EXTENDED REPORT

PXK locus in systemic lupus erythematosus: fine mapping and functional analysis reveals novel susceptibility gene *ABHD6*

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ABSTRACT

Objectives To perform fine mapping of the *PXK* locus associated with systemic lupus erythematosus (SLE) and study functional effects that lead to susceptibility to the disease.

Methods Linkage disequilibrium (LD) mapping was conducted by using 1251 SNPs (single nucleotide polymorphism) covering a 862 kb genomic region on 3p14.3 comprising the *PXK* locus in 1467 SLE patients and 2377 controls of European origin. Tag SNPs and genotypes imputed with IMPUTE2 were tested for association by using SNPTEST and PLINK. The expression QTLs data included three independent datasets for lymphoblastoid cells of European donors: HapMap3, MuTHER and the cross-platform eQTL catalogue. Correlation analysis of eQTLs was performed using Vassarstats. Alternative splicing for the *PXK* gene was analysed on mRNA from PBMCs.

Results Fine mapping revealed long-range LD (>200 kb) extended over the *ABHD6*, *RPP14*, *PXK,* and *PDHB* genes on 3p14.3. The highly correlated variants tagged an SLE-associated haplotype that was less frequent in the patients compared with the controls (*OR=0.89,* p=*0.00684*). A robust correlation between the association with SLE and enhanced expression of *ABHD6* gene was revealed, while neither expression, nor splicing alterations associated with SLE susceptibility were detected for *PXK*. The SNP allele frequencies as well as eQTL pattern analysed in the CEU and CHB HapMap3 populations indicate that the SLE association and the effect on *ABHD6* expression are specific to Europeans.

Conclusions These results confirm the genetic association of the locus 3p14.3 with SLE in Europeans and point to the *ABHD6* and not *PXK*, as the major susceptibility gene in the region. We suggest a pathogenic mechanism mediated by the upregulation of *ABHD6* in individuals carrying the SLE-risk variants.

INTRODUCTION

PXK (PXK domain-containing serine/threonine kinase) is an ubiquitously expressed protein that binds to and modulates the plasma membrane Na, K-ATPase.[1] Genetic variation at the *PXK* gene locus was associated with the susceptibility to develop systemic lupus erythematosus (SLE) by a genome-wide association study (GWAS) conducted in European individuals.[2] The SLE-associated variant *rs6445975* is located in intron 4 of the *PXK* gene, and no functional mechanism by which this single nucleotide polymorphism (SNP), or another variant in LD with it, affects gene function has been characterised yet. Moreover, while the association with this locus was corroborated in SLE[3 4] and other autoimmune diseases, such as systemic sclerosis[5] and rheumatoid arthritis in several European populations and north Indians,[6 7] no association has been observed in either GWAS or single SNP replication studies in Chinese, Korean, African–American and Finnish populations.[8–15] Apart from genetic heterogeneity observed for the *PXK* association, the function of the encoded kinase remains largely unknown, as well as the pathogenic pathway where it might take part in.[16]

In order to confirm and characterise the association of *PXK* in more detail, we performed a fine mapping of the gene locus and neighbouring regions in a collection of European SLE samples, and performed functional analysis of genes in the 3p14.3 region.

METHODS

Patients and controls

After quality control of the data, the study sample consisted of 1467 patients with SLE and 2377 ethnicity-matched healthy control subjects. A total of 1118 cases and 1526 controls belong to the European multicentre collaboration network BIOLUPUS, comprising individuals from Argentina, Belgium, Germany, Hungary, Italy, Portugal, Spain and Sweden. An independent set of 349 cases and 851 Spanish controls recruited at the GENyO in Granada, Spain, were also included. All patients fulfilled at least four of the American College of Rheumatology 1982 criteria for the classification of

SLE.[17] All participating individuals provided informed consent for this study. The institutional review boards (IRB) and ethical committees of each participating organisation approved the study. Samples with an individual genotyping rate lower than 90%, duplicated, and/or related were excluded. We also removed individuals with <90% of European ancestry, as estimated by using STRUCTURE V.2.3.3[18] and 350 ancestry informative markers (AIMs).

Single-nucleotide polymorphisms (SNPs) selection and genotyping

From the linkage disequilibrium (LD) structure of the *PXK* locus in the HapMap CEU population (data release 27; available at http://hapmap.ncbi.nlm.nih.gov/), tag SNPs capturing more than 90% of common variation at the gene locus (minor allele frequency greater than or equal to 5%) including 50 kb upstream and downstream of the gene, at a r^2 threshold of greater than or equal to 0.8, were selected using the tagger algorithm implemented in Haploview V.4.1.[19] The SNP *rs6445975*, previously reported to be associated with SLE,[2] was also genotyped. After genotyping and quality control of the data, 12 tag SNPs were used to guide the imputation.

