DOI:10.1111/j.1365-2125.2006.02771.x

Distribution of the FcγRIIIa 176 F/V polymorphism amongst healthy Chinese, Malays and Asian Indians in Singapore

K. T. Chong, W. F. Ho, S. H. Koo, Peter Thompson & Edmund J. D. Lee
Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

What is already known about this subject

- Genetic variability of the FcγRIIIa 176 F/V polymorphism has been widely studied in patients with systemic lupus erythomatosus and rheumatoid arthritis, as well as the general population of various White groups.
- Its implications in disease pathogenesis and response to therapeutics have been well documented.
- This study aimed to profile its polymorphism pattern in the Asian population, thus it serves as useful reference controls in disease association studies and tailoring therapy to ethnic-specific populations.

What this study adds

- In this study, we have established the genetic variability profile of the FcγRIIIa 176 F/V polymorphism in three distinct Asian groups (Chinese, Malays and Indians) and also supplemented existing data based on ethnic-specific healthy controls.
- The polymorphism pattern of Malays was found to differ significantly from Chinese and Indians, which was also extended to almost all other healthy controls of various ethnic groups published elsewhere.
- Such differences could have implications in disease susceptibility and pathogenesis, as well as response to drug therapeutics.

Correspondence
Professor Edmund Jon Deoon Lee, Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, 18 Medical Drive, Building MD2, Singapore 117597.
Tel: + 65 6874 3677
Fax: + 65 6873 7690
E-mail: edlee@nus.edu.sg

Keywords
disease associations, ethnicity, FcγR, genetic polymorphism, therapeutics

Received
16 March 2006
Accepted
19 June 2006
Published *OnlineEarly*
19 September 2006

Aims
To determine and compare the distribution of the FcγRIIIa 176 F/V polymorphism across three ethnically distinct populations (Chinese, Asian Indians and Malays) in Singapore.

Methods
The FcγRIIIa 176 F/V polymorphism was genotyped by direct sequencing from genomic DNA samples obtained from normal healthy Chinese, Asian Indians and Malays (n = 192 from each population).

Results
The allelic frequencies of the high binding affinity FcγRIIIa 176 V allele for Chinese, Asian Indians and Malays were 35%, 33% and 46%, respectively (F allele frequencies were 65%, 67% and 54%, respectively). Genotype distributions were found to conform to the Hardy–Weinberg law ($P > 0.05$) in each group. χ^2 comparisons revealed significant differences in the genotype distributions of the FcγRIIIa 176 V/F polymorphism of Malays from the other two populations (Chinese and Asian Indians). However, no significant difference in the genotype distributions of the FcγRIIIa 176 V/F polymorphism was observed between Chinese and Asian Indian populations.

Conclusions
The genotype distributions of the FcγRIIIa 176 V/F polymorphism in healthy Malays are significantly different from both Chinese and Indians. These observations provide the fundamentals on which future disease associations may be built and also present important implications for the design of therapeutic regimens amongst various ethnic groups.

Introduction

Receptors with innate affinities for the Fc domain of IgG (FcγR) are important components in the link between the humoral immune response and cell-mediated defence system. These IgG receptors consist of three major classes encompassing eight genes (CD64: FcγR1A/1B/1C; CD32: FcγRIIa/IIb/IIc; CD16: FcγRIIa/IIIb) and are localized on the long arm of chromosome 1 (1q21, 23–24) [1, 2]. FcγRIIIa, expressed predominantly on macrophages, natural killer (NK) cells, monocytes and T cells, binds complexed IgG and is responsible for the clearance of immune complexes. Located on 1q23, FcγRIIIa has a common biallelic polymorphism [phenylalanine (F) or valine (V)] at amino acid position 176 (rs396991) [3] resulting in variable binding phenotypes (F/F: low binding affinity; V/V: high binding affinity). As such, the FcγRIIIa 176 F/V polymorphism has been closely associated with autoimmune diseases such as systemic lupus erythomatosus (SLE) [4, 5], rheumatoid arthritis (RA) [6–8], idiotypic thrombocytopenia (ITP) [9, 10] and Guillain–Barré disease [11, 12].

