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Evidence for association between a Toll-like receptor 4 gene polymorphism and moderate/severe periodontitis in the Japanese population

T. Fukusaki[1], N. Ohara[2], Y. Hara[1], A.Yoshimura[1], K. Yoshiura[3,4]
Departments of [1]Periodontology, [2]Microbiology and Oral Infection and [3]Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan and [4]Solution Oriented Research of Science and Technology (SORST), Japan Science and Technology Agency (JST), Kawaguchi, Japan

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Background and Objective: Chronic periodontitis is an inflammatory disease caused by bacteria in subgingival pockets. Because Toll-like receptor 2 and Toll-like receptor 4 have been shown to play an important role in the recognition of periodontal pathogens, we investigated the relevance of genetic variations in *TLR2* and *TLR4* to susceptibility to periodontitis.

Material and Methods: A total of 97 patients with chronic periodontitis and 100 control subjects were examined for mutations in *TLR2* and *TLR4*. Case-control analysis was performed using individual single nucleotide polymorphisms detected during the mutation search.

Results: The missense mutations reported previously in *TLR2* (677 Arg > Trp and 753 Arg > Gln) and in *TLR4* (299 Asp > Gly and 399 Thr > Ile) were not detected in 97 of the Japanese patients with chronic periodontitis or in 100 of the Japanese control subjects. Nine single nucleotide polymorphisms were identified in exons of *TLR2* and *TLR4*. The case-control analysis revealed that the frequency of the C/C genotype at base-pair position +3725 in *TLR4* was significantly higher in both the moderate and the severe periodontitis patient group than in the control group.

Conclusion: A genetic variation of *TLR4* might be associated with moderate and severe periodontitis in the Japanese population.

Atsutoshi Yoshimura, DDS, PhD, Department of Periodontology, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki 852–8588, Japan
Tel: +81 95 849 7682
Fax: +81 95 849 7684
e-mail: ayoshi@nagasaki-u.ac.jp

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Chronic periodontitis is an inflammatory disease caused by bacterial colonization in the subgingival area (1). The bacteria and their cell wall components can trigger activation of the host immune system through pattern-recognition receptors to induce inflammatory mediators, leading to the destruction of periodontal tissue. Toll-like receptor 2 and Toll-like receptor 4 are two principal pattern-recognition receptors dedicated to the recognition of bacterial cell wall components, such as lipoproteins and lipopolysaccharides (2,3). We previously demonstrated that Toll-like receptor 2 and Toll-like receptor 4 are involved in the recognition of periodontopathic bacteria, such as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans* and *Fusobacterium nucleatum* (4), and that the expression of those two receptors is augmented in connective tissue subjacent to the periodontal epithelium in patients with severe periodontitis (5). These findings suggest that Toll-like receptor 2 and Toll-like

receptor 4 may be involved in the pathogenesis of periodontal diseases.

It has been reported that two missense mutations in *TLR4* (D299G and T399I) are associated with endotoxin hyporesponsiveness. The allele frequency of the D299G mutation was demonstrated to be 3–8% in European and American populations, and these mutations were associated with a blunted response of the subjects to inhaled lipopolysaccharide (6). Two missense mutations in *TLR2* (R677W and R753Q) were later identified (7,8). Although the R753Q mutation occurred in $\approx 3\%$ of healthy subjects, the mutation was detected at a higher frequency (9%) in patients with gram-positive septic shock in France and was associated with a significantly reduced response to bacterial lipoprotein. The R677W mutation was detected in 10 of 45 lepromatous leprosy patients in Korea, but not found in 41 tuberculoid leprosy patients or in 45 healthy controls.

Recently, the association between these four mutations and periodontitis has been investigated. Folwaczny *et al.* found no association between chronic periodontitis and the missense mutations in *TLR2* and *TLR4* (9), and Laine *et al.* also demonstrated that the missense mutations in *TLR4* were not associated with severe periodontitis (10). On the other hand, Schröder *et al.* reported a positive association between periodontitis and the missense mutations in *TLR4* (11). They reported that patients suffering from chronic periodontitis showed a higher frequency of the missense mutations (D299G/T399I) than controls, and that the genotypes with D299G or T399I were found exclusively in patients, whereas no difference was observed for *TLR2* (11). Brett *et al.* reported, conversely, that the *TLR4* T399I minor allele was more frequent in controls than in patients with aggressive and chronic periodontitis (12). These complicated results might be explained by differences between populations.