All samples from the BIOLUPUS cohort were genotyped at the Feinstein Institute for Medical Research (Manhasset, NY) using a GoldenGate Custom Genotyping Assay and a BeadXpress Reader from Illumina (San Diego, California). Additionally, the GENyO samples were genotyped using the Immunochip Bead Array from Illumina.[20]

Imputation

Imputation was performed by using IMPUTE2 V.2.2.2[21] across a 1.1Mb genomic region (chr3 : 57 800 000–58 900 000) (GRCh37/hg19) that includes the *PXK* locus. As reference genotypes, we used the phased haplotypes from a multipopulation reference panel from the 1000 Genomes Project Phase 1 (downloaded from (http://mathgen.stats.ox.ac.uk/impute/data_download_1000G_phase1_interim.html) on 11 November 2012).[22] Cases and controls were imputed in a single run using recommended parameters. After imputation, only imputed genotypes with a probability equal to or greater than 0.9, imputation INFO score equal to or greater than 0.5, MAF equal to or greater than 0.005, genotyping rate equal to or greater than 0.95, and following Hardy–Weinberg equilibrium ($p>0.001$) were retained for association analysis.

Statistical analyses

Genetic association and conditional analyses of directly typed and imputed genotypes were carried out by using SNPTEST V.2.4.1.[23] For single-marker tests, we performed a meta-analysis adjusting by the country of origin as a covariate. The 'score' method implemented in SNPTEST (a missing data likelihood score test) was used to deal with genotype uncertainty. All SNPs with a genotype call rate <90% or not in the Hardy–Weinberg equilibrium ($p<0.001$) were excluded. The p values were corrected for the number of tests performed by using the false discovery rate (FDR) method implemented in PLINK V.1.07.[24] Haplotype analysis was conducted by testing each haplotype against all others pooled together (1 df) using PLINK.

eQTL analysis

The three independent resources with European eQTLs were analysed: HapMap3 dataset (109 samples),[25] MuTHER (856 twins samples),[26] and the cross-platform MRCA/MRCE dataset (405 and 550 samples for each platform).[27] Only cis-eQTLs within 1 Mb flanking the studied markers and/or annotated gene transcription start sites were taken into account. Preloaded eQTL p values were extracted for the 3p14.3 region from each independent eQTL data resource (10 000 permutations were applied for correction of each SNP values). Further statistical analyses, including estimation of correlation between expression-related and SLE-related values, were performed using VassarStats (http://vassarstats.net) and PRISM V.6 (http://www.graphpad.com).

PXK transcripts annotation

The alternative splicing of *PXK* was analysed by PCR with primers matching to different exons. Total RNA and genomic DNA were purified from peripheral blood mononuclear cells (PBMC) obtained from 84 healthy donors collected at Uppsala University Hospital as described elsewhere.[28] The PCR products were purified from gel using QIAquick Gel extraction kit (Qiagen), sequenced, and the sequences were deposited to GenBank with the following accession numbers KF774202, KF774203 and KF774204. The samples' DNA was genotyped for the top 10 associated SNPs (table 1).

RESULTS

Fine mapping of the PXK region revealed extensive LD

We performed an association analysis of SNPs across a 232 kb genomic region surrounding the *PXK* locus, in which polymorphisms had been previously reported as associated with susceptibility to develop SLE in Europeans.[2] After quality control of the genotyped and imputed variants, 417 SNPs located in *ABHD6*, *RPP14*, *PXK* and *PDHB* genes were tested for association adjusting by the country of origin. Meta-analysis of the *PXK* region revealed strong long-range LD across and beyond the *PXK* locus (figure 1A). The strongest, single, associated marker was located within the *RPP14* gene (*rs6445969*, $P_{meta\text{-}analysis}=6.43\times10^{-4}$); however, multiple correlated SNPs ($r^2>0.8$) also displayed similar association along the neighbouring genes *ABHD6*, *RPP14*, *PXK* and *PDHB* (table 1, see online supplementary table S1). The association analysis conditioned on *rs6445969* did not provide evidence in favour of an independent association of any other SNP. The previously GWAS SLE-associated variant *rs6445975*[2] was not associated in our study.

Given the extensive LD across the region, we decided to verify whether the association was extending beyond the previously analysed 232 kb region. We analysed genotype data covering a 862 kb region around the *PXK* locus, which was available for the 349 patients and 851 controls from the Spanish cohort. In total, 1251 SNPs were tested for association with the strongest signal located within the intergenic region *ABHD6/RPP14* (*rs9857570*, $P=8.3x10^{-4}$) (see online supplementary figure 1). Consistent with our initial results, long-range LD was observed with multiple correlated SNPs displaying similar association across the region. Only nominal association was observed upstream of the *ABHD6* locus with some SNPs within the *DNASE1L3* gene ($0.02< p<0.05$). These SNPs were not associated after adjusting by the top SNP *rs9857570*. Therefore, we concluded that the association was limited to the genomic region containing the *ABHD6*, *RPP14*, *PXK* and *PDHB* genes.

