

Role of B cell inhibitory receptor polymorphisms in systemic lupus erythematosus: a negative times a negative makes a positive

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Abstract B lymphocytes play a pivotal role in the pathogenesis of systemic lupus erythematosus (SLE). Here, we will review our studies on the role of polymorphisms of two genes coding for B cell inhibitory receptors, *FCGR2B* and *CD72*. In *FCGR2B*, a single nucleotide polymorphism leading to a nonsynonymous substitution, Ile232Thr, within the transmembrane domain was identified, and a significant association of the 232Thr/Thr genotype with SLE was observed in Japanese, Thai and Chinese populations, while this allele was found to be rare in Caucasians. On the other hand, the association of *FCGR2B* promoter polymorphism with SLE in Caucasians has been reported by two independent groups, but this allele was not found to be present in Japanese. These observations demonstrate that the association of *FCGR2B* polymorphisms with SLE is common to multiple populations, but the alleles associated with SLE depend upon the genetic background of each population. Functional analyses using a human B cell line lacking endogenous Fc γ RIIb revealed that SLE-associated 232Thr allele product was partially excluded from membrane lipid rafts under resting conditions and after coligation with B cell receptor, and was significantly less potent at

inhibiting B cell activation. Two haplotypes were identified in *CD72*, one of which was associated with increased production of an alternative splicing isoform that substantially alters the extracellular region of *CD72*. Interestingly, the presence of the haplotype significantly decreased the risk of SLE conferred by *FCGR2B*-232Thr in an epistatic manner. These observations emphasize the need to understand human immune system diversity if we are to improve our understanding of the pathogenesis of autoimmune diseases.

Keywords Systemic lupus erythematosus · Polymorphism · *FCGR2B* · *CD72* · Lipid raft · Alternative splicing · Epistasis · Inhibitory receptor

Genetic background of systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a prototype of systemic autoimmune diseases. Epidemiological data indicate that genetic factors play a substantial role in the disease, and intensive searches for susceptibility genes are underway by genome-wide as well as candidate gene approaches (Tsao 2004).

Genome-wide linkage analyses have been reported from five groups from North America and Europe. Collectively, more than 50 regions have been identified as meeting the suggestive level of linkage (LOD \geq 1.9 or P <0.01), including seven regions showing a significant level of linkage (LOD \geq 3.3), 1q22-23, 1q41-42, 2q37, 4p16, 6p21-11, 16q13 and 17p13 (Kelly et al. 2002).

Studies on biological and positional candidate genes have reported a number of potential susceptibility genes. Among these, the *HLA-DRB1* (*DRB1**1501 in

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Asians and Caucasians, *DRB1*1503* in African-Americans and *DRB1*0301* in Caucasians) and *C4A*Q0* alleles, both located within the MHC complex at 6p21.3, are the most well-established. As we will see later, genes within the low affinity *FCGR* cluster at 1q23 (*FCGR2A*, *3A*, *3B*) have frequently been reported to be associated with SLE, although the results are not very consistent; as is the case with mannose-binding lectin (10q11.2-12). *PDCDI*, coding for an inhibitory receptor PD-1 on the T cells, has been identified as being associated with SLE through a positional candidate approach (Prokunina et al. 2002). Recently, an association of *IRF5* polymorphism was reported in northern European populations (Sigurdsson et al. 2005), and this has been convincingly replicated in a large-scale study of four cohorts (Sweden, Spain, Argentina and USA) (Graham et al. 2006), suggesting that *IRF5* might be a susceptibility gene common to various populations.

We have been trying to identify susceptibility genes for rheumatic diseases through polymorphism screening and association studies of the functional genes in the immune system (Tsuchiya et al. 2002). The role of B lymphocytes in the pathogenesis of SLE has been established from a number of observations, such as the production of an array of autoantibodies and abnormalities in the peripheral blood B cell subsets, as well as a therapeutic effect of the B cell-depleting anti-CD20 monoclonal antibody (mAb) rituximab (Anolik and Sanz 2004); thus, genes coding for B cell regulatory molecules are considered to be strong candidates for susceptibility genes. Here, we will review a series of studies performed by us on the association of polymorphisms of two B cell inhibitory receptors with SLE.