The aim of this study was to determine whether these polymorphisms have any association with periodontitis in the Japanese population. We were unable to detect any of these four missense mutations in *TLR2*/*TLR4* in 197 Japanese subjects. Therefore, we attempted to identify single nucleotide polymorphisms in *TLR2* and *TLR4* in Japanese periodontitis patients, and we performed association analysis, using single nucleotide polymorphisms in *TLR2*/*TLR4*, to periodontitis. We show here the association between one single nucleotide polymorphism in *TLR4* and severe/moderate periodontitis in Japanese subjects.

Table 1. Classification of periodontitis patients and controls

		Classification of periodontitis		
	Controls	Mild	Moderate	Severe
Bone loss $\geq 50\%$	–	–	$\leq$ 3 teeth	$\geq$ 4 teeth
No. of subjects	100	16	65	16
Maximum PPD (mm)	2.95 ± 0.22	4.13 ± 0.50 ($p < 0.01$)	6.15 ± 0.71 ($p < 0.001$)	10.50 ± 1.46 ($p < 0.001$)

Periodontitis patients were assigned to one of three groups of disease severity on the basis of the above criteria.
p-values were calculated in comparison to the control group.
PPD, probing pocket depth.

Material and methods

Subjects

Patients with chronic periodontitis (59 women and 38 men) and healthy subjects (53 women and 47 men), who visited Nagasaki University Hospital, were enrolled in this study. All of the subjects were Japanese, resided in or around Nagasaki, and had more than 20 teeth. Individuals with malignant diseases, immunodeficiencies, pregnancy, diabetes mellitus, or who had infectious diseases, such as acquired immunedeficiency syndrome or adult T-cell leukemia, were excluded. The mean age of the patients was 60 years (range: 36–83 years) and that of the control population was 46 years (range: 25–75 years). The subjects were screened by full-mouth radiographic assessment and periodontal examinations. Subjects who had neither alveolar bone loss of > 25%, nor periodontal attachment loss of > 3 mm at any sites, were classified into the control group. Subjects who had alveolar bone loss of > 25%, or periodontal attachment loss of > 3 mm in at least at one site, were classified into the chronic periodontitis group. Periodontitis patients were further classified into three groups on the basis of the criteria of disease severity described in Table 1. To make a comparison with a group in the same age range, periodontitis patients were classified into two groups. Patients who were < 60 years of age were categorized into the younger periodontitis patient group, and patients ≥ 60 years of age were categorized into the older periodontitis patient group. The mean age of the younger periodontitis patients was 49 years and that of the older periodontitis patients was 70 years. There was no significant difference between the ages of the younger periodontitis patients and those of the control subjects. Written informed consent was obtained from all of the participants in this study.

Detection of missense mutations in *TLR2* (R677W and R753Q) and *TLR4* (D299G and T399I)

DNA was extracted from peripheral blood leukocytes by the phenol–chloroform method and harvested by ethanol precipitation. Genotyping of *TLR2* (R677W and R753Q) and *TLR4* (D299G and T399I) was accomplished with the polymerase chain reaction (PCR) and restriction enzyme digestion, following the procedures described by Schröder *et al.* (13) and Lorenz *et al.* (14), respectively.

Determination of polymorphisms/mutations in *TLR2 and TLR4*

In order to identify single nucleotide polymorphisms peculiar to the Japanese patient group, we performed direct sequencing of all the exons and introns of *TLR2* and *TLR4* (15) in 16 patients with severe periodontitis. PCR ampli-

fication was performed at various annealing temperatures using Takara ExTaq™. PCR products were sequenced using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems, Foster City, CA, USA) and run on an ABI 3100 automated sequencer (Applied Biosystems). Sequencing electropherograms were aligned by ATGC software, version 3.0 (Genetyx, Tokyo, Japan), and base alterations were inspected visually.