Haplotype analysis indicates that the SLE-associated haplotype is under-represented in patients

Haplotype analysis was performed using a representative and highly informative subset of SNPs that were in approximate LD with each other. To generate this set, sliding windows of 50

Table 1 Association analysis of SNPs on 3p14.3 susceptibility locus (only SNPs displaying a p<0.01 are shown)

SNP	BP position (hg19)	Allele A	Allele B	Imputation INFO score	MAF allele A Cases	MAF allele A Controls	p Value CMH	p Value Breslow–Day	r2 rs6445969	UCSC Gene	Function
rs4681842	58 292 579	G	A	0.99572	0.303	0.331	7.08E-04	0.5668	0.999	*RPP14*	Intronic
rs4681676	58 299 797	A	G	0.99586	0.302	0.330	6.43E-04	0.5672	1	*RPP14*	Intronic
rs6445969	58 301 336	C	T	0.99597	0.302	0.330	6.43E-04	0.5663	1	*RPP14*	Intronic
rs4681677	58 302 040	A	G	0.99704	0.303	0.331	7.08E-04	0.5668	0.999	*RPP14*	Intronic
rs2056119	58 309 393	A	G	0.99704	0.303	0.331	7.08E-04	0.5668	0.999	RPP14/PXK	Intergenic
rs6445971	58 314 324	G	T	0.99704	0.303	0.331	7.08E-04	0.5668	0.999	RPP14/PXK	Intergenic
rs6445971	58 319 958	G	A	1	0.304	0.333	7.79E-04	0.5724	0.991	*PXK*	Intronic
rs11713310	58 320 984	G	A	0.99978	0.364	0.391	9.34E-04	0.7011	0.761	*PXK*	Intronic
rs6445972	58 321 707	C	T	1	0.304	0.332	8.13E-04	0.575	0.993	*PXK*	Intronic
rs9817084	58 326 253	T	A	0.99845	0.304	0.332	7.42E-04	0.5427	0.994	*PXK*	Intronic
rs6445975*	58 370 177	G	T	0.99992	0.265	0.250	9.49E-02	0.1066	0.155	*PXK*	Intronic

CMH: Cochran-Mantel-Haenszel meta-analysis done after adjusting the association by the country of origin. The correspondent Breslow–Day test for assessing the homogeneity of the OR is shown. Linkage disequilibrium (shown as r^2 values) with the top associated variant rs6445969 (bold) is indicated. Association analysis was conducted by using SNPTEST and the imputation INFO score obtained after imputation with IMPUTE2 was incorporated in the analysis.
*rs6445975 was previously associated with SLE in GWAS.[2]

SNPs were considered at a time, then pairwise LD between each pair of SNPs within the window was calculated, and finally, one SNP of every pair was removed if the r^2 was equal to or greater than 0.8. A representative set of 48 SNPs was obtained, which formed five common haplotypes (frequency >0.05). Only one haplotype was reliably associated with SLE after testing each haplotype against all others (figure 1B). The minor alleles of *rs4681842* and *rs7610449* are complete proxies of *rs6445969* (r^2>0.99), and a minor allele of *rs11713310* (r^2>0.83) occurred in this haplotype. The SLE-associated haplotype was under-represented in the patients with a frequency of 29.7% compared with the 32.8% observed in the controls, and this difference was statistically significant (p=*0.0068*, OR 0.89 (0.79–0.99)).

Correlation analysis of eQTLs and SLE association points at ABHD6 as the only gene in the locus significantly affected by SLE-associated variants

In order to uncover the potential functional mechanism underlying the SLE association in the studied locus, we analysed three independent expression quantitative trait loci (eQTLs) databases for lymphoblastoid cell lines (LCLs) obtained from donors of European ancestry (HapMap3, MuTHER and a recent study by Liang *et al*).[25–27] A 1 Mb distance between the target gene transcription start site and the putative eQTL marker was chosen, as recommended.[29]

Preliminary analysis allowed us to identify that *DNASE1L3*, *ABHD6*, *RPP14*, *PXK* and *PDHB* gene expression was potentially influenced by SNPs in the studied region on chromosome 3. No significant *cis*-eQTLs were found for other genes. We compared the polymorphisms studied in the current SLE genetic association analysis and those characterised as potential eQTLs in the HapMap3 project (figure 2, see online supplementary table S2). Among those SNPs, significant eQTLs were detected only for *ABHD6* and *PXK* genes. While the majority of eQTL markers for *ABHD6* were associated with SLE, there was only one associated SNP among the eQTLs for *PXK*, *rs11713310*. However, this polymorphism was also an eQTL for *ABHD6* (p=0.01), suggesting a long-distance effect due to extended LD (*D'1.0*, R^2=*0.737* between eQTL marker for *ABHD6 rs4681677* and *rs11713310*

