European Journal of Human Genetics (2001) 9, 849–854

www.nature.com/ejhg

ARTICLE

Familial Mediterranean Fever: association of elevated IgD plasma levels with specific MEFV mutations

Myrna Medlej-Hashim[1], Isabelle Petit[2], Salim Adib[3], Eliane Chouery[1], Nabiha Salem[1], Valérie Delague[1], Mohammed Rawashdeh[4], Issam Mansour[5], Gérard Lefranc[6], Roger Naman[5], Jacques Loiselet[1], Jean-Claude Lecron[2], Jean-Louis Serre[7] and André Mégarbané*[,1]

[1]Unité de Génétique Médicale, Faculté de Médecine, Université Saint Joseph, Beirut, Lebanon; [2]Faculté de Médecine et de Pharmacie. Laboratoire cytokines, IBMIG, Université de Poitiers, France; [3]Department of Community Medicine, Kuwait University, Kuwait; [4]Division of Gastroenterology, Department of Pediatrics, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan; [5]Service d'Immunologie, Hôtel-Dieu de France, Beirut, Lebanon; [6]Laboratoire d'Immunogénétique Moléculaire, Institut de Génétique Humaine, CNRS UPR 1142 et Université Montpellier II, France; [7]Laboratoire de Cytogénétique et de Génétique Moléculaire Humaine, Université de Versailles/Saint Quentin en Yvelines, France

Familial Mediterranean Fever (FMF) is a recessively inherited disorder, characterized by episodic fever, abdominal and arthritic pain, as well as other forms of inflammation. Some FMF patients present higher IgD serum levels, and it is not yet known whether such an elevation is related to specific genotypes or correlated with a specific phenotype. In order to evaluate the association between known FMF-related mutations and IgD levels in confirmed patients, as well as the correlation between those levels and the presence of specific clinical signs, genotypic analysis and IgD plasma measurements were performed for 148 Lebanese and Jordanian FMF patients. Most common mutational patterns were M694V heterozygotes (19%) and homozygotes (17%), and V726A heterozygotes (18%) and homozygotes (5%), with an additional 11% combining both mutations. Twenty-one patients had higher IgD levels (superior to 100 μg/ml). The risk for higher IgD levels was significantly associated with M694V homozygote status (OR = 6.25) but not with heterozygotic one (OR = 1). Similarly, the risk for higher IgD was also found with V726A homozygotes (OR = 2.2) but not with heterozygotes (OR = 1.05). The use of colchicine was not statistically associated with IgD levels. Clinically, hyper IgD was also found significantly associated with arthritis (OR = 18). Thus, homozygotic status for M694V, and to a lesser extent V726A, is associated with increased risk for higher IgD plasma levels, regardless of colchicine use. Elevated IgD plasma levels are also correlated with the severity of FMF manifestations, and especially with arthritis. *European Journal of Human Genetics* (2001) **9,** 849–854.

Keywords: FMF; genotype; IgD; mutation; phenotype; arthritis

Introduction

Periodic fever syndromes are a family of diseases characterized by common symptoms, namely, episodic crisis of fever, abdominal pain and/or arthritic problems, and various other more specific clinical and genetic characteristics.[1–3] Of those disorders, Familial Mediterranean Fever (FMF), frequently found in populations of the Mediterranean area,[4] and the Hyperimmunoglobulinemia D Syndrome (HIDS), diagnosed mainly in Western European countries,[1,5] are recessively inherited disorders. MEFV, the gene responsible for the Mediterranean disorder, was cloned on chromosome 16p13.3 in 1997.[6,7] Mutations in the mevalonate kinase

*Correspondence: André Megarbané MD, PhD, Unité de Génétique Médicale, Faculté de Médecine, Université Saint-Joseph, 42, rue de Grenelle, 75007 Paris, France. Fax: 00 961 1 614 054;
E-mail: megarban@ dm.net.lb
Received 23 May 2001; revised 21 August 2001; accepted 28 August 2001

gene, MVK, on chromosome 12q24, were found responsible for HIDS.[8,9] Changes in serum immunoglobulin D (IgD) levels are also features of these diseases.[2]

