Research article

Amyloidosis in familial Mediterranean fever patients: correlation with MEFV genotype and SAA1 and MICA polymorphisms effects

Myrna Medlej-Hashim1, Valérie Delague1, Eliane Chouery1, Nabiha Salem1, Mohammed Rawashdeh2, Gérard Lefranc3, Jacques Loiselet1 and André Mégarbané*1

Address: 1Unité de Génétique Médicale. Faculté de Médecine, Université Saint Joseph, Beirut, Lebanon, 2Division of Gastroenterology. Department of Pediatrics, Faculty of Medicine, Jordan University of Science and Technology, Irbid, Jordan and 3Laboratoire d’Immunogénétique Moléculaire, Institut de Génétique Humaine, CNRS UPR 1142 et Université Montpellier II, France

Email: Myrna Medlej-Hashim - myrnahachem@hotmail.com; Valérie Delague - Valerie.Delague@medecine.univ-mrs.fr; Eliane Chouery - eliane.chouery@usj.edu.lb; Nabiha Salem - nabiha.salem@usj.edu.lb; Mohammed Rawashdeh - rawashd@next.jo; Gérard Lefranc - glefranc@srvmail.univ-montp2.fr; Jacques Loiselet - jacques.loiselet@usj.edu.lb; André Mégarbané* - megarban@dm.net.lb

* Corresponding author

Abstract

**Background:** Familial mediterranean fever (FMF) is a recessively inherited disease characterized by recurrent crises of fever, abdominal, articular and/or thoracic pain. The most severe complication is the development of renal amyloidosis. Over 35 mutations have been discovered so far in the gene responsible for the disease, MEFV. This article aims at determining a correlation between the MEFV genotype and the occurrence of amyloidosis in FMF patients, in addition to the study of the modifying effects of the SAA1 (type I serum amyloid A protein) and MICA (Major Histocompatibility Complex (MHC) class-I-chain-related gene A) genes on this severe complication.

**Methods:** Fourteen MEFV mutations were screened and the SAA1 and MICA polymorphisms tested in 30 FMF patients with amyloidosis and 40 FMF patients without amyloidosis.

**Results:** The M694V and V726A allelic frequencies were, respectively, significantly higher and lower in the group with amyloidosis, compared to the control FMF group. The beta and gamma SAA1 alleles were more frequently encountered in the group without amyloidosis, whereas the alpha allele was significantly more observed in FMF patients with amyloidosis (p < 0.025). All the MICA alleles were encountered in both patients’ groups, but none of them was significantly associated with amyloidosis.

**Conclusions:** The results suggest a protective effect of the SAA1 beta and gamma alleles on the development of amyloidosis and show the absence of a MICA modifying effect on amyloidosis development. Testing these polymorphisms on a larger sample will lead to more definite conclusions.

**Background**

Familial mediterranean fever (FMF, MIM 249100) is a recessively inherited disease mostly prevalent in Jews, Armenians, Arabs and Turks [1]. It is characterized by...
recurrent bouts of fever, accompanied by serosal inflammation, leading to abdominal, articular and/or thoracic pain. The most severe complication of FMF is the development of secondary renal amyloidosis, and two disease phenotypes have been identified, whether amyloidosis appears years after the appearance of the other clinical signs (phenotype I), or whether it is the first or the only FMF presenting sign (phenotype II), which is less frequent [1,2]. Over 35 mutations [3] have been discovered so far, in the gene responsible for the disease, MEFV [4,5]. The 5 most frequent mutations are M694V, M694I, V726A, M680I and E148Q.

Correlation of FMF patients genotypes with the various phenotypes of the disease, especially amyloidosis, has been often studied. The latter complication has been often associated with a specific mutation in exon 10, M694V, especially at the homozygous state [6-11], while others found that there was no specific genotype correlated with the development of amyloidosis [12]. Moreover, 2 genes, namely SAA1 (type I serum amyloid A protein) and MICA (Major Histocompatibility Complex (MHC) class-I-chain-related gene A) have been investigated in FMF patients and were found to have an effect on amyloidosis and on the course of the disease respectively. The genotype alpha/alpha of the SAA1 gene was associated with a sevenfold increase in the incidence of renal amyloidosis, especially in patients homozygous for M694V [13,14], whereas the MICA alleles A4 and A9 were associated with a diminished attacks frequency and an earlier age of onset, respectively, notably in patients homozygous for M694V [15].