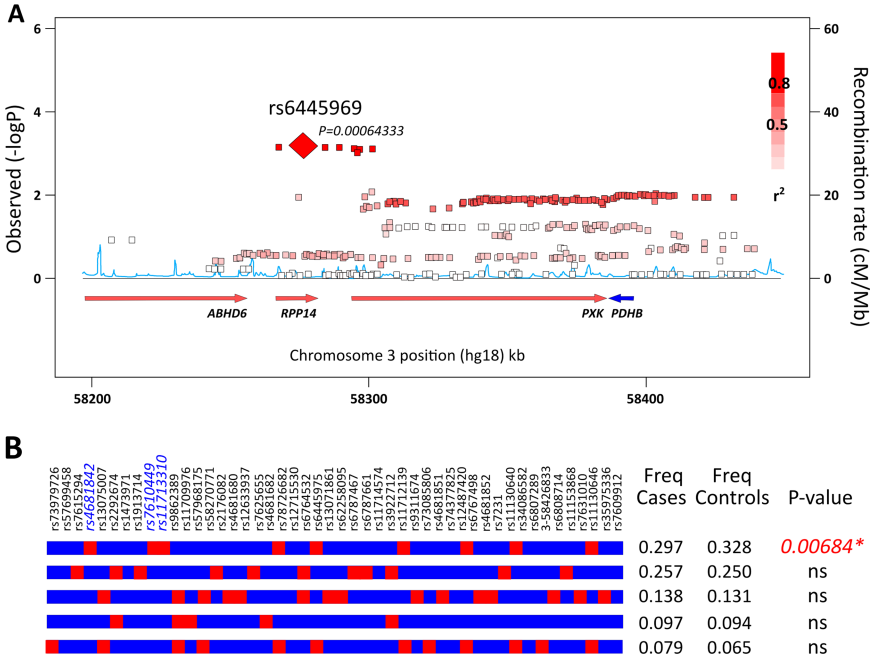

Figure 1 The *PXK* locus in SLE. (A) The association analysis of 417 SNPs covering a 232kb genomic region surrounding the *PXK* locus. The strongest association was located within the *RPP14* gene (*rs6445969*, $P_{meta\text{-}analysis}=6.43\times10^{-4}$); however, multiple correlated SNPs (r^2>0.8) also displayed similar association along the neighbouring genes *ABHD6*, *RPP14*, *PXK* and *PDHB* reflecting long-range linkage disequilibrium in the region. (B) Haplotype analysis indicates that the SLE-associated haplotype is under-represented in the patients with a frequency of 29.7% compared to the 32.8% observed in the controls.

according to the CEU subset of 1000 Genomes data). Three more eQTLs, *rs17059171*, *rs7643185* and *rs13082278*, showing an effect only on the *PXK* gene were not associated with SLE. The low r^2=0.080 between these markers and *rs11713310* argues against their role in the association with SLE. Despite the location of the top associated SNP *rs6445969* in intron of *RPP14*, no significant eQTLs were found for this gene.

As the number of individuals with European ancestry (CEU) in the HapMap3 eQTL dataset was rather limited and contained only 109 samples,[25] we decided to verify the results (figure 3A) by using two additional high-coverage representative resources focused on European samples—multiple tissue MuTHER,[26] and the latest highly representative cross-platform eQTL catalogue published by Liang *et al*.[27] The *ABHD6* gene remained the only gene with positive correlation between SLE association and eQTL values in all these datasets. Moreover, the increase in coverage and representation significantly improved the *ABHD6* gene eQTL values and correlation with SLE association (R^2=0.76 for HapMap3 and R^2=0.85 for Liang *et al*[27] dataset) (figure 3B). The potential regulatory SNPs according to the Regulome database[30] were more frequent among the markers characterised both as significant for SLE association and *ABHD6* expression. Of note, the genetic effect on the *PXK* levels though not related to SLE association, was detected in all databases analysed. No other studied genes showed correlation between SLE association and expression changes, although we observed strong eQTLs for *DNASE1L3* (figure 4).

The MuTHER project contains expression data for lymphoblastoid, adipose and skin tissues obtained from the same individuals.[26] Interestingly, we found that the SNPs associated with SLE correlated with expression of *ABHD6* only in LCLs, but not in the heterologous adipose or skin tissues (see online supplementary figure 2). Such correlation detected only in the highly relevant to immunity lymphoblastoid cells may indirectly support our claim about the role of *ABHD6* in SLE.

Next, in order to exclude alternative splicing that may affect the gene function significantly, but its effect could be missed out in the expression studies, we characterised all isoforms transcribed from the *PXK* gene in PBMCs. We detected several transcripts, but found no correlation of any of them with any genotype or with *PXK* expression levels (data not shown).