Immunoglobulins D are a class of antibodies discovered in myeloma patients in 1964[10] and, subsequently detected as membrane bound Ig, present predominantly on the mature lymphocyte B cells surface in association with IgM.[11] The function of secreted IgD is still unclear. The distribution of IgD in normal adult sera is non-gaussian and extremely heterogeneous (0.1–213 μg/ml, mean: 36 μg/ml).[12]

While most HIDS patients have elevated IgD serum levels, some FMF patients show elevated IgD sera levels, as reported by a previous study.[2] However, since that study was conducted prior to the cloning of the MEFV gene, it is not possible to ascertain whether various mutations leading to FMF had varying effects on IgD levels. Similarly, elevated plasma IgD was not correlated with any specific phenotypic FMF sign.

The present study aims at determining the association of various FMF mutations and IgD plasma levels and correlating those levels with specific phenotypic characteristics.

Materials and methods

Participants and materials

The sample included in this analysis was composed of 148 Lebanese and Jordanian FMF patients (all unrelated except one), diagnosed according to Heller's criteria.[13] Data obtained included clinical symptoms and colchicine intake at the time of the study. The clinical severity score was evaluated for each participant according to the Tel Hashomer classification.[14] Thirteen patients had an incomplete clinical investigation form, and one could not then evaluate their severity scores.

Blood samples were collected from patients with their full consent. At the time of blood donation, none of the patients was undergoing an FMF attack. For comparison, blood was also drawn from 81 unrelated consenting individuals, matched relatively to sex and whose ages vary between 18 and 50 years old, after ensuring they were free of any apparent inflammatory symptoms or allergy. Plasma was obtained by instant centrifugation and stored at -80°C, then DNA was extracted from leucocytes by standard techniques.[15] IgD measurements in plasma were performed using a previously described[12] ELISA technique with a detection threshold of 0.1 μg/ml.

FMF mutations screening

Five most frequent FMF mutations (M694V, V726A, M680I, M694I, E148Q), as well as eight rarer ones (E167D, T267I, R761H, P369S, A744S, F479L, I692del, M694del) were investigated as described elsewhere.[16] E230K, a newly discovered mutation,[17] was tested using PCR same procedures as for other mutations of exon 2 and the restriction enzyme Taq I. Mutation K695R was also tested by amplification refractory mutation screening (ARMS) with special PCR conditions (100 ng genomic DNA, 0.2 mM dNTPs, 4 mM MgCl2, 1 pmol of each primer, 1$\times$ PCR buffer and 0.04 U AmpliTaq Gold in 25 μl mix).

Statistical analysis

Chi-squared test was performed to test the significance of differences in the prevalence of higher IgD values in various comparison groups. A *P*-value of 0.05 or less meant that the difference was statistically significant. The association between a putative risk factor and higher IgD levels was measured using the odds-ratio (OR) and its corresponding 95% confidence interval (95% CI). While an OR$>$1 suggests the existence of an association, the inclusion of 1 in the 95% CI indicates that the estimation of risk is not significant. Computations were conducted using SPSS and EPI-6.

Results

Both alleles were identified for 99 patients, 42 homozygous and 57 compound heterozygous, whereas 33 patients were heterozygous for one mutation and 16 had none of the tested mutations. Most commonly found mutations within 147 unrelated patients were M694V (32%), V726A (20%), M694I (11.6%), M680I (6.8%) and E148Q (5%), while other rare and non-identified mutations totaled 24.5%.

