P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

World J Gastroenterol 2003;9(7):1541-1544
World Journal of Gastroenterology
 ISSN 1007-9327

• BASIC RESEARCH •

Polymorphism in transmembrane region of MICA gene and cholelithiasis

Shou-Chuan Shih, Yann-Jinn Lee, Hsin-Fu Liu, Ching-Wen Dang, Shih-Chuan Chang, Shee-Chan Lin, Chin-Roa Kao

Shou-Chuan Shih, Division of Gastroenterology, Department of Medicine, Mackay Memorial Hospital; Mackay Junior School of Nursing, Taipei, Taiwan
Yann-Jinn Lee, Department of Pediatrics and Medical Research, Mackay Memorial Hospital; College of Medicine, Taipei Medical University, Taipei, Taiwan
Hsin-Fu Liu, Ching-Wen Dang, Shih-Chuan Chang, Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan
Shee-Chan Lin, Chin-Roa Kao, Division of Gastroenterology, Department of Medicine, Mackay Memorial Hospital, Taipei, Taiwan
Supported by MMH Grant 8844 from Mackay Memorial Hospital
Correspondence to: Dr. Yann-Jinn Lee, Departments of Pediatrics and Medical Research, Mackay Memorial Hospital, 92, Section 2, Chung-Shan North Road, Taipei, Taiwan. yannlee@ms2.mmh.org.tw
Telephone: +886-2-25433535 **Fax:** +886-2-25433642
Received: 2003-04-02 **Accepted:** 2003-04-24

Abstract

AIM: To study the significance of polymorphism of MHC class I chain-related gene A (MICA) gene in patients with cholelithiasis.

METHODS: Subjects included 170 unrelated adults (83 males) with cholelithiasis and 245 randomly selected unrelated adults (130 males) as controls. DNA was extracted from peripheral leukocytes and analyzed for polymorphism of 5 alleles (A4, A5, A5.1, A6 and A9) of the MICA gene.

RESULTS: There was no significant difference in phenotype, allele, and genotype frequencies of any of the 5 alleles between cholelithiasis patients and controls.

CONCLUSION: This study demonstrates that MICA alleles studied bear no relation to cholelithiasis.

Shih SC, Lee YJ, Liu HF, Dang CW, Chang SC, Lin SC, Kao CR. Polymorphism in transmembrane region of MICA gene and cholelithiasis. *World J Gastroenterol* 2003; 9(7): 1541-1544
http://www.wjgnet.com/1007-9327/9/1541.asp

INTRODUCTION

Molecular genetics has a substantial impact on our understanding of inherited susceptibility to many diseases. There is an increased familial frequency of cholelithiasis[1], which is a prevalent disorder in Taiwan[2]. HLA gene is associated with many human diseases. It is reasonable to speculate on whether there is a correlation between cholelithiasis and HLA gene.

MICA gene is located near HLA-B on chromosome 6, and is by far the most divergent mammalian MHC class I gene known[3]. It lies 46.4 kb centromeric to HLA-B gene, and they are oriented head-to-head. It has a triplet repeat microsatellite polymorphism $(GCT)_n$ in the transmembrane region. This polymorphism consists of five alleles, with 4, 5, 6, and 9 repetitions of GCT or 5 repetitions of GCT with 1 additional nucleotide insertion (G), designated as A4, A5, A6, A9, and A5.1, respectively[4]. The alleles vary among individuals, and hence this microsatellite can be used as an informative polymorphic marker for genetic mapping and for analysis of disease susceptibility.

To date, there have been no reports investigating an association between MICA gene and cholelithiasis. We designed this study to compare MICA polymorphism among Chinese with cholelithiasis, their family members, and unaffected controls.

MATERIALS AND METHODS

Patients with cholelithiasis and controls

One hundred and seventy unrelated adult patients (83 males) with cholelithiasis were enrolled. The average age at diagnosis was 50.5±3.1 years. Cholelithiasis was documented ultrasonographically and/or after operation (75 patients underwent cholecystectomy). The ultrasonographic criterion for diagnosis of cholelithiasis was echogenic material with a postural shift and/or casting an acoustic shadow in the gallbladder. According to their number and size, ultrasonographic patterns of gallbladder stones were divided into 3 types: single, particle (multiple, larger size) and sandy (multiple, sand-like appearance). Control subjects consisted of 245 unrelated subjects (130 males) from the same area where the patients resided. They came to the hospital for physical checkups, minor operations or injuries, or evaluation of fever or abdominal pain. Those with autoimmune disorders, liver diseases, or blood diseases were excluded from the study. In addition, a total of 144 family members of 75 patients with cholelithiasis were included for further comparison. Blood was drawn from all the subjects to extract genomic DNA. All the subjects were Chinese, and they all gave written consent. The study was approved by the local ethics committee.

