In situ hybridization and immunohistochemistry

In situ hybridization was performed as previously described[50]. Probes were obtained from PCR products using the sequence of *FcRH3* (nt 2052–2490, comprising the intracellular unique region that is poorly conserved among members of this family). An additional probe of the 5′-UTR sequence yielded similar results. Control probes were also examined, and yielded no specific hybridization (data not shown). Antibodies to CD3 (clone PS-1, Nichirei) and CD20 (clone L26, Zymed) were used for immunohistochemistry with an ABC Elite kit (Vector Labs) according to the manufacturer's instructions. No specific staining was detected using mouse isotype IgG (data not shown).

Measurement of autoantibodies

Rheumatoid factor in sera of RA patients was measured using latex-enhanced immunonephelometric assay. Anti-DNA antibody in sera of SLE patients was measured by radioimmunoassay. RA patients (n=147, 81.1% women; mean age, 63.9 ±10.6 years; 87.8% rheumatoid factor positive; mean Steinbrocker radiographic stage, 3.2) and SLE patients (n=120, 92.6% woman; mean age, 36.6 ±12.7 years) were part of the cohorts and from a single medical institute, respectively. For each patient, the maximum value of rheumatoid factor and anti-DNA antibody during the treatment period in the medical center or outpatient clinic was used. Anti-cyclic citrullinated peptide antibody was detected at a single time point using enzyme-linked immunosorbent assay, as previously described[38].

Statistical analysis

LD index Δ[28] was calculated and Figure 1a drawn using Excel software (Microsoft). Haplotype frequencies were estimated using HAPLOTYPER software. The χ^2 test was applied for contingency table tests for associations between allele/genotype distribution and phenotypes. *FcRH3* expression in B-cells and autoantibody production were regressed on the number of susceptible alleles (coded 0, 1, and 2). All other statistical analyses, unless otherwise stated, were performed using STATISTICA software (StatSoft).

URLs

JSNP database (http://snp.ims.u-tokyo.ac.jp/index.html)

TRANSFAC (http://www.gene-regulation.com).

HAPLOTYPER (http://www.people.fas.harvard.edu/~junliu/Haplo/docMain.htm)

GenBank accession number

FcRH3 mRNA, NM_052939.