The FcγRIIIa 176 F/V polymorphism has been found to influence therapeutic outcome. Recent studies have suggested that such polymorphisms affect the efficacy and toxicity of monoclonal antibody-based immunotherapy [13, 14]. In addition, several studies have also found ethnic variation in the distribution of various FcγR genotypes [15, 16]. This study aimed to determine and compare the distribution of the FcγRIIIa 176 F/V polymorphism in healthy donors across three ethnically distinct populations (Chinese, Malays and Asian Indians). The results derived here supplement current knowledge of ethnic variability in disease pathogenesis and drug response therapeutics. These population frequency profiles could also serve as useful reference controls in association studies involving the FcγRIIIa 176 F/V polymorphism.

Methods and results

One hundred and ninety-two healthy individuals (aged 15–60 years at the time of study) from each of the three major ethnic groups (Chinese, Malays and Asian Indians) in Singapore were recruited with local institutional ethics approval (Institutional Review Board, National University Hospital, Singapore) and written informed consent. For inclusion in the study, donors were required to declare a medical history free of major illness and grandparents of identical ethnicity. Median ages for Chinese, Malays and Asian Indians were 22 (99 males, 93 females), 22 (90 males, 102 females) and 21 years (118 males, 78 females), respectively. Venous blood (10 ml) was sampled and genomic DNA isolated from peripheral leucocytes via a standard desalting method. FcγRIIIa 176 V/F genotyping was performed by direct sequencing on a 162-bp polymerase chain reaction product amplified from the extracted DNA using flanking primers as previously described [17]. No other novel single nucleotide polymorphisms were uncovered in the samples.

The genotypic distributions of the FcγRIIIa 176 F/V polymorphism in each of the three populations studied here ($n = 192$ each) were found in full concordance with Hardy–Weinberg equilibrium ($P > 0.05$) using a χ^2 test statistic with one degree of freedom. Frequencies of the high binding affinity V allele for Chinese, Indians and Malays were 35%, 33% and 46%, respectively. F allele frequencies were 65%, 67% and 54% in Chinese, Asian Indians and Malays, respectively (Table 1). Differences in genotypic distributions of the FcγRIIIa 176 F/V polymorphism were compared in a pair-wise manner across the three populations using χ^2 test statistics (degree of freedom = 2). There was no significant difference in genotypic distributions of the FcγRIIIa 176 F/V polymorphism between Chinese and Asian Indians in Singapore ($\chi^2 = 0.86$, $P = 0.651$, $P > 0.05$). However, χ^2 test comparisons of Malays *vs.* either Chinese

Table 1
Allele frequencies and genotype distribution of the FcγRIIIa 176 F/V polymorphism in Chinese, Malays and Asian Indians.

Race	Sample size	TT (F/F)	TG (F/V)	GG (V/V)	Allele frequency T (F)	Allele frequency G (V)	HWE χ^2	HWE *P*
Chinese	192	79	90	23	0.65	0.35	0.12	0.942
Malay	192	63	82	47	0.54	0.46	0.05	0.153
Asian Indian	192	88	83	21	0.67	0.33	3.76	0.975

All three races were in Hardy–Weinberg equilibrium (HWE) (P > 0.05).

($\chi^2 = 10.40$, $P = 0.006$, $P < 0.05$) or Asian Indians ($\chi^2 = 14.09$, $P = 0.009$, $P < 0.05$) revealed significant differences in the genotypic frequencies of FcγRIIIa 176 F/V polymorphism.

Discussion

The genotype frequencies of the Fcγ receptor family (e.g. FcγRIIa and FcγRIIIb) have been shown extensively to exhibit significant interethnic variability [18–20]. Our analysis of the FcγRIIIa 176 F/V polymorphism across three ethnic groups in Singapore revealed a genotype distribution that was consistent in both Chinese and Asian Indians. However, there was a significant difference of this distribution observed in Malays. Extended analysis by comparing our genotype data with work reported previously elsewhere (Table 2) did not suggest significant variance in genotype distribution of the FcγRIIIa 176 F/V polymorphism. However, the observed genotype distribution of Malays was significantly different from that of almost all populations compared. Such observations may have obvious implications for the development of autoimmune diseases such as SLE and RA in Malays compared with Chinese and Asian Indians in Singapore, even though the specific roles this polymorphism plays in disease pathogenesis remain highly contentious [24, 27]. Unfortunately, there have been no reports dedicated to the incidence of such diseases amongst the specified ethnic groups either in Singapore or elsewhere. Haplotype structure and population migration profiles have also not been studied. Nonetheless, we present data here depicting the distribution of the FcγRIIIa 176 F/V genotypes in ethnic-specific control populations that provide an important basis for the interpretation of future association studies.