DNA extraction

Genomic DNA was extracted from fresh or frozen peripheral blood leukocytes by standard techniques[5, 6].

Determination of the polymorphism of $(GCT)_n$ microsatellite

Primers for analysis of microsatellite polymorphism in the transmembrane region of MICA gene, PCR primers flanking the transmembrane region (MICA5F, 5'-CCTTTTTTTCAGGGAAAGTGC-3'; MICA5R, 5'-CCTTACCATCTCCAGAAACTGC-3') were designed according to the reported sequences[3, 4]. The MICA5F primer corresponded to the intron 4 and exon 5 boundary region and MICA5R was located in intron 5[4]. MICA5R was 5' end-labelled with fluorescent dye (Applied Biosystems)[7, 8].

Polymerase chain reaction (PCR) The amplification reaction mixture (15 μl) contained 50 ng genomic DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatin, 0.1 % Triton X-100, 0.2 mM of each dNTP, 0.5 mM of each primer and 0.5 unit Pro Taq DNA polymerase (Protech Technology Enterprise). PCR amplification was carried out in a GeneAmp PCR system (Applied Biosystems). The mixture

was subjected to denaturation at 95 ℃ for 5 min, followed by 10 cycles at 94 ℃ for 15 sec, at 55 ℃ for 15 sec, at 72 ℃ for 30 sec, then by an additional 20 cycles at 89 ℃ for 15 sec, at 55 ℃ for 15 sec, at 72 ℃ for 30 sec, and by a final extension at 72 ℃ for 10 min.

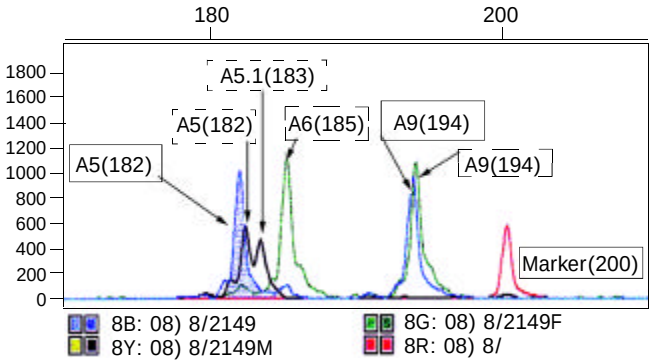
Analysis of triplet repeat polymorphism The amplified products were denatured at 100 ℃ for 5 min, mixed with formamide-containing stop buffer, and subjected to electrophoresis on 4 % polyacrylamide gels containing 8-M urea in an automated DNA sequencer (ABI Prism 377-18 DNA sequencer, Applied Biosystems). The number of microsatellite repeats was estimated automatically with Genescan 672 software (Applied Biosystems) by means of the local Southern method with a size standard marker of GS-350 TAMRA (Applied Biosystems)[8]. Alleles were designated according to Mizuki *et al.*[4] and Perez-Rodriguez *et al.*[9], with amplified sizes of 179 bp (A4), 182 bp (A5), 183 bp (A5.1), 185 bp (A6), 194 bp (A9), and 197 bp (A10).

Statistical analysis

Evaluation of the Hardy-Weinberg equilibrium was performed by comparing observed and expected heterozygotes and homozygotes, as well as observed and expected genotypes, using the χ^2 test[10]. Phenotype, allele, or genotype frequencies of patients and controls were compared by the χ^2 test with Yates' correction where appropriate (one expected number <5). Patients and controls positive for a factor were compared using a free statistical program on the internet[11]. *P* values were corrected using the Bonferonni inequality method for the number of comparisons[12]. Statistical significance was defined as $P<0.05$.

RESULTS

Figure 1 shows the electrophoretograms of the PCR products of 3 subjects. We did not detect A10 in any of the patients or controls. The distribution of MICA genotypes in the two groups was in Hardy-Weinberg equilibrium, i.e. observed and expected figures did not differ. No significant difference was found between patients with cholelithiasis and controls in phenotype, allele, or genotype frequencies. There were also no differences between male and female patients or between patients with different sonographic stone types. Age at diagnosis was also not correlated with allele frequency (Tables 1, 2 3). As the difference between patients and controls was not remarkable, it was not meaningful to compare the results between patients and their family members (data not shown).