Using these allele frequencies, expected genotypes were calculated under Hardy–Weinberg panmixia hypothesis (Table 1) and showed a significant departure. Since 23 patients out of 147 unrelated participants were issued from first cousins parents, allele frequencies and expected number of genotypes were also calculated once homozygote inbred patients had been removed. The comparison between observed and expected genotypes showed that homozygotes for M694V are in excess, and M694V carriers are less than expected in FMF patients, even when inbred patients were removed.

IgD levels in the patients group varied between 0.07 and 337 μg/ml, with a median of 28 μg/ml, and only 14% ($n=21$) had IgD levels superior to 100 μg/ml. Remarkably, in the control group, values varied between 0.2 and 218 μg/ml, with a median of 25 μg/ml. The measurements in the latter group included eight values superior to 100 μg/ml (10%). As the distributions of IgD levels in both the patients and control series are non-gaussian, these values were transformed to logarithms and the histograms obtained (Figure 1) showed a distribution quite similar to the multimodal distribution previously observed in sera of healthy subjects.[18] Some prevalence of higher IgD values in apparently healthy subjects is not unusual.[10,12]

All patients with higher IgD levels had two identified mutations. Detailed clinical, genotypic and serological features of those 21 FMF patients are presented in Table 2. The association between specific genotypes and higher IgD levels was analysed and presented in Table 3. Compared to

Table 1 Observed and expected number (frequencies) of genotypes among unrelated FMF patients with either removing or not homozygote inbred patients

n (%)	*M694V/ M694V*	*M694V/ V726A*	*M694V/ others*	*V726A/ V726A*	*V726A/ others*	*Other genotypes*	*Total*	*Chi-square (df=3)**
Observed genotypes	25 (17)	16 (11)	28 (19)	8 (5.5)	27 (18.5)	43 (29)	147 (100)	
Expected under Hardy–Weinberg	15.05 (10)	18.91 (13)	45.06 (31)	5.94 (4)	28.31 (19)	33.73 (23)	147 (100)	16.80 ($p \sim 0.001$)
Observed genotypes once inbred removed	20 (15)	16 (12)	28 (21)	7 (5)	27 (20.5)	35 (26.5)	133 (100)	
Expected genotypes once homozygotes inbred removed	13.28 (10)	17.99 (13.5)	39.50 (30)	6.09 (4.5)	26.75 (20)	29.39 (22)	133 (100)	8.17 ($p \sim 0.004$)

*df: degrees of freedom.