The purpose of the present article is to determine a correlation between the MEFV genotype and the occurrence of amyloidosis in FMF patients, in addition to the study of the modifying effects of the SAA1 and MICA genes on this severe complication.

**Methods**

The main study group was composed of thirty Lebanese and Jordanian amyloidosis affected FMF patients from 24 non-related families. Renal amyloidosis was detected either by biopsy or persistent proteinuria. Thirteen of these patients were either having regular renal dialysis or had a renal transplantation. A comparative group was composed of 40 FMF patients, either homozygous or compound heterozygous for MEFV mutations, having an age superior to 20 years old. These patients did not have amyloidosis, nor did they have any sign of proteinuria, and were not under any medication, including colchicine, before the time of the study. Patients of both groups willingly filled up a questionnaire form where clinical and social information were recorded.

Blood samples were withdrawn from all the patients on EDTA, and DNA was extracted from leucocytes by standard technique [16].

Fourteen mutations previously detected in the Lebanese and Jordanian populations were screened (M694V, M694I, V726A, M680I, E148Q, E167D, T267I, R761H, P369S, A744S, F479L, I692del, M694del, K695R) as previously described [11,17].

SAA1 and MICA genes polymorphisms were tested in the 2 FMF patients’ groups. The DNA fragment including the SAA1 gene polymorphisms was amplified using the Saa1F (5’-GCCAATTACATCGGCCTCAG-3’) and Saa1R (5’-TGGCCAAAGAATCTCTGGAT-3’) primers. The 2 polymorphisms of the SAA1 gene were determined with 2 restriction enzymes, BanI and Bell, that allowed the identification of the amino acids at positions 52 and 57, respectively [18]. Valine/Alanine, Alanine/Valine and Alainine/Alainine amino acids at positions 52/57 defined respectively the alpha, beta and gamma SAA1 alleles.

The MICA transmembrane region polymorphism, which is a polyalanine repeat, was tested by genotyping using fluorescent dUTP (2’-deoxyuridine 5’-triphosphate) on an ABI 310 genetic analyzer. Sequencing was performed whenever needed to determine the different MICA alleles. The primers used to amplify the exon 5 polymorphism containing DNA fragment were Mica-5F (5’-CCITTITTTTCAGGAAAGTGC-3’) and Mica-5R (5’-CCITTACCATCCCAAGAACTGC-3’).

Chi-squared test was performed to test the significance of differences in FMF crises frequency and in SAA1 genotypes and MICA alleles distribution between the 2 groups of FMF patients, with and without amyloidosis. A P value of 0.05 or less meant that the difference was statistically significant.

**Results**

Fifty percent and 20 % of the 24 unrelated amyloidosis affected FMF patients and of the control FMF group, respectively, were consanguineous (p < 0.02). All amyloidosis affected FMF patients in this study were of the phenotype I. Twelve of the unrelated amyloidosis affected patients never took colchicine before the development of renal amyloidosis, one patient was not taking it regularly, 3 of them stopped taking it after having taken it for some years, in one case because of the inefficacy of the drug in relieving the patient from painful crises.

Ages at the moment of diagnosis of FMF varied from 3 to 50 years, and from 7 to 44 years, in the groups of patients with and without amyloidosis, respectively. Diagnosis was mainly made between 20 and 35 years of age in

**Correlation of FMF patients genotypes with the various phenotypes of the disease, especially amyloidosis, has been often studied.**

The latter complication has been often associated with a specific mutation in exon 10, M694V, especially at the homozygous state [6-11], while others found that there was no specific genotype correlated with the development of amyloidosis [12]. Moreover, 2 genes, namely SAA1 (type I serum amyloid A protein) and MICA (Major Histocompatibility Complex (MHC) class-I-chain-related gene A) have been investigated in FMF patients and were found to have an effect on amyloidosis and on the course of the disease respectively. The genotype alpha/alpha of the SAA1 gene was associated with a sevenfold increase in the incidence of renal amyloidosis, especially in patients homozygous for M694V [13,14], whereas the MICA alleles A4 and A9 were associated with a diminished attacks frequency and an earlier age of onset, respectively, notably in patients homozygous for M694V [15].