We also verified if any detrimental non-synonymous SNPs in the *PXK* gene were reported in the 1000 Genomes project and other databases, and if these could be in LD with associated variants that might explain the association of *PXK*. Only two nsSNPs with moderate LD (R^2=0.7) with SNP *rs4681677* were reported for *PXK*. Both are, however, predicted to be benign by SIFT and PolyPhen-2 (see online supplementary table S3).[31 32]

eQTL analysis supports the population-specific nature of the ABHD6-PXK region association with SLE

The genetic association of the *PXK* region with SLE in Europeans was not confirmed in either Asians or African–Americans.[8–11] This prompted us to compare the expression data for Europeans (CEU) and Chinese (CHB) populations available in HapMap3 dataset. The Chinese eQTL dataset included 45 unrelated Bejing individuals. In contrast with Europeans, we detected no significant eQTLs for *ABHD6* and *PXK* in the Chinese population data (figure 4, see online supplementary table S4). While the strongest eQTL signals located in the promoter of *DNASE1L3* were found in both populations, none of them were associated with SLE, neither in the CEU nor CHB sets. The eQTL signals for *PXK* were also significant in Europeans, but not in Chinese, however, as mentioned above, they are not associated with SLE (figures 2 and 3). This finding allowed us to conclude that changes in *ABHD6* gene expression could be important for SLE risk only in Europeans, and this is further supported by the lack of an association signal in the 3p14.3 locus with SLE in Chinese GWAS.[13]

SLE risk alleles are strongly associated with ABHD6 overexpression in LCLs

Further, in order to study the effect of polymorphisms associated with SLE on *ABHD6* gene expression, we focused on the top associated SNPs (table 1) and analysed *ABHD6* expression in HapMap3 CEU dataset in more detail. We found that *ABHD6* mRNA level was higher in the samples with the major allele T of *rs6445969*, while the minor allele C correlates with lower transcript levels (p=*0.02*) (figure 5A). Moreover, stratification by the SLE-associated haplotype made of the minor alleles of *rs6445969* (genotypes for *rs4681842* were not available), *rs7610449* and rs11713310 revealed significantly lower gene expression in homozygotes CC-GG-GG compared with TT-AA-AA (p=0.04) (figure 5B). Interestingly, the GWAS-associated SNP *rs6445975* whose minor allele is also present in the SLE-associated haplotype (figure 1B) does not correlate with *ABHD6* expression (data not shown). These data indicate that the *ABHD6* gene is upregulated in the risk for SLE, and downregulated in the protective haplotype.

DISCUSSION

We have shown here that a comprehensive analysis of expression QTL markers may help to pinpoint the culprit gene in large associated regions with extensive LD. The *PXK* region is one of the uncertain genetic associations of SLE due to the lack of replication in several ethnicities, or of a clear understanding of the disease pathogenic pathways that might involve the PXK kinase itself.

The entire locus from *DNASE1L3* to *PDHB* is characterised by lower recombination rate in comparison to its flanking regions, thus making it difficult to narrow down the culprit variant(s) (see online supplementary figure 1A). Since we detected neither splicing nor expression level changes, and no detrimental non-synonymous SNPs in the *PXK* gene that could be correlated with SLE association, this prompted us to extend the functional analysis and include more genes in the region, *DNASE1L3*, *ABHD6*, *RPP14*, *PXK* and *PDHB*, in order to examine for correlation between polymorphism(s) associated with SLE susceptibility and gene expression changes.

The gene expression analysis in LCLs using three independent resources suggests that neither *PXK* nor *RPP14* genes expression regulation is the mechanism underlying the SLE association with this locus. Although we could not completely rule out that the associated variants influence *PXK* or *RPP14* transcription in a tissue-specific manner, we believe that blood cells and LCLs derived from them represent the most relevant tissues to study expression of the genes in the locus. Among the tissues and cells analysed and available at BioGPS (http://biogps.org/#goto=welcome), high levels of *PXK* were detected in CD34+ cells, CD19+, CD33+, dendritic cells and CD56+ NK cells, and in several zones of the brain, while *PXK* expression in all other tissues was at a very low, yet detectable level.

Further, among the genes in the 1 Mb region surrounding *PXK*, only *DNASE1L3* and *ABHD6* loci show notable eQTLs in LCLs from individuals of European ancestry. The linear regression analysis revealed significant correlation between SLE association and eQTLs for *ABHD6* only. The observed strong eQTL signals for *DNASE1L3* and several eQTLs for *PXK* and *PDHB*