Detection of *FCGR2B* polymorphism in the transmembrane region and its association with SLE in Asian populations

FCGR2B codes for a receptor for the Fc portion of IgG expressed on B cells and monocytes, Fc γ receptor IIb (Fc γ RIIb). Fc γ RIIb contains immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic region, which becomes phosphorylated when Fc γ RIIb is coligated with B cell receptor (BCR) by antigen–IgG complexes and recruits SH2-containing 5'-inositol-phosphate phosphatase (SHIP). SHIP hydrolyzes phosphatidylinositol-3,4,5-triphosphate (PIP3) and attenuates activation and survival signals in B cells (Takai 2002). Thus, Fc γ RIIb represents one of the molecules that prevents excessive B cell activation.

Indeed, *Fcgr2b*-deficient mice become susceptible to autoimmune diseases such as collagen-induced arthritis

(Yuasa et al. 1999) and Goodpasture's syndrome (Nakamura et al. 2000). Moreover, depending on their genetic backgrounds, they spontaneously develop lupus-like disease (Bolland and Ravetch 2000; Yajima et al. 2003) with the production of rheumatoid factor and anti-DNA antibody (Moll et al. 2004). Such mice exhibit an increased frequency of anti-DNA antibody-producing plasma cells, suggesting a role of Fc γ RIIb in the maintenance of peripheral tolerance (Fukuyama et al. 2005). Furthermore, mice strains which spontaneously develop lupus or autoimmune disease, such as NZB, MRL and NOD, all share *Fcgr2b* polymorphisms (Jiang et al. 1999, 2000), and the development of autoimmunity is inhibited in such mice by the transfer of the wild-type *Fcgr2b* gene (McGaha et al. 2005).

Human *FCGR2B* gene and other classical *FCGR* genes such as *FCGR2A*, *2C*, *3A* and *3B* form a gene family clustered within ~200-kb of 1q23 (Su et al. 2002). These genes, which are presumed to have developed through unequal crossover events, are extremely homologous to one another, not only in exons but also in introns and intergenic regions.

Association with *FCGR2A*, *3A* and *3B* polymorphisms as well as linkage to this region has been reported on multiple occasions (Hatta et al. 1999; Tsuchiya and Kyogoku 2005). Unlike *FCGR2B*, *FCGR2A*, *3A* and *3B* mediate activation signals and phagocytosis in cell lineages such as neutrophils, monocytes and NK cells. All of these genes have functional polymorphisms, and, interestingly, alleles that are reported to be associated with SLE at each locus (*FCGR2A*-131Arg, *FCGR3A*-176Phe [also referred to as 158Phe], *FCGR3B*-NA2) uniformly exhibit lower affinity to IgG or decreased phagocytotic potential compared with the nonassociated alleles. The mechanism of association has been explained by defective clearance of the immune complex (Dijstelbloem et al. 2001). However, prior to our studies, linkage disequilibrium (LD) among these genes and *FCGR2B* had not been investigated; therefore, the possibility that *FCGR2B* might be the causative susceptibility gene that explains the linkage and association signals of this region had not been formally excluded.

Based on such a background, *FCGR2B* was considered a strong candidate susceptibility gene to SLE. However, polymorphism screening of *FCGR2B* proved to be extremely difficult because the nucleotide sequence of *FCGR2B* was almost identical to *FCGR2C* from the 5' flanking region to exon 6, in terms of both exons and in introns, and the specific amplification of *FCGR2B* was not successful. Furthermore, some of the single nucleotide differences between *FCGR2B* and *2C* found in the database turned out to be polymorphisms.

We employed nested PCR to specifically amplify the *FCGR2B* gene, and identified a single nucleotide polymorphism (SNP) (c.695T>C, rs1050501) which led to an amino acid substitution within the transmembrane domain at position 232, Ile232Thr (Kyogoku et al. 2002). (The same SNP is also referred to as Ile187Thr by excluding the signal sequence; Li et al. 2003.) A case-control association study on Japanese indicated that there was a significant association of the 232Thr/Thr genotype with SLE (Kyogoku et al. 2002). We subsequently replicated the association of this polymorphism with SLE in Thai (Siriboonrit et al. 2003) and Chinese populations (Chu et al. 2004). When the data from these three Asian populations were combined using meta-analysis, the odds ratio of 232Thr/Thr for developing SLE was 2.45 (95% confidence interval 1.49–4.02, $P=0.0004$) against 232Ile/Ile genotype (Table 1a). This association was predominantly observed in the patients with lupus nephritis (Chu et al. 2004).