Genotypes of a subject (2149), his father (2149F), and mother (2149M)

Subject	Line	Genotype
2149	solid	A5 / A9
2149F	dash	A6 / A9
2149M	dot	A5 / A5.1

Figure 1 Electrophoretograms of the PCR products for three subjects. Amplified sizes of the alleles were 182 bp (A5), 183 bp (A5.1), 185 bp (A6), and 194 bp (A9).

Table 1 Phenotype frequencies of the polymorphism in transmembrane region of MICA gene in patients with cholelithiasis and controls

Phenotype	Patient[a]		Control[a]		Single[b]		Particle[b]		Sandy[b]		Male[c]		Female[c]	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
A4	49	28.8	69	28.2	10	35.7	24	30.0	15	24.2	30	36.1	19	21.9
A5	85	50.0	133	54.3	15	53.6	43	53.8	27	43.6	42	50.6	43	49.4
A5.1	72	42.4	113	46.1	9	32.1	32	40.0	31	50.0	33	39.8	39	44.8
A6	23	13.5	30	12.2	4	14.3	10	12.5	9	14.5	9	10.8	14	16.1
A9	64	37.7	79	32.2	11	39.3	31	38.8	22	35.5	30	36.1	34	39.1
Total	170	100	245	100	28	100	80	100	62	100	83	100	87	100

[a]$P=0.81$, [b]$P=0.441$, [c]$P=0.316$.

Table 2 Allele frequencies of the polymorphism in transmembrane region of MICA gene in patients with cholelithiasis and controls

Allele	Patient[a]		Control[a]		Single[b]		Particle[b]		Sandy[b]		Age at Dx≤50		Age at Dx≤30		Age at Dx ≤40[d]		Male[c]		Female[c]	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
A4	53	15.6	74	15.1	11	19.6	26	16.3	16	12.9	21	13.8	3	15.0	5	8.1	34	20.5	19	10.9
A5	105	30.9	162	33.1	18	32.1	56	35.0	31	25.0	49	32.2	6	30.0	19	30.6	51	30.7	54	31.0
A5.1	85	25.0	135	27.6	11	19.6	35	21.9	39	31.5	39	25.7	5	25.0	18	29.0	38	22.9	47	27.0
A6	24	7.1	32	6.5	4	7.1	10	6.3	10	8.1	13	8.6	2	10.0	6	9.7	10	6.0	14	8.0
A9	73	21.5	87	17.8	12	21.4	33	20.6	28	22.6	30	19.7	4	20.0	14	22.6	33	19.9	40	23.0
Total	340	100	490	100	56	100	160	100	124	100	152	100	20	100	62	100	166	100	174	100

[a]$P=0.71$, [b]$P=0.541$, [c]$P=0.415$, [d]$P=0.501$ (age ⩽ 40 *vs.* control), Dx=Diagnosis.

Table 3 Genotype frequencies of the polymorphism in transmembrane region of MICA gene in patients with cholelithiasis and controls

Genotype	Patient[a]		Control[a,b]		Single[b]		Particle[b]		Sandy[b]		Age at Dx ⩽ 50		Male[c]		Female[c]	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
A4 / A4	4	2.4	5	2.0	1	3.6	2	2.5	1	1.6	2	2.6	4	4.8	0	0
A4 / A5	14	8.2	22	9.0	3	10.7	8	10.0	3	4.8	6	7.9	8	9.6	6	6.9
A4 / A5.1	13	7.6	23	9.4	2	7.1	6	7.5	5	8.1	6	7.9	7	8.4	6	6.9
A4 / A6	2	1.2	8	3.3	0	0	1	1.3	1	1.6	0	0	2	2.4	0	0
A4 / A9	16	9.4	11	4.5	4	14.3	7	8.8	5	8.1	5	6.6	9	10.8	7	8.0
A5 / A5	20	11.8	29	11.8	3	10.7	13	16.3	4	6.5	8	10.5	9	10.8	11	12.6
A5 / A5.1	21	12.4	37	15.1	2	7.1	8	10.0	11	17.7	10	13.2	11	13.3	10	11.5
A5 / A6	10	5.9	11	4.5	4	14.3	3	3.8	3	4.8	7	9.2	3	3.6	7	8.0
A5 / A9	20	11.8	34	13.9	3	10.7	11	13.8	6	9.7	10	13.2	11	13.3	9	10.3
A5.1 / A5.1	13	7.6	22	9.0	2	7.1	3	3.8	8	12.9	6	7.9	5	6.0	8	9.2
A5.1 / A6	8	4.7	7	2.9	0	0	5	6.3	3	4.8	3	3.9	3	3.6	5	5.7
A5.1 / A9	17	10.0	24	9.8	3	10.7	10	12.5	4	6.5	8	10.5	7	8.4	10	11.5
A6 / A6	1	0.6	2	0.8	0	0	0	0	1	1.6	1	1.3	1	1.2	0	0
A6 / A9	2	1.2	2	0.8	0	0	1	1.3	1	1.6	1	1.3	0	0	2	2.3
A9 / A9	9	5.3	8	3.3	1	3.6	2	2.5	6	9.7	3	3.9	3	3.6	6	6.9
Total	170	100	245	100	28	100	80	100	62	100	76	100	83	100	87	100