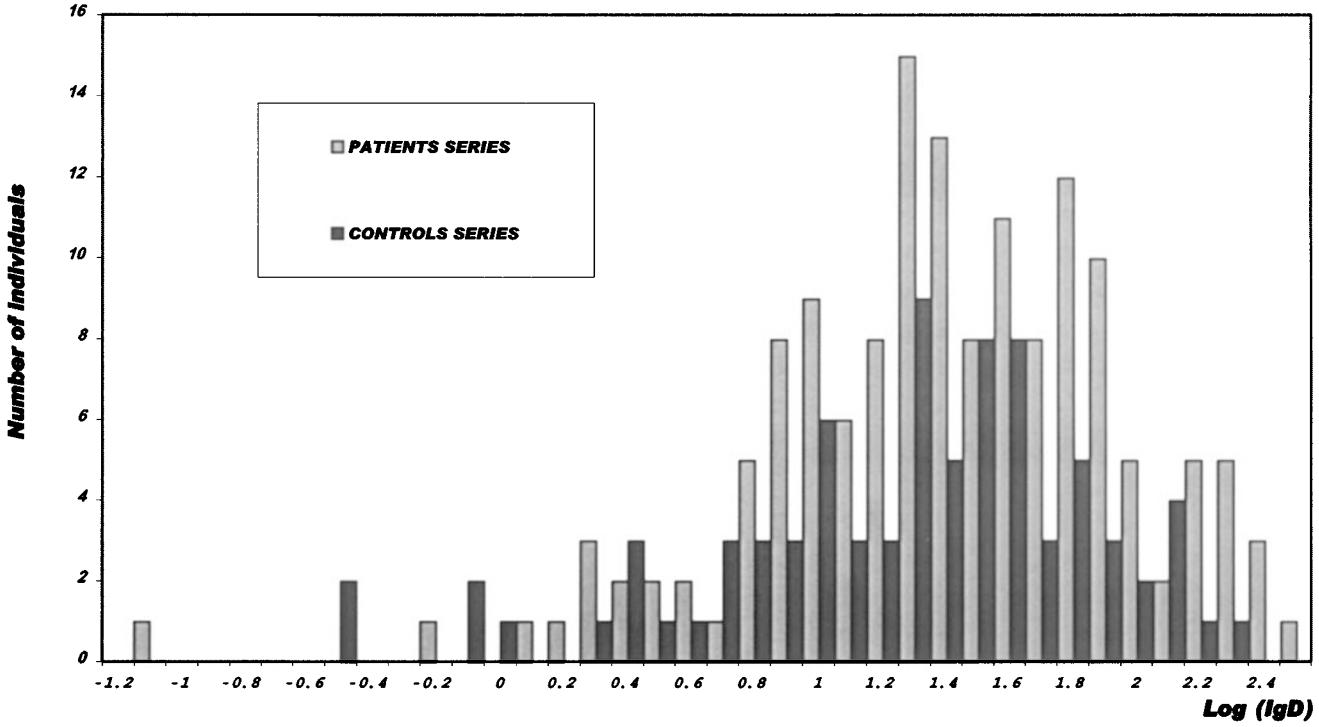

Figure 1 Plasma IgD values distribution among 148 FMF patients and 81 healthy individuals.

those not carrying any M694V mutation, the homozygotic pattern of this mutation was associated with highest risk for higher IgD (OR = 6.25), followed by the combination M694V/V726A (OR = 2.3), while other heterozygotic patterns were not associated with higher IgD levels. Compared to those not carrying any V726A mutation, the homozygotic pattern of V726A was associated with some risk for higher IgD (OR = 2.2), while heterozygotic patterns were not. Patients with a combined M694V/V726A genotype were about five times more likely to present higher IgD levels than all other genotypes (OR = 4.7).

Average severity scores of the high and low IgD plasma levels group show significant differences (8.1 ± 5.1 for the former versus 5.5 ± 4.9 for the latter). Moreover, differences in specific clinical features were detected between the two groups. Compared to those with lower IgD levels, patients with higher levels were more likely to suffer from articular crisis (OR = 18, CI(2.33 – 139.31)), amyloidosis (OR = 3.44, CI(0.78 – 15.13)), thoracic crisis (OR = 1.5, CI(0.55 – 3.93)), and erysipelas-like erythema (OR = 1.25, CI(0.25 – 6.19)) (Data not shown).

About 58% of the sample were using colchicine at the time of the study. The proportion of colchicine users having elevated IgD levels (67%) was more important than that of colchicine users in the group with lower IgD levels (57%). However, no significant association was found between colchicine and lower IgD levels.

Discussion

The aim of this study was mainly to clarify the association of specific genotypes with IgD levels in FMF patients, and to

Table 2 Clinical and serological characteristics of 21 FMF patients with higher IgD plasma