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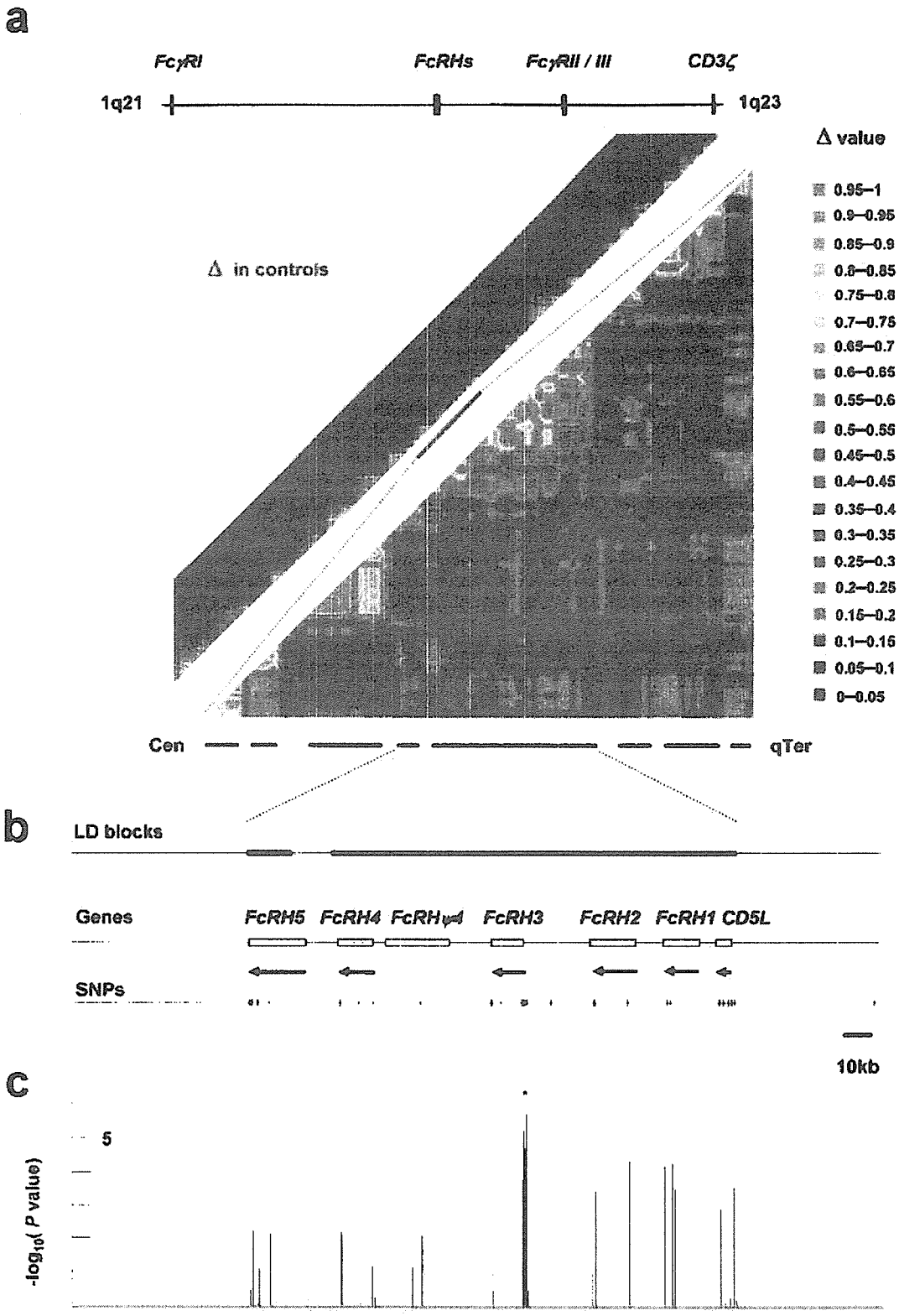

Figure 1.
LD and association of the FcRH gene cluster. (**a**) Pairwise LD between SNPs, as measured by Δ in 658 controls. The 16-Mb region in 1q21-23 (upper left) and the 2-Mb region around the FcRH gene cluster (lower right) were evaluated. (**b**) Location of LD blocks, genes and 41 SNPs in the FcRH gene cluster. (**c**) Case-control association test with 41 SNPs in the FcRH gene cluster using 830 patients and 658 controls. *Peak association.

