed
rs142652405	78607839	A	T	6.49E-02	0.998	Imputed
rs181375019	78617580	G	A	1.20E-01	0.996	Imputed
rs145573290	78619199	A	G	5.50E-02	0.998	Imputed
rs147509745	78621192	A	G	4.79E-02	0.998	Imputed
rs182270935	78629183	G	T	2.87E-01	1	Imputed
rs142644914	78630328	T	A	6.00E-01	0.999	Imputed
rs186828020	78630957	G	A	5.00E-01	0.998	Imputed
rs12008882	78636781	T	A	1.09E-01	0.997	Imputed
rs55634143	78638058	C	A	1.09E-01	0.997	Imputed
rs7065711	78641381	G	A	8.96E-02	0.998	Imputed
rs16979234	78643846	A	G	1.21E-01	0.997	Imputed
rs73630304	78644967	C	T	1.23E-01	0.997	Imputed
rs144797567	78646124	C	G	9.12E-02	0.998	Imputed
rs141016332	78646514	A	G	9.11E-02	0.998	Imputed
rs143120918	78648073	C	T	8.92E-02	0.998	Imputed
rs185784362	78649875	C	A	1.30E-01	0.999	Imputed
rs7049731	78649882	T	C	1.20E-01	0.997	Imputed
rs12011129	78651604	A	G	1.18E-01	0.997	Imputed
rs12015087	78651925	G	C	1.17E-01	0.997	Imputed

Table S2 continued

rs12012232	78652059	T	C	8.49E-02	0.998	Imputed
rs7890116	78653712	G	A	8.41E-02	0.998	Imputed
rs7880446	78654031	A	G	8.39E-02	0.998	Imputed
rs181218133	78654097	G	T	1.31E-01	0.999	Imputed
rs67515309	78655806	T	C	1.16E-01	0.997	Imputed
rs55962854	78655881	C	T	1.16E-01	0.997	Imputed
rs144117435	78657776	G	A	1.17E-01	0.999	Imputed
rs55999824	78659523	G	T	1.20E-01	0.997	Imputed
rs116812070	78665131	C	T	1.19E-01	0.999	Imputed
rs55982355	78666224	G	A	1.29E-01	0.997	Imputed
rs182920109	78667796	C	T	2.87E-01	1	Imputed
rs6652734	78670461	G	A	1.27E-01	0.997	Imputed
rs147070784	78671405	G	A	1.00E-01	0.998	Imputed
rs58274731	78671810	T	C	1.26E-01	0.997	Imputed
rs2205675	78674839	C	T	1.23E-01	0.997	Imputed
rs150916921	78678879	C	T	1.14E-01	0.999	Imputed
rs141656198	78683164	C	G	1.29E-01	0.999	Imputed
rs114687936	78684111	C	T	1.15E-01	0.999	Imputed
rs115171060	78686069	T	C	1.16E-01	0.999	Imputed
rs183360582	78687339	C	T	2.86E-01	1	Imputed
rs2411895	78687466	G	A	1.14E-01	0.997	Imputed
rs150455921	78698403	G	C	6.27E-01	0.998	Imputed
rs183114030	78718017	A	T	8.13E-01	0.999	Imputed
rs144758609	78720145	T	C	4.18E-01	0.995	Imputed
rs186204017	78720620	C	A	4.00E-01	0.999	Imputed
rs1588835	78737104	A	G	2.00E-01	0.996	Imputed
rs148470936	78778026	T	A	1.39E-01	0.998	Imputed
rs140163495	78786856	C	A	1.90E-01	0.997	Imputed
rs147109787	78803446	A	T	4.50E-01	0.997	Imputed
rs4590575	78806968	G	T	4.00E-04	0.901	Imputed
rs1554088	78827778	C	A	3.17E-01	0.998	Imputed
rs112702388	78848264	C	T	1.06E-01	0.999	Imputed
rs147010403	78856415	A	G	4.98E-02	0.998	Imputed
rs189901332	78875057	G	A	2.30E-01	0.993	Imputed
rs139958324	78875684	T	A	4.57E-01	0.999	Imputed
rs144926270	78876459	T	A	4.57E-01	0.999	Imputed
rs183851017	78876465	A	G	4.57E-01	0.999	Imputed
rs149516430	78878192	C	G	4.57E-01	0.999	Imputed
rs11798519	78881800	G	A	3.54E-01	0.999	Imputed
rs141216595	78885465	T	A	5.29E-01	0.999	Imputed
rs140904305	78886434	A	T	5.41E-01	0.999	Imputed
rs144579391	78904712	G	A	9.80E-01	0.999	Imputed
rs138649072	78917998	C	T	3.40E-01	0.998	Imputed
rs148000831	78922639	T	A	2.29E-01	0.993	Imputed
rs182189643	78926924	C	T	8.10E-01	0.998	Imputed