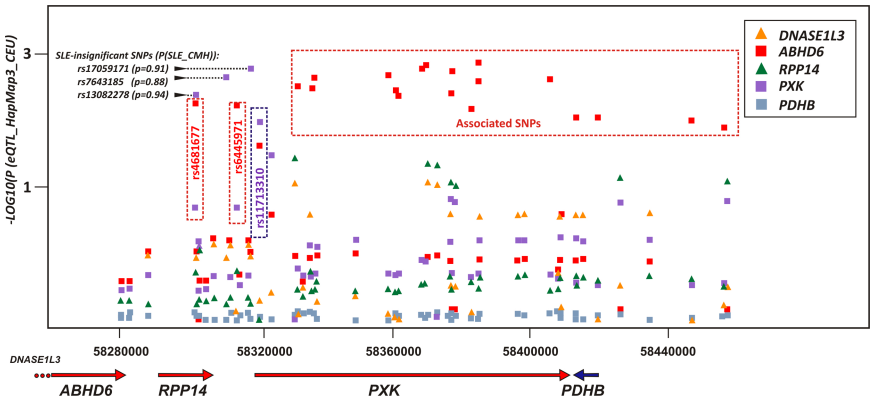

Figure 2 Comparative mapping of eQTL markers in the *PXK* locus. The identical set of SNPs was analysed for their effect on expression of studied genes in HapMap3 CEU dataset. The p values for eQTLs are shown in -log scale, SNPs associated with SLE are depicted with red dotted boxes. The SLE-associated SNP *rs11713310* shows slightly higher effect on *PXK* gene comparing with *ABHD6* and enclosed in violet dotted box. Three SNPs (depicted by arrows) showing the profound effect on *PXK* gene expression are not associated with SLE.

were neither associated with SLE nor lie in LD with other associated SNPs (figures 3 and 4). The initial better correlation of the SNP *rs11713310* with *PXK* than *ABHD6* eQTL in HapMap3 dataset (figure 2) was not validated in the other two independent resources with more individuals included. This variant was a better eQTL for *ABHD6* in MuTHER and the cross-platform eQTL catalogue.

Moreover, those eQTL signals are common for Europeans and Chinese, while the association with SLE is rather population-specific. The variants that define the SLE-associated haplotype identified in the present study are not polymorphic in Asians, therefore, we hypothesise that this is the reason why no association with *PXK* variants has been replicated in patients with SLE from this population. Furthermore, the positive