*Patients**	*Fever*	*Abdominal attacks*	*Thoracic attacks*	*Articular attacks*	*Amyloidosis*	*Erysipela-like erythema*	*Severity score*	*Genotype*	*IgD plasma level (μg/ml)*
1	+	+	+	+	–	–	7	M694V/M694V	182
2	+	+	+	+	–	–	7	M694V/M694V	236
3	+	+	–	+	+	–	12	M694V/M694V	169
4	+	+	–	+	–	–	8	M694V/M694V	201
5	+	+	+	+	–	–	11	M694V/M694V	162
6	+	+	+	+	–	–	6	M694V/M694V	250
7	+	+	–	+	+	+	12	M694V/M694V	149
8	+	+	–	+	–	+	8	M694V/M694V	139
9	+	+	+	+	–	–	10	M694V/M694V	105
10	–	+	–	+	–	–	7	M694V/M694V	106
11	+	+	+	+	–	–	10	M694V/V726A	198
12	+	+	?	?	?	?	—	M694V/V726A	301
13	+	+	–	+	–	–	4	M694V/V726A	123
14	+	+	?	?	?	?	—	M694V/E148Q	247
15	+	+	+	+	–	–	6	V726A/V726A	284
16	+	+	–	+	–	–	4	V726A/V726A	281
17	+	+	+	+	+	–	12	V7826A/M680I	175
18	+	+	–	+	–	–	8	V726A/M680I	224
19	+	+	+	+	–	–	8	V726A/T267I	337
20	+	+	+	+	–	–	9	M694I/M694I	108
21	+	+	+	–	–	–	5	M694I/E148Q	111

*All these patients, except 2 (numbers 2 and 4), are non consanguineous.
Patients' numbers 3, 4, 5, 17, 18 and 19 are Jordanian and the others are Lebanese.

Table 3 Risk for higher IgD serum levels associated with selected types of mutations in FMF patients

n (%)	*IgD < 100 μg/ml*	*IgD > 100 μg/ml*	*Total*	*OR (95% CI)**
Total	127 (85.8)	21 (14.2)	148	—
M694V carriers				
M694V/M694V	16 (61.5)	10 (38.5)	26	6.25 (1.8–25)
M694V/other	27 (96.4)	1 (3.6)	28	0.4 (0.02–3.3)
M694V/V726A	13 (81.3)	3 (18.7)	16	2.3 (0.4–12.2)
All M694V heterozygotes	40 (90.9)	4 (9.1)	44	1 (0.24–4.31)
All M694V carriers	56 (80)	14 (20)	70	2.5 (0.96–6.71)
All others**	71 (91)	7 (9)	78	reference
V726A carriers				
V726A/V726A	6 (75)	2 (25)	8	2.2 (0.3–14)
V726A/other	24 (88.9)	3 (11.1)	27	0.8 (0.2–3.4)
M694V/V726A	13 (81.3)	3 (18.7)	16	1.5 (0.3–6.8)
All V726A heterozygotes	37 (86)	6 (14)	43	1.05 (0.4–3)
All others**	84 (86.6)	13 (13.4)	97	reference
Mixed carriers				
M694V/V726A	13 (81.3)	3 (18.7)	16	4.7 (0.6–46.7)
Other carriers (excluding M694V or V726A)	41 (95.3)	2 (4.7)	43	reference

*Limits of confidence intervals are not always precise because of the small numbers involved.
**All those not carrying this specific mutation.

determine whether a specific phenotype characterises patients with an elevated plasma IgD level. Higher IgD values were found in 14% of the FMF patients involved in this study. In contrast, the distribution of IgD levels in patients and controls in this study were largely similar (Figure 1), which is not in favour of a direct effect of MEFV mutations on serum IgD levels. However, the analysis of the association between specific mutational genotypes and IgD levels suggests that homozygotic genotypes for M694V and to a lesser extent V726A increase the risk of higher IgD levels when compared to other genotypes (Table 3). But the association with the risk for higher IgD decreased considerably when all M694V carriers were considered against non-M694V carriers (OR = 2.5). Such a result is especially interesting for it is in accordance with the significantly high proportion of M694V/M694V on a genetic populational point of view.[16,19] Indeed, although Lebanese and Jordanian people are known to display a high level of consanguinity (mean value of 1% in our sample),[20–22] the difference between observed and expected number of homozygotes is far too high to be explained only by consanguinity. On the other hand, the genetic differences between Lebanese and Jordanian people,

although present,[16,19] are too slight to induce a strong Walhund effect. Thus, due to the correlation between M694V/M694V and clinical status of FMF, especially through the high level of IgD, one can postulate that the excess of these homozygotes is mainly due to an assessment bias and partially due to consanguinity and Walhund effect. In fact, the M694V mutation was previously correlated with severe phenotypes,[19,23] and the recruitment of more patients whose genotypes carry M694V, especially homozygous ones, may be due to the severity of their crises.