The use of genetic markers as a tool in predictive pharmacotherapy has gained considerable interest over the past decade. An area that has become increasingly popular is the use of target-specific immunoglobulins in treating B-cell malignances. Rituximab (Rituxan® and Mabthera®), a chimeric human immunoglobulin G_1 monoclonal antibody, has recently been used with reasonable success for the treatment of patients with low-grade B-cell lymphomas, including Waldenström's macroglobulinaemia (WM). While 30–50% of patients receiving such treatment showed little or no clinical response, studies have shown that a higher response rate of WM patients to rituximab was observed in patients haboring the FcγRIIIa 176 V/V (high binding affinity)

Table 2
χ^2 comparisons of the genotype distributions in healthy control populations reported elsewhere

Race	Sample size	Allele frequencies		Genotypes			Pair-wise χ^2 (*P*-value)			Reference
		V	F	F/F	F/V	V/V	Chinese	Malay	Asian Indian	
Chinese	311	0.34	0.66	133	146	32	>0.05	<0.05	>0.05	[21]
Norwegian	270	0.3	0.7	132	101	37	>0.05	<0.05	>0.05	[15]
Samis	193	0.2	0.8	122	62	9	<0.05	<0.05	<0.05	
Dutch	176	0.34	0.66	74	85	17	>0.05	<0.05	>0.05	[17]
Japanese	104	0.26	0.74	54	46	4	<0.05	<0.05	>0.05	
Korean	197	0.38	0.62	71	104	22	>0.05	<0.05	>0.05	[23]
African-Americans	152	0.33	0.67	64	76	12	>0.05	<0.05	>0.05	[16]
Whites	181	0.3	0.7	91	71	19	>0.05	<0.05	>0.05	
Whites	581	0.31	0.69	275	249	57	>0.05	<0.05	>0.05	[8]
Korean	144	0.37	0.63	52	77	15	>0.05	<0.05	>0.05	[24]
Dutch	514	0.38	0.62	197	243	74	>0.05	<0.05	>0.05	[12]
UK	111	0.48	0.52	29	57	25	<0.05	>0.05	<0.05	
Norweigian	89	0.38	0.62	197	243	74	>0.05	<0.05	>0.05	
Whites	420	0.33	0.67	172	213	35	>0.05	<0.05	>0.05	[22]
Indians	93	0.37	0.63	44	35	14	>0.05	<0.05	>0.05	
Dutch	514	0.38	0.62	197	243	74	>0.05	<0.05	>0.05	[25]
Japanese	149	0.27	0.73	83	53	13	>0.05	<0.05	>0.05	
Thais	187	0.4	0.6	64	96	27	>0.05	<0.05	>0.05	[26]

Not significant (P > 0.05); significant (P ≤ 0.05).

vs. 176 F/F polymorphism (low binding affinity) [13, 14]. An association between the high binding affinity polymorphism and response to active idiotypic immunization has also been reported [28]. These observations are important, as they suggest a predictive role of the FcγRIIIa 176 F/V polymorphism in anticipating therapeutic response that could revolutionalize conventional monoclonal antibody-based strategies in cancer treatment. Already, predictive FcγRIIIa genotyping has aided clinicians in deciding whether rituximab is to be used as a monotherapy (in V/V homozygotes) or in combination with chemotherapeutic drugs (in F/F homozygotes). Moreover, other drugs could also be applied to regulate the immune and Fc response in anticipation of potential therapeutic failure/poor response or adverse drug reactions associated with unfavourable FcγRIIIa genotypes. In addition, therapeutic regimes requiring the use of intact IgGs, such as Herceptin® or Erbitux®, could also benefit from preprescription FcγRIIIa genotyping. The data presented here could serve as initial prognostic markers in determining drug response.

In summary, we have presented the allele frequencies and genotype distributions of the FcγRIIIa 176 F/V polymorphism in Chinese, Malays and Asian Indians. Ethnic differences were evident in our study population. Differences in genotype distributions could suggest ethnic-related variability in disease susceptibility, even though such claims remain to be fully elucidated. Nonetheless, our data provide a useful resource on which future case–control analysis may be based. More importantly, the prognosis of therapeutic effectiveness among different populations may be prospectively predicted from existing data.