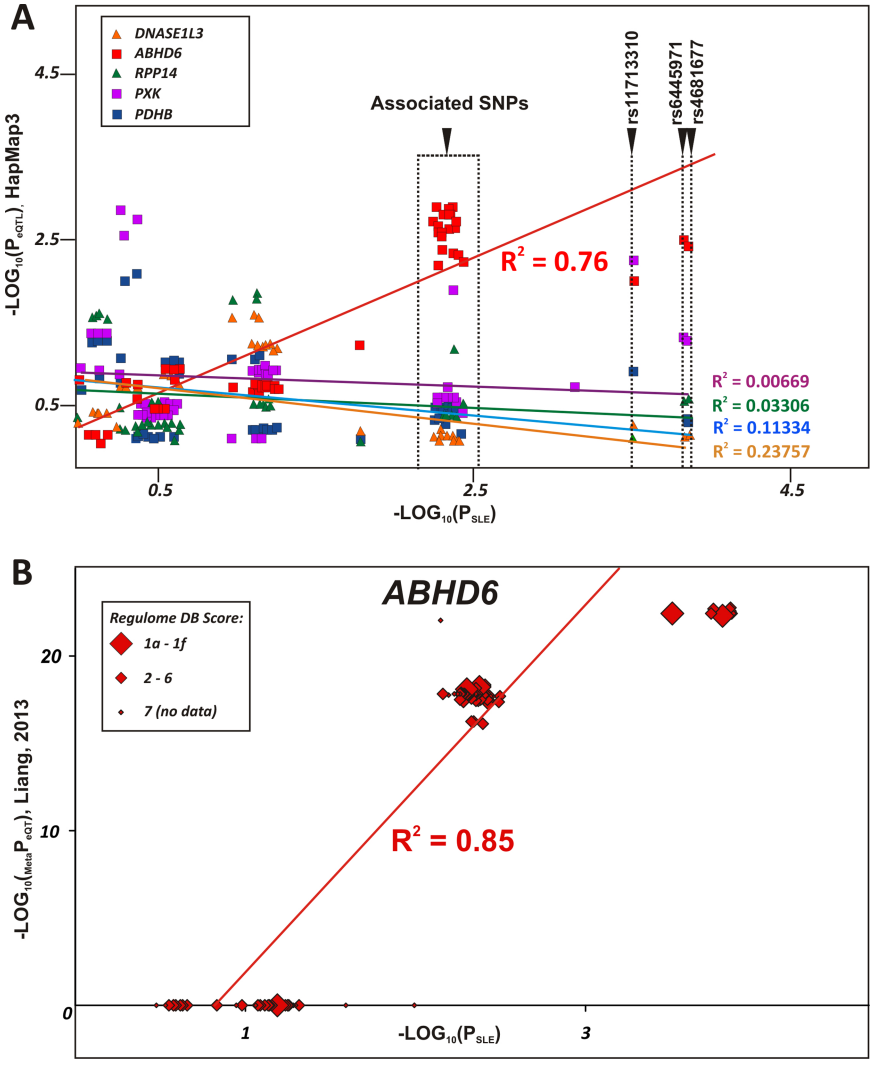

Figure 3 Linear regression analysis of association with SLE and gene expression in lymphoblastoid cell lines. (A) Correlation of SLE-associated polymorphisms and eQTLs from CEU HapMap3. p values are presented in –log scale. *ABHD6* is the only gene characterised with positive correlation between SLE association and expression effect. (B) The positive correlation between *ABHD6* expression and association with SLE is significantly improved when using the high-coverage cross-platform eQTL dataset.[27] Other studied genes demonstrated lack of any correlations between SLE association and eQTLs. The functionally relevant SNPs are depicted according to their Regulome database score,[30] shown as diamonds of decreasing size. The declining order from 1 to 7 reflects the presence of either a full set of features (1a contains: eQTL, TF binding, matched TF motif, matched DNase footprint and DNase peak) or absence of some of them or all (7). Many *ABHD6* SNPs have high potential to be functionally important for gene regulation.

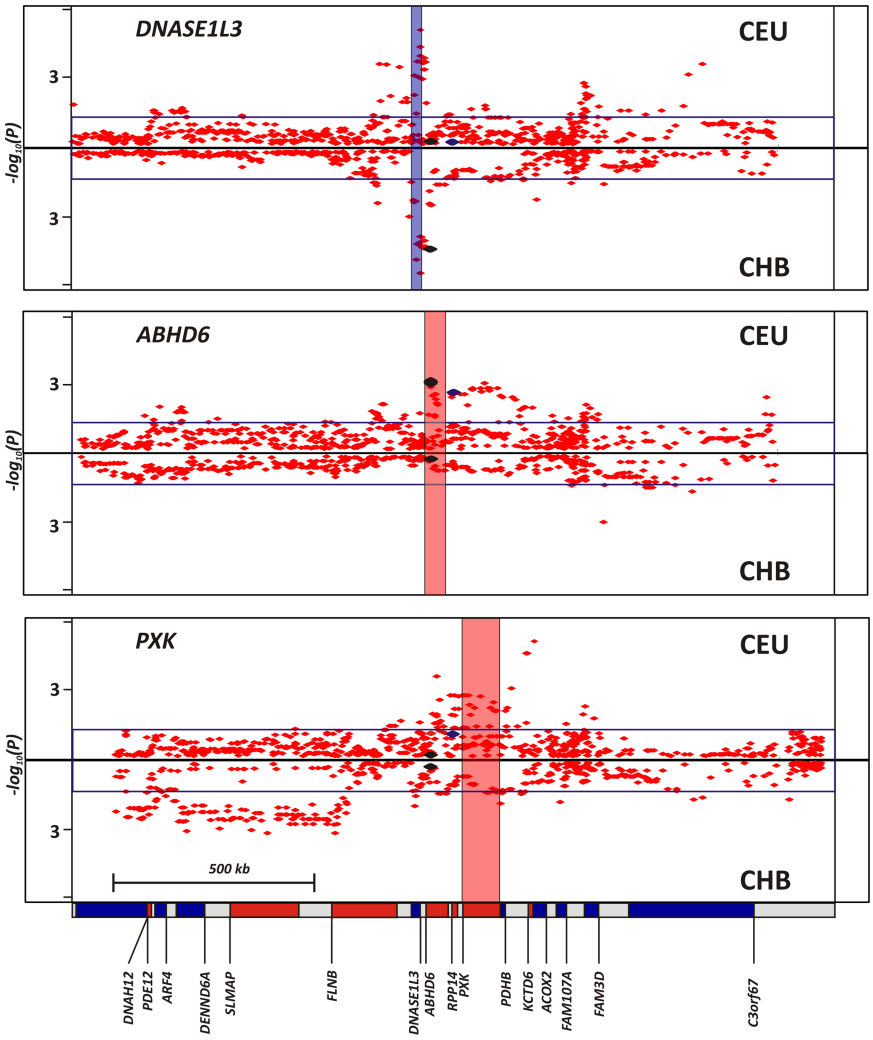

Figure 4 Population-specific nature of *ABHD6* expression changes in LCLs. The distribution of eQTL p values for *DNASE1L3*, *ABHD6* and *PXK* in European (CEU) and Chinese (CHB) HapMap3 datasets shown as normalised fluorescence expression values. No significant eQTL signals were detected for *ABHD6* in CHB. The transparent boxes corresponding to the each gene are shown. The cut-off lines depicted at each plot correspond to p=*0.05*. The three SLE-associated SNPs rs4681677, rs6445971 and rs11713310 are depicted as black diamonds.

correlation between *ABHD6* expression changes and association with SLE was detectable in most of the data obtained on LCLs from European donors, while no significant *cis*-eQTL signals for *ABHD6* in HapMap3 Chinese population were found.