The estimated association of V726A with higher IgD values did not achieve statistical significance, most likely because of the small numbers involved. However, when M694V homozygotes were excluded from the reference group, the homozygotic pattern of V726A was found associated with a high risk for higher IgD (OR = 7.5, CI(1.05 – 54.41)), which suggests that M694V homozygotes were overwhelming the V726A homozygotic effect. As for M694I, small numbers also precluded any meaningful statistical association between this mutation and IgD levels.

Colchicine is, since 1972, the only therapeutic agent used to decrease FMF attacks frequencies and prevent amyloidosis, the most severe complication of this disease.[24] According to the results, colchicine use may be discarded as a potential confounding variable.

The clinical significance of higher IgD levels is not clear. In the present study, higher IgD levels were associated with high severity scores and particular clinical signs or symptoms, namely articular pain, amyloidosis, and to a lesser extent thoracic crisis, and erysipelas-like erythema. Indeed, all except one patient (95%) with higher IgD levels reported articular pain crises, whereas this type of pain was present in only 50% of patients with lower IgD plasma level. In a previous study,[2] 78% of FMF patients having an IgD value superior to 100 IU/ml (equivalent of 141 μg/ml)[25] had attacks of monoarthritis. As a matter of fact, arthralgia is a common feature of HIDS crisis, as well as arthritis involving large joints.[2,5] Thus, the severity of attacks was noticed in FMF patients having high IgD plasma levels, especially regarding joints pain, whereas the absence of relationship between serum IgD level and the frequency and severity of attacks had been reported in HIDS patients.[5]

In conclusion, the most solid finding here is the association between M694V homozygotic genotypes and higher IgD levels. The fact that this association seems confined only to one specific genotype may explain the low though apparently persistent incidence of higher IgD in FMF patients. The mechanism that governs the effect of M694V mutation on the serum IgD level is currently unknown and may probably be indirect. On the other hand, the correlation established between high IgD levels and the severity of FMF manifestations, especially arthritis may reveal a novel aspect of IgD effects. However, the functions of IgD remain to be investigated as well as the mechanism of the IgD-phenotype-genotype association revealed in this analysis.

Acknowledgments

We thank Mrs Adriana Delwail and Dr Franck Morel for their help, as well as Professors Josue Feingold for his review and Patrice Bouvagnet for his suggestions. This work was supported by Scientific Research grants from the Saint Joseph University and the University of Poitiers, and the Lebanese-NCSR.