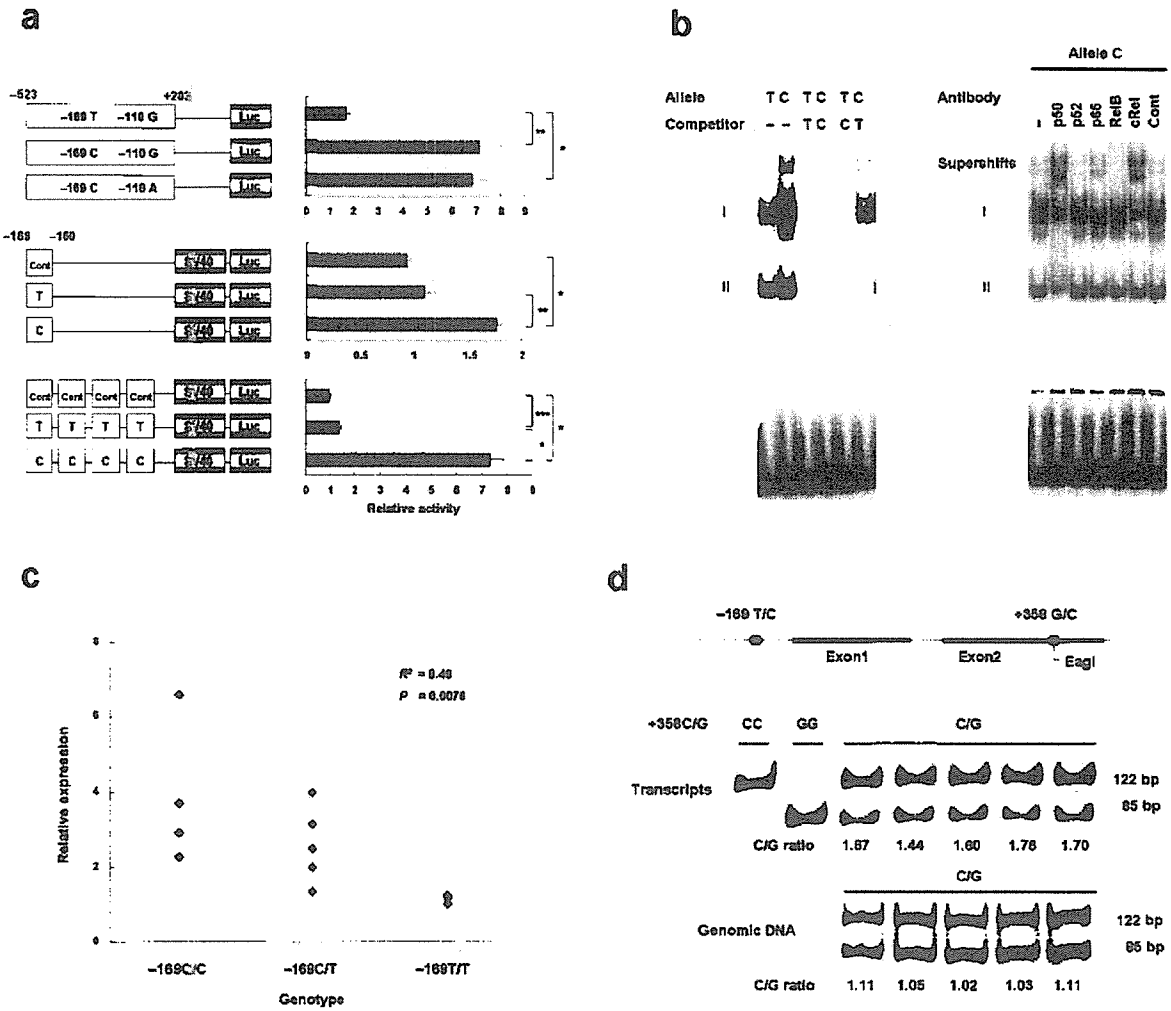

Figure 2.

Correlation of *FcRH3* expression with allele and genotype. (**a**) Promoter activity of haplotypes in *FcRH3* (top) and enhancing activity of the 30-bp promoter region around -169 C/T (middle and bottom), as evaluated by luciferase assay. Data represent mean ±SEM. Representative data from three experiments performed in quadruplicate. $^{*}P<0.0001$, $^{**}P<0.001$, and $^{***}P<0.01$ by Student's *t*-test. (**b**) Binding affinity of nuclear factors to the 30-bp promoter region around -169C/T, evaluated by EMSA. Allelic difference and competition experiment (left) and supershift experiment using antibodies for NF-κB components (right). (**c**) Expression of *FcRH3* measured by quantitative Taqman PCR of RNA purified from CD19-positive B-cells obtained from 13 healthy volunteers (C/C, n=4; C/T, n=5; T/T, n=4). (**d**) Allele-specific transcript quantification (ASTQ). *FcRH3* transcripts in B cells and genomic DNA from individuals (n=5) with heterozygous genotype (−169C+358C/−169T+358G) were amplified and quantified using an Eag I restriction fragment length polymorphism located at position +358. The 122-bp and 85-bp bands represent transcripts of the +358C allele and +358G allele, respectively. Transcripts from homozygous individuals (+358C/C and +358G/G) are shown as controls for digestion.