FCGR2B was found to be in LD with *FCGR3B*, *3A* and *2A*. In Asian subjects, association with SLE was also found for *FCGR3B*-NA2 and *3A*-176Phe. The association of each of the three *FCGRs* could not be explained solely by LD with other *FCGR*; thus, each of the *FCGRs* appeared to have its own contribution (Kyogoku et al. 2002; Chu et al. 2004).

Population difference

It is becoming increasingly clear that susceptibility genes are often different among populations. For example, the association of *PTPN22*-Arg620Trp with rheumatoid arthritis (RA) has now been replicated in multiple Caucasian populations (Gregersen and Batliwalla 2005), but this allele is not found in Japanese (Mori et al. 2005).

Although the results are not consistent, many studies have demonstrated associations of *FCGR2A*, *3A* and *3B* with SLE. Recent meta-analyses have demonstrated that *FCGR2A* is associated with the development of SLE (Karassa et al. 2002), while *FCGR3A* is associated with the presence of nephritis (Karassa et al. 2003). These meta-analyses are mostly based on data from Caucasian populations. As described above, we observed associations of *FCGR3A* and *3B* (in addition to *FCGR2B*) in three Asian populations, but not an association of *2A* (Chu et al. 2004). This difference was not caused by differences in the genotyping methods, because we observed an association of *FCGR2A*, but not *3A*, with SLE in Caucasians (Kyogoku et al. 2004). Thus, this differ-

ence seems to be caused by differences by the populations.

As for *FCGR2B*, association with *FCGR2B*-232Thr was found in three Asian populations. However, this allele was rare in Caucasians, and significant association with SLE was not detected (Li et al. 2003; Kyogoku et al. 2004; Magnusson et al. 2004) (Table 1a).

On the other hand, two groups demonstrated an association of *FCGR2B* promoter polymorphisms in Caucasians (Su et al. 2004a; Blank et al. 2005). However, this promoter allele was not found in Japanese (unpublished results). This might be another example of a difference between populations, but it also may represent an allelic heterogeneity between populations (Table 1b). The finding that two different polymorphic sites of the same gene are associated with the same disease in different populations may provide strong evidence to support the role of the gene in the disease. Differences between the alleles associated with SLE among populations have also been reported in *PDCDI*. While the A allele at an intron 4 SNP called PD1.3 has been shown to be significantly increased among Swedish, European American and Mexican patients (Prokunina et al. 2002), the same allele was significantly decreased in Spanish SLE (Ferreiros-Vidal et al. 2004).

The two groups reported conflicting results regarding the activity of the SLE-associated promoter allele. While Su et al. (2004a, 2004b) reported a SLE-associated promoter haplotype containing the –386C allele (rs3219018), Blank et al. (2005) reported a lower promoter activity for the –386C allele (they referred to the same SNP as –343G>C by counting from the transcription start site). This discrepancy may be caused by differences between the experimental systems used, although lower promoter activity is in better agreement with other lines of evidence, including our own, as described later.

A copy number polymorphism (CNP) is known to exist for *FCGR3B*. In addition to the common haplotype carrying one *FCGR3B* locus, haplotypes carrying no or two *FCGR3B* loci are present. Thus, the number of *FCGR3B* loci in each individual varies from 0 to 4. *FCGR3B* CNP appears to be common in Caucasians, and it was recently reported that low copy number of *FCGR3B* is associated with the presence of nephritis in SLE in the northern European population (Aitman et al. 2006). Detection of *FCGR3B* CNP is technically challenging, and population data are not available for other populations. However, we found that homozygosity of the reduction-of-function allele *NA2* is associated with nephritis in SLE in Asians (Chu et al. 2004), supporting the idea that a reduction of function