References

1 Centola M, Aksentijevich I, Kastner DL: The hereditary periodic syndromes: molecular analysis of a new family of inflammatory diseases. *Hum Mol Genet* 1998; **7**: 1581 – 1588.
2 Livneh A, Drenth JPH, Klasen IS *et al*: Familial Mediterranean Fever and Hyperimmunoglobulinemia D Syndrome: two diseases with distinct clinical, serologic, and genetic features. *J Rheumatol* 1997; **24**: 1558 – 1563.
3 McDermott MF, Aksentijevich I, Galon J *et al*: Germline mutations in the extracellular domains of the 55 kDa TNF receptor, TNFR1, define a family of dominantly inherited autoinflammatory syndromes. *Cell* 1999; **7**: 133 – 144.
4 Sohar E, Gafni J, Pras M, Heller H: Familial Mediterranean Fever. A survey of 470 cases and review of the literature. *Am J Med* 1967; **43**: 227 – 253.
5 Drenth JPH, Haagsma CJ, van der Meer JWM: The International Hyper-IgD Study Group. Hyperimmunoglobulinemia D and Periodic Fever Syndrome: The clinical spectrum in a series of 50 patients. *Medicine* 1994; **73**: 133 – 144.
6 French FMF Consortium: A candidate gene for familial Mediterranean Fever. *Nat Genet* 1997; **17**: 25 – 31.
7 International FMF Consortium: Ancient missense mutations in a new member of the RoRet family are likely to cause familial Mediterranean fever. *Cell* 1997; **90**: 797 – 807.
8 Drenth JPH, Cuisset L, Grateau G *et al*: Mutations in the gene encoding mevalonate kinase cause hyper-IgD and periodic fever syndrome. *Nat Genet* 1999; **22**: 178 – 181.
9 Houten SM, Kuis W, Duran M *et al*: Mutations in MVK, encoding mevalonate kinase, cause hyperimmunoglobulinaemia D and periodic fever syndrome. *Nat Genet* 1999; **22**: 175 – 177.
10 Rowe DS, Fahey JL: A new class of human immunoglobulins. *J Exp Med* 1965; **121**: 171 – 199.
11 Preud'homme JL, Petit I, Barra A *et al*.: Review Structural and functional properties of membrane and secreted IgD. *Mol Immunol* 2001; **37**: 871 – 887.
12 Levan-Petit I, Cardonna J, Garcia M *et al*: Sensitive ELISA for human immunoglobulin D measurement in neonate, infant, and adult sera. *Clin Chem* 2000; **46**: 876 – 878.
13 Heller H, Sohar E, Sherf L: Familial Mediterranean Fever. *Arch Intern Med* 1958; **102**: 50 – 71.
14 Sohar E, Gafni J, Pras M, Freund (eds): Proceedings 1st International Conference on FMF, Jerusalem. 1997; pp. 207 – 208.
15 Miller SA, Dynes DD, Polesky F: A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988; **16**: 1215.
16 Medlej-Hashim M, Rawashdeh M, Chouery E *et al*: Genetic screening of fourteen mutations in Jordanian Familial Mediterranean Fever patients. *Hum Mutat* (Online) 2000; **15**: 384.
17 Torosyan T, Aksentijevich I, Sarkisian T *et al*: Role of complex alleles and gender in susceptibility to Familial Mediterranean fever in the Armenian population. *Am J Hum Genet* 2000; **67**(supp.2): 404.
18 Dunette SL, Gleish GJ, Drew Miller R, Kyle RA: Measurement of IgD by a double antibody radioimmunoassay: demonstration of an apparent trimodal distribution of IgD levels in normal human sera. *Journal Immunol* 1977; **119**: 1727 – 1731.

19 Mansour I, Delague V, Cazeneuve C *et al*: Familial Mediterranean fever in Lebanon: mutation spectrum, evidence for cases in Maronites, Greek catholics, Syriacs and Chiites and for an association between amyloidosis and M694V and M694I mutations. *Eur J Hum Genet* 2001; **9**: 51 – 55.
20 Khlat M: Consanguineous marriage and reproduction in Beirut, Lebanon. *Am J Hum Genet* 1988; **43**: 188 – 196.
21 Al-Salem M, Rawashdeh N: Consanguinity in North Jordan: prevalence and pattern. *J Biosoc Sci* 1993; **25**: 553 – 556.
22 Khoury SA, Massad D: Consanguineous marriage in Jordan. *Am J Med Genet* 1998; **43**: 769 – 775.
23 Dewalle M, Domingo C, Rozenbaum M *et al*: Phenotype-genotype correlation in Jewish patients suffering from familial Mediterranean fever (FMF). *Eur J Hum Genet* 1998; **6**: 95 – 97.
24 Goldfinger SE: Colchicine for familial Mediterranean fever. *N Engl J Med* 1972; **287**: 1302.
25 Rowe DS, Anderson SG, Tackett L: A research standard for human serum immunoglobulin D. *Bull WHO* 1970; **43**: 607 – 609.