Statistical calculation for the case-control study

As a result of single nucleotide polymorphism/mutation detection, we found five single nucleotide polymorphisms in *TLR2* and four single nucleotide polymorphisms in *TLR4*. Next, we performed genotyping of all of those single nucleotide polymorphisms in the remaining 81 patients and 100 controls. Individual single nucleotide polymorphisms were tested for Hardy–Weinberg distribution and linkage disequilibrium using SNPALYZE software (Dynacom, Yokohama, Japan). Case-control analysis was performed at individual single nucleotide polymorphisms using SNPALYZE software. The values of D′ and r^2 were calculated and referred for haplotype analysis.

Results

We failed to identify any of the reported mutations – R677W and R753Q in *TLR2* and D299G and T399I in *TLR4* – in 197 Japanese subjects, comprising 97 patients with periodontitis and 100 healthy controls.

To examine an association between *TLR2*/*TLR4* and periodontitis, we needed to find single nucleotide polymorphisms in *TLR2* and *TLR4*. Therefore, we decided to perform direct sequencing of *TLR2* and *TLR4* in 16 of the patients with severe periodontitis in the present study. Three point mutations in the 5′-untranslated region, and two synonymous mutations in the coding region of *TLR2*, were identified at base-pair positions −183, −148, −146, +1350 (corresponding to rs3804100) and +2343 (corresponding to rs5743709). Four point mutations were identified in the 3′-untranslated region of *TLR4* at base-pair positions +3528, +3725 (corresponding to rs11536889), +4022 and +4529 bp (Table 2). None of the mutations resulted in amino acid substitution. Seven of the nine single nucleotide polymorphisms were present at a frequency of <2% in patients and therefore would provide only a low power of association, but we performed association analysis using all of the nine single nucleotide polymorphisms. The case-control analysis revealed a significant difference between the genotype frequency of the mutation at base-pair position +3725 in *TLR4* of the patient group with that of the control group ($p = 0.043$) (Table 3). There was no significant difference between the frequencies of the other eight single nucleotide polymorphisms. Next, the genotype frequency at base-pair position +3725 in *TLR4* of the control group was compared with the genotype frequencies in the groups of patients with mild, moderate and severe periodontitis. There was no significant difference between the control group and the mild periodontitis patient group, but significant differences were found between the control group and the moderate/severe periodontitis patient groups, as

Table 2. Minor allele frequencies of single nucleotide polymorphisms in TLR2 and TLR4 genes

Gene and SNP position	SNP ID in NCBI	Nucleotide change in major/minor alleles	Minor allele frequency		HWE
			Controls	Patients	
TLR2					
−183	[a]	A/G	0	0.0053	
−148	[a]	C/T	0	0.0053	
−146	[a]	T/G	0	0.0053	$p > 0.05$
+1350	rs3804100	T/C	0.2447	0.1684	
+2343	rs5743709	G/A	0	0.0156	
TLR4					
+3528	[a]	C/G	0	0.0105	$p > 0.05$
+3725	rs11536889	G/C	0.1414	0.1959	
+4022	[a]	C/G	0	0.0053	
+4529	[a]	G/C	0.0104	0.0053	

[a]Novel single nucleotide polymorphism (SNP).
HWE, *p*-value of the Hardy–Weinberg equilibrium test in the control population. (The Hardy–Weinberg equilibrium test was performed for only two single nucleotide polymorphisms because minor allele frequencies of other single nucleotide polymorphisms were < 0.05.); ID, identity; NCBI, National Center for Biotechnology Information.