Table 1 Population differences in the *FCGR2B* alleles associated with SLE

References	Population	SLE		Controls		OR (95% CI) (Thr/Thr versus Ile/Ile)	P
		Ile/Ile	Ile/Thr	Ile/Ile	Ile/Thr		
(a) Association studies on c.695T>C (Ile232Thr)	Kyogoku et al. (2002)	106 (54.9)	66 (34.2)	183 (60.4)	104 (34.3)	2.27 (1.15–4.48)	0.018
	Siriboonrit et al. (2003)	38 (48.1)	29 (36.7)	97 (58.8)	56 (33.9)	2.55 (1.07–6.05)	0.033
	Chu et al. (2004)	49 (45.4)	48 (44.4)	51 (60.0)	30 (35.3)	2.86 (0.71–11.5)	NS
	Meta-analysis of the above three studies					2.45 (1.49–4.02)	0.0004
Li et al. (2003)	Caucasian	112 (75.7)	30 (20.3)	106 (77.4)	27 (19.7)	1.42 (0.39–5.16)	NS
Kyogoku et al. (2004)	African-American	97 (60.6)	49 (30.6)	79 (53.0)	53 (35.6)	0.67 (0.31–1.44)	NS
	Caucasian	TDT (Thr allele transmitted 16, non-transmitted 18)					NS
(b) Association studies on –386G>C							
References	Population	SLE		Controls		OR (95% CI)	P
		G/G	C/C	G/G	C/C		
Su et al. (2004a)	Caucasian	180 (74.1)	56 (23.0)	300 (82.0)	63 (17.2)	1.62 (1.14–2.31) ^a	0.007 ^a
Blank et al. (2005)	Caucasian	146 (76.8)	29 (15.3)	100 (76.9)	29 (22.3)	11.1 (1.7–469) ^b	0.003

–386G>C was not detected in 50 Japanese subjects (25 healthy individuals and 25 patients with SLE). Genotype frequencies are shown in parentheses (%)

^aComparison of allele frequencies

^bComparison between C/C genotype versus (G/G + G/C)

for Fc γ RIIb is somehow associated with development of nephritis.

***FCGR2B-232Thr* is a reduction-of-function inhibitory receptor**

Since homozygous deletion of *FCGR2B* in mice has been repeatedly shown to be susceptible to autoimmune diseases (Takai 2002; Nimmerjahn and Ravetch 2006), functional difference between the polymorphic *FCGR2B* allele products was the next focus of interest. Human Fc γ RIIb exists in two isoforms, Fc γ RIIb-1 and b-2, and in the latter, the first cytoplasmic exon that inhibits receptor internalization is spliced out. It is generally accepted that the b-1 isoform is expressed in B cells, and the b-2 isoform in myeloid cell lineage including dendritic cells (Daeron 1997). Recent works highlighted the critical role of Fc γ RIIb in B cells in preventing tolerance breakdown, based on the observations that retroviral Fc γ RIIb gene transfer to B cells effectively suppresses autoimmunity associated with reduced Fc γ RIIb expression in NZB, MRL and NOD mice (McGaha et al. 2005).

In order to characterize the polymorphic human *FCGR2B* allele products in the context of B cells, we took advantage of the ST486 human B cell line, which is inherently devoid of Fc γ RIIb expression (Koncz et al. 1998). The IIA1.6 subline of mouse A20 cells, which also lacks Fc γ RIIb, has so far been utilized as a competent cell line. However, IIA1.6 is obviously inappropriate in this setting for studying Ile232Thr single amino acid substitution, in light of the modest amino acid identity (~60%) of human and mouse Fc γ RIIb. Indeed, a recent functional analysis of the polymorphic Fc γ RIIb in IIA1.6 (Li et al. 2003) has led to different conclusions from ours using ST486 human B cells (Kono et al. 2005), and also from those using human macrophages (Floto et al. 2005).

Each allele of the human Fc γ RIIb-1 isoform was introduced into ST486 cells, and two groups of cells with equivalent Fc γ RIIb-1 expression were established. First we determined ligand-binding parameters. The results showed that the dissociation constants of human Fc γ RIIb for IgG are in the order of 10^{-7} M, values comparable with those in the previous studies (Nimmerjahn and Ravetch 2006), and that Ile232Thr substitution does not significantly affect the binding affinity.