Table S2 continued

rs150556097	78935223	G	T	9.90E-01	0.999	Imputed
rs5958923	78950061	G	A	2.00E-01	0.994	Imputed
rs141274994	78979930	T	C	4.00E-01	0.998	Imputed
rs143133242	78988784	G	A	6.10E-01	0.998	Imputed
rs1000530	78124078	T	C	4.98E-02		Typed
rs5912686	78191390	A	G	3.04E-02		Typed
rs2858575	78203241	T	G	7.91E-01		Typed
rs5959212	78219460	A	G	2.63E-03		Typed
rs11799022	78246472	A	C	3.75E-09		Typed
rs5912206	78279211	T	C	3.37E-02		Typed
rs5959225	78299831	T	C	1.38E-08		Typed
rs5912749	78353334	G	A	1.09E-06		Typed
rs2411976	78383858	C	T	1.07E-08		Typed
rs2411975	78395070	C	A	6.15E-09		Typed
rs17317518	78402918	C	T	3.83E-01		Typed
rs5912785	78420331	C	T	5.68E-09		Typed
rs3827440	78426988	T	C	5.53E-09		Typed
rs5959276	78496118	G	T	3.13E-02		Typed
rs5912838	78497118	A	C	1.67E-09		Typed
rs2411961	78508481	C	T	1.99E-01		Typed
rs17324573	78520702	C	A	1.21E-07		Typed
rs10521391	78522635	C	T	2.10E-06		Typed
rs12012447	78544341	G	A	7.18E-03		Typed
rs1736673	78559535	G	T	3.91E-02		Typed
rs5912878	78565662	A	G	2.71E-06		Typed
rs1736661	78576827	C	T	8.71E-01		Typed
rs5959303	78586744	T	G	5.31E-05		Typed
rs6619915	78587587	G	A	5.71E-06		Typed
rs13441063	78587795	T	C	1.43E-01		Typed
rs1736646	78596496	G	A	7.71E-06		Typed
rs1751107	78601102	G	A	2.01E-07		Typed
rs1751105	78604660	A	G	3.76E-06		Typed
rs1040408	78609267	T	C	6.21E-02		Typed
rs2056918	78629178	T	C	3.54E-01		Typed
rs5912919	78644267	C	A	4.79E-01		Typed
rs1474563	78649193	C	T	2.53E-01		Typed
rs5959348	78670840	C	A	2.07E-01		Typed
rs5959349	78671087	T	G	2.19E-01		Typed
rs5912942	78679237	G	A	4.52E-01		Typed
rs5959353	78683339	T	C	4.35E-01		Typed
rs1120643	78734143	C	T	7.74E-02		Typed
rs5958896	78761046	G	A	1.06E-04		Typed
rs5912953	78773099	C	T	3.69E-03		Typed
rs7059686	78806589	T	C	1.65E-01		Typed
rs11796452	78810199	T	C	1.65E-01		Typed

Table S2 continued

rs5959388	78832608	C	T	1.10E-03	Typed
rs1353452	78848072	A	C	1.21E-01	Typed
rs1496186	78872882	C	T	9.89E-02	Typed
rs12014367	78874921	C	T	1.79E-01	Typed
rs1496210	78882885	A	G	7.41E-02	Typed
rs12012345	78885537	G	A	7.94E-02	Typed
rs5959408	78914271	A	G	8.09E-02	Typed
rs5958916	78929109	A	C	7.84E-02	Typed
rs2132476	78938976	T	G	7.87E-02	Typed
rs1353456	78942721	C	A	7.08E-02	Typed
rs5913021	78944759	C	T	1.99E-01	Typed
rs5959428	78946422	G	A	2.51E-01	Typed
rs7063238	78953383	T	C	9.07E-01	Typed
rs5912329	78956405	A	G	3.53E-01	Typed
rs5912331	78957027	C	T	1.56E-01	Typed
rs1948538	78978879	C	T	2.96E-01	Typed

Table S3. Primers used for quantitative real-time PCR assays of *GPR174* and *GAPDH*

Genes	Primers	Sequences
GPR174	Forward	5'-TTGCATGACAGCATCCAACT-3'
	Reverse	5'-AAGTTCTTCCCTGTGGCTTG-3'
GAPDH	Forward	5'-AAGGTCGGAGTCAACGGATT-3'
	Reverse	5'-CTCCTGGAAGATGGTGATGG-3'

Table S4. Primers used for resequencing of *GPR174*

Fragment	Primers	Sequences
GPR174_1	Forward	5’- gtcccagagggccttaaaat -3’
	Reverse	5’- TACACAGGCAAGGCAGATGA -3’
GPR174_2	Forward	5’- GCCCTGTGGGTATTCTATGG -3’
	Reverse	5’- CTAGCAAGACACAATGCCACA -3’
GPR174_3	Forward	5’- CCTGTGCAGGGGTATTCCTA -3’
	Reverse	5’- catttcctctgaacataaagactca -3’

Figure S1 Tissue expression patterns of *GPR174* and *GAPDH*. cDNA samples of 15 tissues were from the Human Immune System MTC Panel (spleen, lymph node, thymus, leukocyte, bone marrow and fetal liver) and Human MTC Panel I (heart, brain, placenta, lung, liver, skeletal muscle kidney and pancreas). The cDNA of thyroid was reverse-transcribed from Human Thyroid Total RNA (Clontech). The PCR products were loaded in 3% agarose gel.