**Correlation of FMF patients genotypes with the various phenotypes of the disease, especially amyloidosis, has been often studied.**

The latter complication has been often associated with a specific mutation in exon 10, M694V, especially at the homozygous state [6-11], while others found that there was no specific genotype correlated with the development of amyloidosis [12]. Moreover, 2 genes, namely SAA1 (type I serum amyloid A protein) and MICA (Major Histocompatibility Complex (MHC) class-I-chain-related gene A) have been investigated in FMF patients and were found to have an effect on amyloidosis and on the course of the disease respectively. The genotype alpha/alpha of the SAA1 gene was associated with a sevenfold increase in the incidence of renal amyloidosis, especially in patients homozygous for M694V [13,14], whereas the MICA alleles A4 and A9 were associated with a diminished attacks frequency and an earlier age of onset, respectively, notably in patients homozygous for M694V [15].
patients without amyloidosis, whereas the disease manifested earlier, mostly between 10 and 20 years, in patients with amyloidosis (p < 0.005). Moreover, patients with amyloidosis had a significantly higher crises frequency, compared to the FMF control group (p < 0.02).

The genotypes of the FMF patients with and without amyloidosis are shown in table 1. The 6 siblings in the group with amyloidosis were homozygous for M694V. The M694V, V726A and M694I allelic frequencies were respectively 67%, 12%, and 8% in the group of patients with amyloidosis, and 25%, 39% and and 15% in patients without amyloidosis (p < 0.001).

The alpha, beta and gamma SAA1 alleles were encountered in patients without amyloidosis, whereas only the alpha and beta alleles were detected in amyloidosis patients. Table 2 shows the allele combinations detected in the patients. Once the beta/beta and beta/gamma genotypes were grouped, the difference between the 2 groups was significant (p < 0.05). Comparison of the alpha and beta allelic frequencies in the 2 groups of patients was also significant (p < 0.025). However, the distribution of the SAA1 alleles among the M694V homozygous FMF patients with amyloidosis was not different from the M694V homozygous patients without amyloidosis.

All the different MICA alleles were encountered in both patients’ groups. Once the 2 groups were combined, no specific MICA allele was significantly associated neither with the age of onset of the disease, nor with the attacks frequency. Moreover, no specific MICA genotype or allele was significantly associated with the development of amyloidosis (Table 3), even in the M694V homozygotes subgroup.

Discussion
Familial mediterranean fever is a clinically heterogeneous disease. The quite variable phenotypes among FMF patients have suggested specific mutational effects as well
as the presence of some genes that would affect the appearance of the different clinical signs, especially amyloidosis. The significantly more important consanguinity in the group with amyloidosis (p < 0.02), compared with the control group, suggests a recessive modifying aspect of this phenotype. The present study associates amyloidosis with some aspects of disease severity, and with the specific mutation M694V, although it points out the involvement of other mutations in the occurrence of this severe phenotype. Moreover, it associates SAA1 alleles with the presence or absence of amyloidosis, whereas MICA was not found to have any association with amyloidosis.

The development of glomerular renal amyloidosis in FMF patients, unlike the secondary amyloidosis that occurs in other different diseases, was not previously associated with the frequency, nor the severity of the febrile crises [19]. The development of amyloidosis in patients having never experienced FMF attacks (phenotype II) is in favor of the lack of association between amyloidosis and FMF severity. However, in the present study, amyloidosis development in FMF patients has been significantly associated with a beginning of the disease prior to 20 years old (p < 0.005), and with a higher FMF crises frequency (p < 0.02), compared with the control FMF group, which allowed the association of amyloidosis with FMF severity in phenotype I patients.

Contradictory genotype-phenotype studies tried to associate amyloidosis with a specific mutation or genotype. The M694V mutation, that has often been correlated with a severe FMF phenotype, has also been correlated, especially at the homozygous state, with amyloidosis development [6,7,9,14], whereas a study on Turkish families presenting FMF patients with amyloidosis revealed that the M694V mutation was not the only mutation presenting a risk for amyloidosis development [12]. In the present study, a significant increase in the number of patients homozygous for the M694V mutation was noted in the group with amyloidosis (p < 0.001), compared to all the other genotypes. In parallel, the M694V allelic frequency was significantly lower in the group with amyloidosis (p < 0.001), compared to the control FMF group. However, the simultaneous occurrence of V726A with another mutation such as M694V or M680I seems to modify the mild effect of V726A, and thus increase the severity of the disease. Indeed, 3 FMF patients with amyloidosis were compound heterozygous for V726A and M680I (Table 1). The latter genotype was also previously encountered in FMF patients with amyloidosis [7].