Recent analysis of receptor-proximal events in a variety of systems has featured a novel view of initial signaling that involves specialized plasma membrane domains, variously referred to as lipid rafts, detergent-

insoluble membrane domains (DRMs) or detergent-insensitive membranes (DIMs) (Simons and Ikonen 1997; Ikonen 2001; London 2002). Lipid rafts are small membrane patches (diameter <100 nm) consisting of a plasma membrane bilayer and constructed with specific lipids including cholesterol and glycosphingolipid. Lipid rafts accumulate receptor-proximal signaling molecules harboring saturated acyl-chain anchors, including Src family kinases, heterotrimeric G proteins and adaptor proteins such as linker for activation of T cells (LAT) and C-terminal Src kinase (Csk) binding protein (Cbp) (Pierce 2002). Receptors such as T and B cell receptors, Fc receptors and TNF family receptors become associated with, or induce the aggregation of, lipid rafts shortly after agonist addition (Fig. 1). It is convincingly argued that this process precedes tyrosine phosphorylation signaling, and, moreover, is required for the de novo generation of signaling (Honda et al. 2000; Suzuki et al. 2000; Kono et al. 2002; Pierce 2002). Since we have unveiled the critical roles of their transmembrane domains in the association of Fc receptor with lipid rafts (Kono et al. 2002), we hypothesized that Ile232Thr substitution influences Fc γ RIIb redistribution to lipid rafts.

Distribution of receptor to lipid rafts is best measured by the surface probing of the receptor with specific antibody Fab labeled with 125 I, followed by its solubilization with nonionic detergents and fractionation under density gradient ultracentrifugation (Field et al. 1997; Holowka et al. 2000). However, we found that it was impossible to prepare anti-human Fc γ RIIb Fab with sufficient affinity for the analysis from the available FL18.26 and AT-10 mAbs. We thus employed two different strategies to perform the assay (Kono et al. 2005). First, we created chimeric Fc γ RIIbs in which extracellular domain of mouse Fc γ RIIb was fused to transmembrane (containing 232Ile or Thr) and to intracellular domain of human Fc γ RIIb, and we probed this with high affinity 125 I-2.4G2 mAb Fab. Second, human Fc γ RIIb was probed with 125 I-AT10 whole IgG. Both systems yielded consistent results; 232Thr was relatively excluded from lipid rafts as compared to 232Ile under resting conditions and after coligation with BCR, and the differences were statistically significant (Kono et al. 2005).

It is widely accepted that Fc γ RIIb is able to attenuate BCR signaling only when it is physically coligated with BCR (Muta et al. 1994; Ravetch and Lanier 2000). These features are different from the other B cell inhibitory coreceptors CD22 and CD72, which also possess ITIM: CD22 and CD72 are able to suppress BCR signaling without coligation (Adachi et al. 2000; Nitschke and Tsubata 2004). Although this concept is

supported by several series of reports, it may be worth bearing in mind that this idea derives from the observations in mouse B cells. In the current study, it was reproducibly observed that human Fc γ RIIb attenuates BCR signaling in ST486 immortalized human B cells even when BCR was ligated separately from Fc γ RIIb (Kono et al. 2005).

With respect to the allelic differences, we found that the extent of either constitutive or coligation-induced inhibition was significantly greater in 232Ile than that in 232Thr by monitoring a variety of signals including phosphatidylinositol 3,4,5-trisphosphate (PIP3) levels, phospholipase C γ 2 (PLC γ 2) and Akt tyrosine phosphorylation and calcium mobilization (Kono et al. 2005). From the functional aspects, therefore, it was concluded that human Fc γ RIIb 232Thr represents a reduction-of-function receptor isoform when taking 232Ile as a standard. This conclusion was supported by Floto et al. (2005), who showed that Fc γ RIIb 232Ile exerts greater inhibitory effects than 232Thr in human macrophages on Fc γ RI- and Fc γ RIII-mediated phagocytosis, superoxide generation, cytokine production and MHC class I and II expression. In addition, it should be noted that, under the experimental conditions of Floto et al., Fc γ RIIb was not coligated with Fc γ RI. These findings indicate common mechanisms underlying constitutive inhibition by Fc γ RIIb in human B cells and macrophages.

Does the altered subcellular distribution of 232Thr, the partial exclusion from lipid rafts, provide a mechanistic basis for its phenotype? Floto et al. (2005)