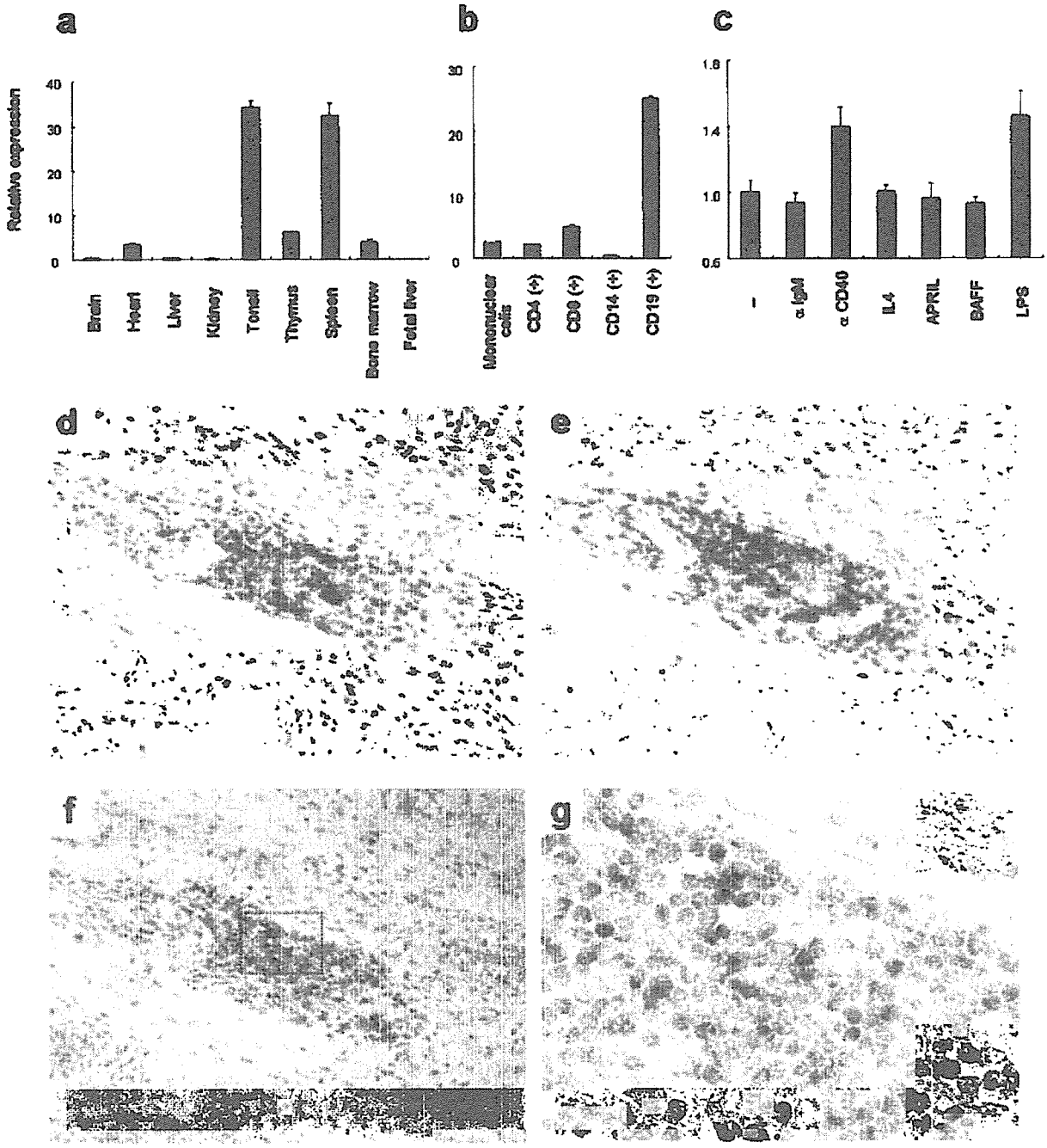

Figure 3.