The serum amyloid protein (SAA) is an acute phase protein which serum level increases considerably during inflammation and leads, by proteolytic cleavage, to amyloid A proteins, the major fibrillar proteins in secondary amyloidosis [20]. Two isotypes, SAA1 and SAA2, with quite homologous sequences have been distinguished, and allelic forms of these isotypes have been identified [21]. The SAA1 allelic forms have been associated with the incidence of amyloidosis in different diseases and various populations. The SAA1 alpha/alpha genotype has already been associated with the occurrence of amyloidosis in Armenian FMF patients [13], whereas both the alpha SAA1 allele and the alpha/alpha genotype were significantly associated with this phenotype in Caucasian juvenile chronic arthritis patients [22]. In the present study, the beta/beta and beta/gamma SAA1 genotypes, as well as the alleles beta and gamma, were more frequently encountered in the group without amyloidosis than in the group with amyloidosis, whereas the alpha/alpha and the alpha/beta genotypes, and hence the alpha allele were much more observed in patients with amyloidosis (Table 2). This study shows therefore the association of the alpha allele with this severe phenotype, as well as the protective effect of the beta and gamma alleles on the development of amyloidosis in FMF patients, an effect that was not mentioned previously.

The MICA gene has been recently associated with different inflammatory diseases. The MICA transmembrane exon 5 polymorphism consisting of a (GCT/AGC)n triplet repetition has been particularly studied. Five alleles have been identified so far: A4, A5, A6, A9, corresponding to 4, 5, 6 and 9 repetitions and A5.1, corresponding to a 5 triplet repetition and the insertion of one nucleotide (G/C). MICA appears to be a susceptibility factor in many multifactorial diseases such as Behcet's disease [23], psoriatic arthritis [24] and diabetes [25,26]. In FMF, the first monogenic disease associated with MICA, the latter was found to have a modifying effect on the course of the disease, namely on the attacks frequency and the age of onset, but

| Table 3: Distribution of MICA alleles in the 2 groups of FMF patients, with and without amyloidosis (Non significant) |
|-----------------|-------|-------|-------|-------|-------|-------|
| MICA alleles    | A4    | A5    | A5.1  | A6    | A9    | Total |
| FMF patients with amyloidosis | 7     | 13    | 12    | 19    | 9     | 60    |
| FMF patients without amyloidosis | 15    | 12    | 13    | 30    | 10    | 80    |
| Total           | 22    | 25    | 25    | 49    | 19    | 140   |

http://www.biomedcentral.com/1471-2350/5/4
not on its clinical manifestations [15]. As it was not, however, tested in FMF patients having developed renal amyloidosis, we tried to investigate any possible correlation between the MICA polymorphism and this specific phenotype. The MICA alleles and genotypes were found in a non-significant way in both FMF groups, with and without amyloidosis. Hence, no clear cut association was detected between any MICA allele or genotype and this severe phenotype (Table 3). Moreover, the lack of association between any MICA allele and the age of onset of the disease on one hand, and the attack frequency on the other hand, in the combined group of FMF patients, is in contradiction with previously published results, that correlated the A4 and the A9 MICA alleles with a reduced frequency of attacks and an early age of onset, respectively [15]. Such observation could suggest either a weak effect of MICA hidden by a stronger effect of MEFV or SAA1 genotypes, or a possible varying effect of MICA on the course of the disease in different populations, which could be expected in the case of genes that have minor modifying effects on a disease phenotype.

Conclusions
With the availability of the genetic FMF test, since the cloning of the MEFV gene in 1997, and the automatic prescription of colchicine to the confirmed patients, amyloidosis is being far less encountered. But the reason why it develops in some patients, not in others, is still unknown. The effect of the MEFV genotypes, mainly the ones homozygous for the M694V mutation, seems evident, but there are definitely other modifying factors or genes that play a role in the manifestation of amyloidosis. In the present study, no association was found between any MICA allele or genotype and the age of onset of the disease, or the attack frequency and an early age of onset, respectively [15]. Such observation could suggest either a weak effect of MICA hidden by a stronger effect of MEFV or SAA1 genotypes, or a possible varying effect of MICA on the course of the disease in different populations, which could be expected in the case of genes that have minor modifying effects on a disease phenotype.

Acknowledgment
This work was supported by Scientific Research grants from the Saint Joseph University, Beirut, the Lebanese National Council for Scientific Research and Agence Universitaire de la Francophonie.