Table 3. Case-control analysis using single nucleotide polymorphisms in TLR2 and TLR4 genes

Gene and SNP position	Genotype frequency (%)						*p*-value
	Controls			Patients			
	MM	Mm	mm	MM	Mm	mm	
TLR2							
−183	93 (100)	0 (0)	0 (0)	90 (99)	1 (1)	0 (0)	0.311
−148	93 (100)	0 (0)	0 (0)	90 (99)	1 (1)	0 (0)	0.311
−146	93 (100)	0 (0)	0 (0)	90 (99)	1 (1)	0 (0)	0.311
+1350	56 (60)	30 (32)	8 (8)	66 (72)	21 (23)	5 (5)	0.214
+2343	100 (100)	0 (0)	0 (0)	93 (97)	3 (3)	0 (0)	0.082
TLR4							
+3528	97 (100)	0 (0)	0 (0)	93 (98)	2 (2)	0 (0)	0.151
+3725	73 (74)	24 (24)	2 (2)	69 (71)	18 (19)	10 (10)	0.043
+4022	92 (100)	0 (0)	0 (0)	92 (99)	1 (1)	0 (0)	0.319
+4529	94 (98)	2 (2)	0 (0)	93 (99)	1 (1)	0 (0)	0.573

SNP, single nucleotide polymorphism.

a recessive effect ($p = 0.016$ for the moderate periodontitis patient group and $p = 0.034$ for the severe periodontitis patient group) (Table 4). No significant difference was found between the control group and the mild/moderate/severe periodontitis patient groups regarding the frequencies of the other eight single nucleotide polymorphisms in *TLR2* and *TLR4*.

Because the mean age of the control group was significantly younger than that of the patient group, we classified the patients into two groups, according to their ages, to enable comparison with the group in the same age range. The frequency of the 'C/C' genotype at the +3725 base-pair position in *TLR4* in the group of younger periodontitis patients was significantly higher than that in the control group ($p = 0.022$), whereas no significant difference was found between subjects in the older periodontitis patient group and the control group (Table 5).

Discussion

The missense mutations, reported previously, in *TLR2* (R677W and R753Q) and *TLR4* (D299G and T399I), were not found in the present study (6–8). These results are consistent with the report that the D299G mutation in *TLR4* is not present in the Japanese (16) and Chinese populations (17). Because three of the four missense mutations (R753Q in *TLR2*, and D299G and T399I in *TLR4*) were reported only in European and American populations, the reason why our present results are inconsistent with previous reports might come from the differences between populations. Although R677W in *TLR2* was identified in Korean lepromatous leprosy patients, it was not detected in 286 Indian lepromatous leprosy patients (18). It was suggested that the R677W mutation might come from the variation in the duplicated region with 93% homology to *TLR2* exon 3 located at ≈23 kb 5′-position to the functional *TLR2* gene (18). Because the primers we used in this study were designed specifically for the functional *TLR2* gene, our results are definitive. Although there are conflicting results in the literature regarding the association between those four missense mutations and the susceptibility to periodontitis (9–12), it is difficult to use those four missense mutations to confirm the association between periodontitis and *TLR2*/*TLR4* in the Japanese population because those missense mutations are very rare in Japanese subjects.

We searched for single nucleotide polymorphisms around exons in *TLR2*/*TLR4* because it is possible that other single nucleotide polymorphisms, previously reported, are associated with periodontitis in Japanese subjects. We found nine single nucleotide polymorphisms in the exons of *TLR2* and *TLR4* in the present study; however, none resulted in amino acid substitution. A missense mutation(s) in *TLR2*/*TLR4* would probably not be found as a common variation in Japanese periodontitis patients. It is possible that periodontitis is based on many rare variants, although we did not perform a mutation search in *TLR2*/*TLR4* in all of the patients. Smirnova *et al*. reported that 11 rare missense mutations in *TLR4* were found in 197 meningococcal patients, but that only one rare missense mutation was identified in 127 controls in the UK (19). This is an example that is consistent with the hypothesis that many rare variants are related to common diseases.