In general, the reason why *rs6445975* was not found associated in several populations including our own study could be that it is not a good tag SNP for the disease-associated haplotype. A fine mapping of the entire locus, including haplotype analysis, is recommended for studies in populations with previously reported lack-of-association instead of a single-SNP replication strategy.

The *ABHD6* gene codes for the abhydrolase domain-containing protein 6, with a function not fully characterised yet. ABHD6 catalyses the hydrolysis of 2-arachidonylglycerol and takes part

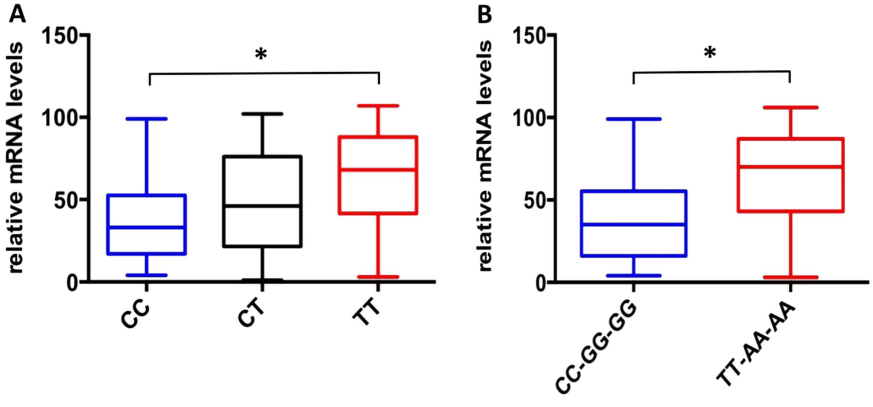

Figure 5 Expression of the *ABHD6* gene in LCLs. (A) The *ABHD6* microarray expression values for 109 HapMap3 European individuals are shown for the top associated single nucleotide polymorphism (SNP) *rs6445969*. The major allele T correlates with higher transcript levels. (B) The upregulation of *ABHD6* gene in a haplotype made of the major alleles of SNPs *rs6445969*, *rs7610449* and *rs11713310*. The median expression levels, first and third quartiles as well as minimum and maximum values are shown. Gene expression was analysed by using Kruskal–Wallis non-parametric analysis of variance (ANOVA). Statistical significance indicated by a star corresponds to p=*0.02* (A) and p=*0.04* (B). The risk is shown in red colour, while the protective is in blue.

in the endocannabinoid signalling regulation.[33] Studies on the protein function have focused on its use as a potential target in drug addiction therapy.[33–35] Besides the proposed neuroregulatory function, the detectable expression of *ABHD6* in tissues such as kidney, liver and spleen, indicate the multifunctionality of this gene's product. For instance, upregulation of *ABHD6* is detected in Ewing tumours.[36 37] Additionally, gene expression studies of B cells induced by Epstein–Barr virus' (EBV) EBNA protein demonstrated that among the top 12 induced genes, there were two genes from the SLE-associated 3p14.3 locus: *ABHD6* and *DNASE1L3*.[38] Another study showed a rapid increase of uterine *ABHD6* mRNA levels upon stimulation with oestradiol.[39] The EBV is known for its role in a variety of human pathologies, including several autoimmune diseases.[40] The role of oestrogen in the pathogenesis of SLE is widely acknowledged and supported also by the prevalently female susceptibility to the disease.[41] Thus, the induction of *ABHD6* by EBV and oestrogen is in line with our finding that enhanced expression of *ABHD6* is associated with increased risk for SLE, and may further advocate the potential role of *ABHD6* in immunity and SLE aetiology.[41 42]

Although our data suggest the strong effect of SLE-associated SNPs on the *ABHD6* gene expression, the direct causative variant(s) is still unknown, and further functional studies are required to uncover the underlying regulatory mechanisms.

In summary, we demonstrated for the first time that the previous genetic association of *PXK* variants with SLE might be attributed to the *ABHD6* gene. The association is population-specific and supported by the gene upregulation in the risk haplotype present in Europeans. While those variants are not polymorphic in Asians, no notable eQTLs were detected in the locus in the Chinese population either.

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Contributors MEAR and SVK conceived the study. AMDV and MMB performed genotyping and genetic analyses; SVK and NYO performed functional experiments and analyses; CMC, CF, ROC, BAPE, SD'A, GDS, TW, BRL, EE, LK, AE, CLP, CV, BMdS, JF, LT, JM, ER, NOC, MdLAA, EdRG, MdJC provided samples; NYO, AMDV, MEAR and SVK wrote the manuscript with input from other authors.

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PXK locus in systemic lupus erythematosus: fine mapping and functional analysis reveals novel susceptibility gene *ABHD6*

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