This work was supported by Phase Solutions Pte Ltd.

References

1 Kimberly RP, Salmon JE, Edberg JC. Receptors for immunoglobulin G. Molecular diversity and implications for disease. Arthritis Rheum 1995; 38: 306–14.

2 Ernst LK, van de Winkel JG, Chui IM, Anderson CL. Three genes for the human high affinity Fc receptor for IgG (FcγRI) encode four distinct transcription products. J Biol Chem 1992; 267: 15692–700.

3 Ravetch JV, Perussia B. Alternative membrane forms of FcγRIII (CD16) on human natural killer cells and neutrophils. Cell type expression of two genes that differ in single nucleotide substitutions. J Exp Med 1989; 170: 481–97.

4 Koene HR, Kleijer M, Swaak AJG, Sullivan KE, Bijl M, Petri MA, Kallenberg CG, von Roos D, dem Borne AE, de Haas M. The Fc gammaRIIIA-158F allele is a risk factor for systemic lupus erythematosus. Arthritis Rheum 1998; 41: 1813–8.

5 Karassa FB, Trikalinos TA, Ioannidis JP, Fc gamma RIIIA-SLE meta-analysis investigators. The FcγRIIIA-F158 allele is a risk factor for the development of lupus nephritis: a meta analysis. Kidney Int 2003; 63: 1475–82.

6 Nieto A, Caliz R, Pascual M, Mataran L, Garcia S, Martin J. Involvement of FcγRIIIA genotypes in susceptibility to rheumatoid arthritis. Arthritis Rheum 2000; 43: 735–9.

7 Brun JG, Madland TM, Vedeler CA. Immunoglobulin G Fc receptor (FcR) IIA IIIA and IIIB polymorphisms related to the severity in rheumatoid arthritis. J Rheumatol 2002; 29: 1135–40.

8 Morgan AW, Keyte VH, Babbage SJ, Robinson JI, Ponchel F, Barrett JH, Bhakta BB, Bingham SJ, Buch MH, Conaghan PG, Gough A, Green M, Lawson CA, Pease CT, Markham AF, Ollier WE, Emery P, Worthington J, Isaacs JD. FcRIIIA-158V and rheumatoid arthritis: a confirmation study. Rheumatology (Oxford) 2003; 42: 528–33.

9 Foster CB, Zhu S, Erichsen HC, Lehrnbecher T, Hart ES, Choi E, Stein S, Smith MW, Steinberg SM, Imbach P, Kuhne T, Chanock SJ, Early Chronic ITP Study Group. Polymorphisms in inflammatory cytokines and Fcgamma receptors in childhood chronic immune thrombocytopenic purpura: a pilot study. Br J Haematol 2001; 113: 596–9.

10 Williams Y, Lynch S, McCann S, Smith O, Feighery C, Whelan A. Correlation of platelet Fc gammaRIIA polymorphism in refractory idiopathic (immune) thrombocytopenic purpura. Br J Haematol 1998; 101: 779–82.

11 Vedeler CA, Myhr KM, Myland H. Fc receptors for immunoglobulin G – a role in the pathogenesis of Guillain–Barre syndrome and multiple sclerosis. J Neuroimmunol 2001; 118: 187–93.

12 van Sorge NM, van der Pol WL, Jansen MD, Geleijins KPW, Kalmijn S, Hughs RAC, Rees JH, Pritchard J, Vedeler CA, Myhr KM, Shaw C, van Schaik IN, Wokke JHJ, van Doorn PA, Jacobs BC, van de Winkel JGJ, van den Berg LH. Severity of Guillain–Barré syndrome is associated with Fcγ receptor III polymorphisms. J Neuroimmunol 2005; 162: 157–64.

13 Treon SP, Hansen M, Branagan AR, Verselis S, Emmanouilides C, Kimby E, Frankel SR, Touroutoglou N, Turnbull B, Anderson KC, Maloney DG, Fox EA. Polymorphisms in FcγRIIIA (CD16) receptor expression are associated with clinical response to Rituximab in Waldenström's macroglobulinemia. J Clin Oncol 2005; 23: 474–81.