We found that the *TLR4* +3725G > C mutation was associated with the whole periodontitis group, and a significant association was also found between the control group and the moderate/severe periodontitis patient groups. Although the mean age of the control group was lower than that of the patient group, the 'C/C' genotype was observed more frequently in the younger periodontitis patient group than in the control group. The positive results from the whole case-control study, and the comparison between the age-matched groups, strongly suggest that the *TLR4* +3725G > C mutation is associated with periodontitis. We did not perform a haplotype association study because the single nucleotide polymorphisms used in this study showed no evidence of linkage disequilibrium (data not shown) with each other. In our next research step, we need to perform a mutation search for other base changes within the genomic region, including *TLR4*. Such a study will uncover the single nucleotide polymorphisms in

Table 4. TLR4 +3725 (rs11536889) genotype frequencies in periodontitis patients (mild, moderate, severe) and control subjects

SNP ID	Subjects	Genotype			
		GG (%)	GC (%)	CC (%)	*p*-value
rs11536889	Mild	9 (63)	6 (32)	1 (5)	$P1 = 0.325$ $P2 = 0.151$
	Moderate	47 (71)	11 (18)	7 (11)	$P1 = 0.016$ $P2 = 0.840$
	Severe	13 (81)	1 (6)	2 (13)	$P1 = 0.034$ $P2 = 0.521$
	Control	73 (74)	24 (24)	2 (2)	

ID, identity; SNP, single nucleotide polymorphism.
*P*1: *p*-value considered as the C allele having a recessive effect (GG + GC vs. CC).
*P*2: *p*-value considered as the C allele having a dominant effect (GG vs. GC + CC).

Table 5. TLR4 +3725 (rs11536889) genotype frequencies in the younger periodontitis patient group (< 60 years of age) and in the older periodontitis patient group (≥ 60 years of age)

Age-group	Genotype			*p*-value
	GG (%)	GC (%)	CC (%)	
Younger	32 (70)	8 (17)	6 (13)	0.022
Older	37 (72)	10 (20)	4 (8)	0.204

p-values were calculated in comparison to the control group.

linkage disequilibrium with +3725G > C of the *TLR4* gene or the disease-associated haplotype within the *TLR4* gene. Considering that the progression of periodontitis is affected by multiple factors, such as oral hygiene and the deposition of calculus, a genetic influence may not be sufficient to distinguish the mild periodontitis group from the control group. Age is also known to be a putative risk factor for periodontitis (20), and older patients, with a relatively low-genetic background of mutations, might be suffering from periodontitis. Those factors may account for the lack of statistical difference between the control group and the mild periodontitis group, and between the control group and the older periodontitis group.

Because the +3725G > C mutation is located in the 3′-untranslated region of *TLR4*, it does not have any direct influence on the conformation of the Toll-like receptor 4 protein molecule, according to our present biological knowledge. However, because single nucleotide polymorphisms in introns and/or untranslated regions may influence transcription and/or translation (21–24), the +3725G > C mutation might have a direct effect on mRNA stability or translation efficiency. Antisense transcripts might be important for regulating *TLR4* transcription. The reported disease-associated single nucleotide polymorphisms or haplotypes are not always found in coding regions in 'common diseases' (25,26). The functional assay of disease association with single nucleotide polymorphisms in introns is the next point requiring investigation. In view of the importance of the Toll-like receptor 4 in the pathogenesis of periodontal diseases, the biological significance of genetic variation, including transcription efficiency of the mutated gene, needs to be elucidated.