References
1. Sohar E, Gefin J, Pras M, Heller H: Familial Mediterranean fever. A survey of 470 cases and review of the literature. Ann J Med 1967, 43:227-253.
2. Konstantopoulos K, Michael S, Kanta A, Pecheur C, Grateau J, Helioti H, Stathakis C: Renal amyloidosis as a first manifestation of Familial Mediterranean Fever. Scand J Rheumatol 2000, 29:129-130.
3. The repertory of Familial Mediterranean Fever (FMF) and Hereditary Autoinflammatory Disorders Mutations [http://fmd.igb.cors.fr/infevers/]
4. French FMF Consortium: A candidate gene for familial Mediterranean fever. Nat Genet 1997, 17:25-31.
5. International FMF Consortium: Ancient missense mutations in a new member of the RoRet gene family are likely to cause familial Mediterranean fever. Cell 1997, 90:797-807.
6. Dewalle M, Domingo C, Rozenbaum M, Ben-Chetrit E, Cattan D, Bernot A, Dross C, Dupont M, Notarnicola C, Levy M, Rosner I, Demaille J, Touitou I: Phenotype-genotype correlation in Jewish patients suffering from familial Mediterranean fever (FMF). Eur J Hum Genet 1998, 6:95-97.
7. Cazeneuve C, Sarkissian T, Pecheur C, Dervichian M, Nédelec B, Reiner P, Ayyazyman A, Kouyoudjian JC, Ajrapetyan H, Delpech M, Goossens M, Dodé C, Amselem S: MEFV-gene analysis in Armenian patients with familial Mediterranean fever: diagnostic value and unfavorable renal prognosis of the M694V homozygous genotype-Genetic and therapeutic implications. Am J Hum Genet 1999, 65:88-97.
8. Shohat M, Magal N, Shohat T, Chen X, Dagan T, Mimouni A, Danon Y, Lotan R, Ogur G, Sirin A, Schlezinge M, Halmpern GJ, Schwabe A, Kastner D, Rotter JL, Fischel-Ghodsi N: Phenotype-genotype correlation in familial Mediterranean fever: evidence for an association between Met694Val and amyloidosis. Eur J Hum Genet 1999, 7:287-292.
9. Mimouni A, Magal N, Stoffman N, Shohat T, Minasian A, Krasnov M, Halmpern GJ, Rotter JL, Fischel-Ghodsi N, Danon YL, Shohat M: Familial Mediterranean fever: Effects of genotype and ethnicity on inflammatory attacks and amyloidosis. Pediatrics 2000, 105(3):E70.
10. Ben-Chetrit E, Backenroth R: Amyloidosis induced, end stage renal disease in patients with familial Mediterranean fever is highly associated with point mutations in the MEFV gene. Ann Rheum Dis 2001, 60(2):146-149.
11. Mansour I, Delague V, Cazeneuve C, Dodé C, Chouery E, Pecheur C, Medlej-Hashim M, Salem N, El Zein L, Levan-Petit I, Lefranc G, Goossens M, Delpech M, Amselem S, Loiselet J, Graeule T, Megarbané A, Namran R: Familial Mediterranean fever in Lebanon: mutation spectrum, evidence for cases in Maronites, Greek orthodoxes, Greek catholics, Syriacs and Chitties and for an association between amyloidosis and M694V and M694I mutations. Eur J Hum Genet 2001, 9:51-55.
12. Tekin M, Yalcinlaya F, Cakar N, Akar N, Misirlioglu M, Tastan H, Toker N: MEFV mutations in multiplex families with familial Mediterranean fever: is a particular genotype necessary for amyloidosis? Clin Genet 2000, 57:430-434.
13. Cazeneuve C, Ajrapetyan H, Papin S, Roudot-Thoraval F, Genevieve D, Mndjoyan E, Papazian M, Sarkissian A, Babloyan A, Boissier B, Duquesnoy P, Kouyoudjian JC, Girodon-Boulandet E, Graeule T, Sarkissian T, Amselem S: Identification of MEFV-independent modifying genetic factors for familial Mediterranean fever. Am J Hum Genet 2000, 67:1136-1143.
14. Gershoni-Baruch R, Brik R, Zachs N, Shinawi M, Lidar M, Livneh A: The contribution of genotypes at the MEFV and SAA1 loci to amyloidosis and disease severity in patients with familial Mediterranean fever. Arthritis Rheum 2003, 48(4):1149-1155.
15. Touitou I, Picot MC, Domingo C, Notarnicola C, Cattan D, Demaille J, Koné-Paut I: The MICA region determines the first modifier locus in familial Mediterranean fever. Arth Rheum 2001, 44(1):163-169.