14 Cartron G, Dacheux L, Salles G, Solal-Celigny P, Bardos P, Colombat P, Watier H. Therapeutic activity of humanized anti-CD20 monoclonal antibody and polymorphism in IgG Fc receptor FcgammaRIIIa gene. Blood 2002; 99: 754–8.

15 Torkildsen Ø, Utsi E, Mellgren SI, Harbo HF, Vedeler CA, Myhr KM. Ethnic variation of Fcγ receptor polymorphism in Sami and Norweigian populations. Immunology 2005; 115: 416–21.

16 Lehrnbecher T, Foster CB, Zhu S, Leitman SF, Goldin LR, Huppi K, Chanock SJ. Variant genotypes of the low-affinity Fc receptors in two control populations and a review of low-affinity Fc receptor polymorphisms in control and disease populations. Blood 1999; 94: 4220–32.

17 Leppers-van de Straat FGJ, van der Pol WL, Jansen MD, Sugita N, Yosie H, Kobayashi T, van de Winkel JG. A novel PCR-based method for direct Fc receptor IIIa (CD16) allotyping. J Immunol Methods 2000; 242: 127–32.

18 van der Pol W, van de Winkel JG. IgG receptor polymorphisms: risk factors for disease. Immunogenetics 1998; 48: 222–32.

19 Rascu A, Repp R, Weaterdaal NAC, Kalden JR, van de Winkel JGJ. Clinical relevance of FcγR polymorphisms. Ann NY Acad Sci 1997; 815: 282–95.

20 Osborne JM, Chacko GW, Brandt JT, Anderson CL. Ethnic variation in frequency of an allelic polymorphism of human FcγRIIa determined with allele specific oligonucleotide probes. J Immunol Methods 1994; 173: 207–17.

21 Chen JY, Wang CM, Tsao KC, Chow JM, Wu JM, Li CL, Ho HH, Jan Wu YJ, Luo SF. Fcγ receptor IIa IIIa, and IIIb polymorphisms of systemic lupus erythematosus in Taiwan. Ann Rheum Dis 2002; 63: 877–80.

22 Milicic A, Misra R, Agrawal S, Aggarwal A, Brown MA, Wordsworth BP. The 158V polymorphism in FcγRIIIA shows disparate associations with rheumatoid arthritis in two genetically distinct populations. Ann Rheum Dis 2002; 61: 1021–3.

23 Yun HR, Koh HK, Kim SS, Chung WT, Kim DW, Hong KP, Song GG, Chang HK, Choe JY, Bae SC, Salmon JE, Yoo DH, Kim TY, Kim SY. FcγRIIa/IIIa polymorphism and its association with clinical manifestations in Korean lupus patients. Lupus 2001; 10: 466–72.

24 Lee EB, Lee YJ, Baek HJ, Kang SW, Chung ES, Shin CH, Hong KM, Tsao BP, Hahn BH, Song YW. Fcγ receptor IIIA polymorphism in Korean patients with systemic lupus erythematosus. Rheumatol Int 2002; 21: 222–6.

25 Van der Pol W, Jansen MD, Sluiter WJ, van de Sluis B, Lepper-van de Straat FGJ, Kobayashi T, Westendorp RGJ, Huizinga TWJ, Van de Winkel JGJ. Evidence for non-random distribution of Fcγ receptor genotype combinations. Immunogenetics 2003; 55: 240–6.

26 Siriboonrit U, Tsuchiya N, Sirikong M, Kyogoku C, Bejrachandra S, Suthipinittham P, Luangtrakool K, Srinak D, Thongpradit R, Fujiwara K, Chandanayingyong D, Tokunaga K. Association of Fcγ receptor IIb and IIIb polymorphisms with susceptibility to systemic lupus erythematosus in Thais. Tissue Antigens 2003; 61: 374–83.

27 Magnusson V, Johanneson B, Lima G, Odeberg J, Alarcón-Segovia D, Alarcón-Riquelme ME and the SLE Genetics Collaboration Group. Both risk alleles for Fcγ RIIA and FcγRIIIA are susceptilbilty factors for SLE: a unifying hypothesis. Genes Immunol 2004; 5: 130–7.

28 Weng WK, Czerwinski D, Timmerman J, Hsu FJ, Levy R. Clinical outcome of lymphoma patients after idiotypic vaccination is correlated with humoral immune response and immunoglobulin G Fc receptor genotype. J Clin Oncol 2004; 22: 4717–24.