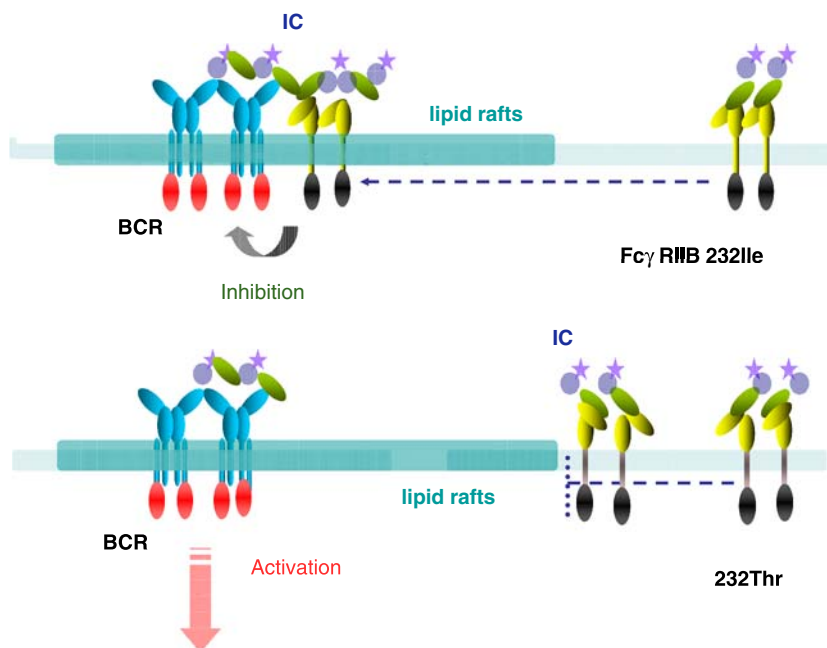
coincidentally showed defective 232Thr distribution to lipid rafts, and concluded it to be responsible for the loss-of-function characteristics of 232Thr. It is certainly attractive to speculate that 232Thr is excluded from lipid rafts, and concurrently from BCR signaling complex (Fig. 1). However, it should be emphasized that no firm evidence exists in support of the idea that association with lipid rafts is a prerequisite for human Fc γ RIIb to inhibit signaling from BCR or activating Fc γ Rs. Indeed, Fas-mediated apoptosis has been shown to depend upon its association with lipid rafts in certain cell types (type I cells), but not in others (type II cells) (Muppidi and Siegel 2004). The determinant in the transmembrane structure required for Fc γ RIIb to associate with lipid rafts, and to efficiently attenuate BCR signaling needs to be elucidated in future studies.

Polymorphisms affecting the alternative splicing of *CD72*

CD72 is a 45 kDa type II transmembrane protein containing a C-type lectin domain expressed in B cells, dendritic cells and macrophages as a homodimer. CD72 contains two ITIMs in its cytoplasmic tail, one of which recruits SH2-containing tyrosine phosphatase (SHP)-1, and functions as a negative regulator of BCR signaling (Adachi et al. 2000; Hokazono et al. 2003; Nitschke and Tsubata 2004).

Human *CD72* gene is encoded on human chromosome 9p13.2. In a murine model of SLE, MRL/lpr,

Fig. 1 Schematic hypothetical representation of the Fc γ RIIb-232Ile redistribution to lipid rafts (*upper panel*) and 232Thr exclusion from lipid rafts (*lower panel*). Note that, even if 232Thr is (partially) excluded from lipid rafts, this does not necessarily mean that 232Thr is concomitantly excluded from B cell receptor (BCR) signaling machinery. IC immune complex



CD72c allele has been shown to be associated with systemic vasculitis phenotype (Qu et al. 2000), indicating that *CD72* accounts for one of the background genes of MRL.

Based on such information, we considered *CD72* as a candidate susceptibility gene for human SLE. Polymorphism screening revealed four polymorphic sites with minor allele frequencies >0.01, two of which were 13-nucleotide repeat polymorphism (once or twice) and four-nucleotide deletion in intron 8. These four polymorphic sites were in LD, forming two major haplotypes. The haplotypes were represented by the number of the 13-nucleotide repeat used as the tag polymorphism (*1 and *2) (Hitomi et al. 2004).

A case-control association study did not show significant association with the development of SLE; however, genotype *1/*1 was increased and *2/*2 was decreased in the SLE patients with nephritis. Furthermore, when the combination of the *FCGR2B* and *CD72* genotypes was examined in the Japanese and Thai populations, it was revealed that the association of *FCGR2B*-232Thr/Thr with SLE was more striking in individuals with *CD72*-*1/*1 genotype ($P=0.009$), while the risk of this *FCGR2B* genotype was substantially decreased and did not reach statistical significance in individuals with *CD72*-*1/*2 and *2/*2 genotypes (Hitomi et al. 2004) (Fig. 2). Thus, *CD72* polymorphisms were shown to affect the risk of *FCGR2B* polymorphisms for SLE, although *CD72* per se had only a slight effect, if any, on the development of SLE. It is interesting to note that *FCGR2B*-232Thr/Thr also showed a preferential association in the subgroup of SLE with nephritis (Chu et al. 2004), suggesting that genetic interaction with *CD72* may play a role.