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References

1. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr. Microbial complexes in subgingival plaque. *J Clin Periodontol* 1998;**25:**134–144.
2. Lien E, Sellati TJ, Yoshimura A *et al.* Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J Biol Chem* 1999;**274:**33419–33425.
3. Hoshino K, Takeuchi O, Kawai T *et al.* Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the *Lps* gene product. *J Immunol* 1999; **162:**3749–3752.
4. Yoshimura A, Kaneko T, Kato Y, Golenbock DT, Hara Y. Lipopolysaccharides from periodontopathic bacteria *Porphyromonas gingivalis* and *Capnocytophaga ochracea* are antagonists for human Toll-like receptor 4. *Infect Immun* 2002;**70:**218–225.
5. Mori Y, Yoshimura A, Ukai T, Lien E, Espevik T, Hara Y. Immunohistochemical localization of Toll-like receptors 2 and 4 in gingival tissue from patients with periodontitis. *Oral Microbiol Immunol* 2003;**18:**54–58.
6. Arbour NC, Lorenz E, Schutte BC *et al. TLR4* mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000;**25:**187–191.
7. Lorenz E, Mira JP, Cornish KL, Arbour NC, Schwartz DA. A novel polymorphism in the Toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infect Immun* 2000;**68:**6398–6401.
8. Kang TJ, Chae GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. *FEMS Immunol Med Microbiol* 2001;**31:**53–58.
9. Folwaczny M, Glas J, Torok HP, Limbersky O, Folwaczny C. Toll-like receptor (TLR) 2 and 4 mutations in periodontal disease. *Clin Exp Immunol* 2004;**135:**330–335.
10. Laine ML, Morre SA, Murillo LS, van Winkelhoff AJ, Pena AS. *CD14* and *TLR4* gene polymorphisms in adult periodontitis. *J Dent Res* 2005;**84:**1042–1046.
11. Schröder NW, Meister D, Wolff V *et al.* Chronic periodontal disease is associated with single-nucleotide polymorphisms of the human *TLR-4* gene. *Genes Immun* 2005;**6:**448–451.
12. Brett PM, Zygogianni P, Griffiths GS *et al.* Functional gene polymorphisms in aggressive and chronic periodontitis. *J Dent Res* 2005;**84:**1149–1153.
13. Schröder NW, Hermann C, Hamann L, Gobel UB, Hartung T, Schumann RR. High frequency of polymorphism Arg753Gln of the Toll-like receptor-2 gene detected by a novel allele-specific PCR. *J Mol Med* 2003;**81:**368–372.
14. Lorenz E, Hallman M, Marttila R, Haataja R, Schwartz DA. Association between the Asp299Gly polymorphisms in the Toll-like receptor 4 and premature births in the Finnish population. *Pediatr Res* 2002;**52:**373–376.
15. Ichikawa E, Watanabe A, Nakano Y *et al. PAX9* and *TGFB3* are linked to susceptibility to nonsyndromic cleft lip with or without cleft palate in the Japanese: population-based and family-based candidate gene analyses. *J Hum Genet* 2006;**51:**38–46.
16. Okayama N, Fujimura K, Suehiro Y *et al.* Simple genotype analysis of the Asp299Gly polymorphism of the *Toll-like receptor-4* gene that is associated with lipopolysaccharide hyporesponsiveness. *J Clin Lab Anal* 2002;**16:**56–58.
17. Hang J, Zhou W, Zhang H *et al. TLR4 Asp299Gly* and *Thr399Ile* polymorphisms are very rare in the Chinese population. *J Endotoxin Res* 2004;**10:**238–240.
18. Malhotra D, Relhan V, Reddy BS, Bamezai R. TLR2 Arg677Trp polymorphism in leprosy: revisited. *Hum Genet* 2005;**116:**413–415.
19. Smirnova I, Mann N, Dols A *et al.* Assay of locus-specific genetic load implicates rare Toll-like receptor 4 mutations in meningococcal susceptibility. *Proc Natl Acad Sci USA* 2003;**100:**6075–6080.
20. Tonetti MS, Claffey N. Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. *J Clin Periodontol* 2005;**32:**210–213.
21. Bream JH, Carrington M, O'Toole S *et al.* Polymorphisms of the human *IFNG* gene noncoding regions. *Immunogenetics* 2000;**51:**50–58.
22. Borrmann L, Wilkening S, Bullerdiek J. The expression of *HMGA* genes is regulated by their 3′UTR. *Oncogene* 2001; **20:**4537–4541.
23. Rousseau P, Le Discorde M, Mouillot G, Marcou C, Carosella ED, Moreau P. The 14 bp deletion-insertion polymorphism in the 3′ UT region of the HLA-G gene influences HLA-G mRNA stability. *Hum Immunol* 2003;**64:**1005–1010.
24. Hesketh J. 3′-Untranslated regions are important in mRNA localization and translation: lessons from selenium and metallothionein. *Biochem Soc Trans* 2004;**32:**990–993.
25. Curran JE, Jowett JB, Elliott KS *et al.* Genetic variation in selenoprotein S influences inflammatory response. *Nat Genet* 2005;**37:**1234–1241.
26. Grant SF, Thorleifsson G, Reynisdottir I *et al.* Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. *Nat Genet* 2006;**38:**320–323.