Expression patterns of *FcRH3* in human tissues and cells. (**a**) Relative expression of *FcRH3* in various tissues. (**b**) Relative expression of *FcRH3* in fractionated leukocytes using MTC panel (Clontech). (**c**) Relative expression of *FcRH3* in response to stimuli (anti-CD40 Ab, 1 μg/ml; anti-IgM Ab, 1 μg/ml; IL-4, 10 ng/ml; APRIL, 10 ng/ml; BAFF, 10 ng/ml; LPS 100ng/ml for 4 h). Representative data from 3 experiments performed in triplicate. (**d, e**) Lymphocyte

aggregates in RA synovium. T-cells and B-cells in serial sections were immunostained using anti-CD3 (d) and anti-CD20 (e) antibodies respectively. (**f, g**) *FcRH3* mRNA expression (blue stain) in RA synovium as analyzed by *in situ* hybridization. Higher magnification views of synovium (g) are denoted by the *box* in f (magnifications: d, e, f, ×100; g, ×400). Counterstaining: d, e, hematoxylin; f, g, nuclear fast red.

Table 1

Case-control analysis of *FcRH3*

SNPs[a] ID	Location	Allele 1/2	Allele1 frequency Patients	Controls	Genotype 11 versus 12+22 OR (95% CI)	χ^2	*P*
fcrh3_3	−169	C/T	0.42	0.35	2.15 (1.58–2.93)	24.3	0.00000085
fcrh3_4	−110	A/G	0.25	0.18	3.01 (1.71–5.29)	16.1	0.000060
fcrh3_5	Exon2	C/G	0.42	0.35	2.05 (1.51–2.78)	21.6	0.0000033
fcrh3_6	Intron3	A/G	0.42	0.34	2.02 (1.49–2.75)	20.8	0.0000052

[a] SNPs with $P<0.0001$ in allele frequency comparison test

Table 2

Haplotype structure and frequency in *FcRH3*

Haplotype[a]	fcrh3_3/4/5/6	Haplotype frequency	
		Patients	Controls
Haplotype1	TGGG	0.58	0.65
Haplotype2	CACA	0.25	0.19
Haplotype3	CGCA	0.17	0.14

[a] Haplotypes with frequency >0.01

Table 3

Genotype and autoantibodies in patients

Genotype	Rheumatoid factor		Anti-CCP antibody	
	n (N=148)	Serum level ±SEM (IU/ml)	n (N=71)	Positivity (%)
−169 C/C	29	479.9 ±91.3[a]	17	100.0[b]
−169 C/T	75	323.7 ±47.3[a]	35	94.3[b]
−169 T/T	44	216.4 ±44.0[a]	19	73.7[b]

[a] R^2=0.049, P=0.0065 by regression analysis.

[b] P=0.029 by Fisher's exact test.

Table 4

Association of SNP -169C/T with AITD and SLE

Disease	Number of subjects	Genotype			Allele C frequency	Recessive-trait comparison		
		CC	CT	TT		OR (95% CI)	χ^2	*P*
GD	351	72	179	100	0.46	1.79 (1.34–2.39)	15.7	0.000074
HT	158	30	74	54	0.42	1.62 (1.07–2.47)	5.2	0.022
AITD total	509	102	253	154	0.45	1.74 (1.35–2.24)	18.5	0.000017
SLE	564	100	259	205	0.41	1.49 (1.16–1.92)	9.8	0.0017
RA+AITD +SLE[a]	2437	438	1167	832	0.42	1.52 (1.29–1.79)	24.2	0.00000084
Controls	2037	257	995	785	0.37			

[a] RA represents sum of three sets (n=1364).

GD = Graves' disease; HT = Hashimoto's thyroiditis; AITD = Autoimmune thyroid disease; SLE = Systemic lupus erythematosus.