In view of the two polymorphic sites within intron 8, we then examined whether the polymorphisms influence splicing. RT-PCR of RNA derived from peripheral blood mononuclear cells using primers placed within exons 6 and 9 revealed two amplification products, one of which was found to encode a novel alternative splicing isoform caused by exon 8 skipping (AS isoform). While the termination codon appears within exon 8 in the common isoform, exon 8 is skipped in the AS isoform. This would result in the translation of exon 9 sequences until the new termination codon appears, leading to the replacement of 42 C-terminal amino acids in the membrane-distal portion of the extracellular region with 49 new amino acids in the AS form. The predicted amino acid sequence maintains the C-type lectin domain, suggesting that the alternative isoform may be functional at the protein level. Indeed, we confirmed that the protein product of this isoform is expressed when the full-

length cDNA is introduced into the COS-7 cells (Hitomi et al. 2004).

We then examined whether the polymorphisms affect the efficiency of alternative splicing, and this was indeed found to be the case. Thus, although both common and AS isoforms were found in each individual, the proportion of AS isoform was clearly increased in individuals with *2/*2 genotype, followed by *1/*2. We confirmed that the 13-nucleotide repeat and four-nucleotide deletion directly regulate the splicing efficiency by minigene assay (Hitomi et al. 2004).

These results imply that the *CD72*-*2 allele increases the relative proportion of the *CD72* AS isoform, and protects its carriers from the risk of SLE conferred by the *FCGR2B*-232Thr/Thr genotype. The molecular mechanisms of this protection are currently being investigated.

Concluding remarks

It is surprising that our studies that focused on two genes provided potential keys to so many new fields of biological significance, such as the relationships between transmembrane polymorphism, lipid raft and signaling, as well as genomic polymorphism, alternative splicing and epistatic interaction.

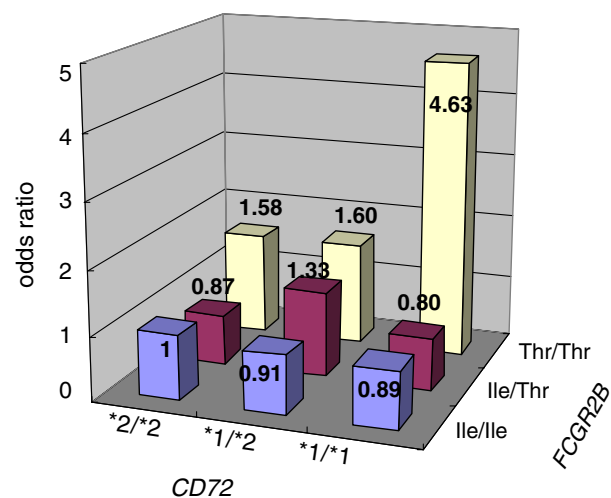


Fig. 2 Genetic interaction between *FCGR2B* and *CD72* polymorphisms against the development of SLE. Risk of SLE conferred by *FCGR2B*-232Thr/Thr genotype only reached statistical significance in individuals with *CD72*-*1/*1 ($P=0.009$), not in individuals with *CD72*-*1/*2 and *2/*2 genotypes. The odds ratios for the *FCGR2B* genotype alone were 2.38 (232Thr/Thr) and 1.10 (232Ile/Thr) versus 232Ile/Ile, and those for *CD72* alone were 1.07 (*1/*1), 1.18 (*1/*2) versus *2/*2. The data were derived from Japanese and Thai association studies combined via meta-analysis (Hitomi et al. 2004)

Human systems are much more complicated than we could possibly imagine, and immune systems are already known to be highly polymorphic, with most genes being subject to alternative splicing (Lynch 2004). Most of our current understanding of the immune system is based on the analysis of the interaction among the major isoform of the major allele of each gene. Although such analysis provides an indispensable scheme for the molecular interaction, in order to fully understand the diversity of immune responses among human populations, and the etiology and pathogenesis of human disease, it will be necessary to study multiple isoforms and alleles of each gene and their interactions as well.

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