Authors' contributions
Myrna Medlej-Hashim looked for the patients, tested the SAA1 and MICA alleles and wrote the article. Valérie Delague helped in the recruitment of Lebanese FMF patients and MICA alleles' analysis. Eliane Chouery and Nabiba Salem helped in DNA extraction from patients' blood leucocytes and testing of the MEFV genotype. Mohammed Rawashdeh helped in the recruitment of Jordanian FMF patients with amyloidosis. Gérard Lefranc participated in the study design and coordination, Jacques Loiselet helped in the results analysis and statistical calculations and André Mégarbané directed and supervised the study. All authors read and approved the final manuscript.
16. Miller SA, Dynes DD, Polesky F: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988, 16:1215.

17. Medlej-Hashim M, Rawashdeh M, Chouery E, Mansour I, Delague V, Lefranc G, Naran R, Loiselet J, Megarbane A: Genetic screening of fourteen mutations in Jordanian familial Mediterranean fever patients. Hum Mutat 2000, 15(4):384.

18. Morishige M, Terai C, Kosuki Y, Usatsu M, Nakajima A, Inada S, Nasheinana M, Uchida S, Nakajima A, Kim SY, Chen CL, Kamatani N: Influence of genotypes at SAA1 and SAA2 loci on the development and the length of latent period of secondary AA-amyloidosis in patients with rheumatoid arthritis. Hum Genet 1999, 105:360-366.

19. Friedman S, Janowitz F: Systemic amyloidosis and the gastrointestinal tract. Gastroenterol Clin North America 1998, 27(3):595-614.

20. Baba S, Takahashi T, Kasama T, Shirasawa H: Identification of two novel amyloid A protein subsets coexisting in an individual patient of AA-amyloidosis. Biochem Biophys Acta 1992, 1180:195-200.

21. Sipe JD, Colten HR, Goldberger G, Edge MD, Tack BF, Cohen AS, Whitehead AS: Human serum amyloid A (SAA): Biosynthesis and post-synthetic processing of pre-SAA and structural variants defined by complementary DNA. Biochemistry 1985, 24:2931-2936.

22. Booth DR, Booth SE, Gillmore JD, Hawkins PN, Pepys MB: SAA1 alleles as risk factors in reactive systemic AA amyloidosis. Amyloid 1998, 5(4):262-265.

23. Mizuki N, Ota M, Kimura M, Ohno S, Ando H, Katsuyma Y, Yamauzaki M, Watanabe K, Goto K, Nakamura S, Bahram S, Inoko H: Triplet repeat polymorphism in the transmembrane region of the MICA gene: a strong association of six GCT repetitions with Behçet disease. Proc Natl Acad Sci USA 1997, 94:1298-1303.

24. Gonzalez S, Martinez-Borra J, Torre-Alonso JC, Gonzalez-Roces S, Sanchez del Rio J, Rodriguez Perez A, Chaim Brautbar, Lopez-Larrea C: The MICA-A9 triplet repeat polymorphism in the transmembrane region of the MICA gene confers additional susceptibility to the development of psoriatic arthritis and is independent of the association of CW*0602 in psoriasis. Arh Dermatol 1999, 42(5):1010-1016.

25. Kawabata Y, Ikegami H, Kawagushi Y, Fujisawa T, Hotta M, Ueda H, Shintani M, Nojima K, Ono M, Nishino M, Taniguchi H, Nosso S, Yariada K, Babaya N, Oghara T: Age-related association of MHC class I chain-related gene A (MICA) with type 1 (insulin-dependent) diabetes mellitus. Hum Immunol 2000, 61:624-629.

26. Lee YJ, Huang FY, Wang CH, Lo FS, Tsan KW, Hsu CH, Huang CY, Chang SC, Chang JG: Polymorphism in the transmembrane region of the MICA gene and type 1 diabetes. J Pediatr Endocrinol Metab 2000, 13:489-496.

Pre-publication history
The pre-publication history for this paper can be accessed here:

http://www.biomedcentral.com/1471-2350/5/4/prepub