[a]P=0.797, [b]P=0.063 (control *vs.* stone types), [c]P=0.337, Dx=Diagnosis.

DISCUSSION

Cholelithiasis is quite prevalent in Taiwan[2] and is easily detected by ultrasonography, which has become a commonly used screening tool for health maintenance exams. There is as yet no well documented method to prevent cholelithiasis formation. However, it is now well known that detecting genetic defects may lead to better surveillance or even avoidance of certain diseases. Thus it seems worthwhile to search for gene disorders in a common disease like cholelithiasis.

MHC class I genes (HLA-A, -B and -C) encode single polypeptides organized into three domains: α_1, α_2, and α_3. Their surface expression on most nucleated cells of the body depends on the noncovalent association with a fourth domain, the non-MHC-encoded polypeptide β_2-microglobulin[13]. MHC class I molecules are important in the efferent limb of immunity, which is designed to destroy cells bearing foreign antigens. Foreign peptides presented within the cell are deposited in the binding groove of MHC class I molecules and expressed on the cell surface. Cytotoxic T cells recognize them and destroy the cell[14]. Certain MHC class I molecules have been found to be associated with various diseases, including type 1 diabetes and cholelithiasis[15-18]. An even stronger association has been found with MHC class II molecules[18, 19]. The initial explanation of HLA class I associations is linkage disequilibrium between certain subtypes of MHC class I and class II molecules[19, 20]. However, other studies have suggested that there may be other loci in the MHC gene complex that also play a role[21, 22]. Further investigation of such loci nearby or within MHC class I genes, such as MICA gene, is necessary to clarify these discrepancies.

MICA gene has recently been found to be more significantly associated with disease susceptibility that had been previously reported to be associated with the HLA-B locus (HLA-B7, -B8, -B15, -B18 in Caucasians; -Bw22, and -Bw54 in Chinese; -B5, -Bw52, and -Bw54 in Japanese are reportedly associated with various diseases)[4, 7, 8, 15, 16, 23-28]. This suggests that susceptibility associated with the HLA-B locus might be due to different genotypes of MICA gene.

MICA is specifically expressed by fibroblasts, epithelial cells, keratinocytes, endothelial cells, and monocytes[3, 29]. The molecule is similar to MHC class I antigens. Thus MICA is thought to represent a second lineage of MHC antigens and possibly to play a specialized or modified role in the immune response[3]. The recruitment of γδ T cells in skin and intestinal mucosa mirrors the pattern of expression of MICA and is consistent with the hypothesis that these molecules may play a role in inflammation and in the response to stress or damage in certain tissues[29]. MICA expression is regulated by promoter heat shock elements similar to those of HSP70 genes[30]. The high levels of MICA expression in epithelial cell lines together with the upregulation of MICA after heat shock may represent a molecular mechanism for exposing stressed epithelial cells to the immune system[31]. Thus MICA may function as an indicator of cell stress and may be recognized by γδ T cells in an unusual interaction. It is possible, therefore, that MICA regulates the immune response when cells are stressed and might be involved in the development of cholelithiasis.

However, our study showed that the polymorphic MICA alleles were not significantly associated with cholelithiasis, nor were there any differences when we looked at subgroups of patients, stratified according to sex, types of gallstone, or age. The latter factor is of interest in considering the contribution of heredity *vs.* environment. These results are perhaps not surprising, since the pathogenesis of cholelithiasis in Taiwan is multifactorial[32]. Nevertheless, our demonstration of the absence of an association between MICA gene and cholelithiasis is useful in pointing future research in other directions. The fact of the increased familial frequency of cholelithiasis[1] thus remains to be explained, perhaps by studying other loci on the human chromosome.

REFERENCES

1 **Gilat T,** Feldman C, Halpern Z, Dan M, Bar-Meir S. An increased familial frequency of gallstones. *Gastroenterol* 1983; **84:** 242-246

2 **Su CH,** Lui WY, P'eng FK. Relative prevalence of gallstone diseases in Taiwan. A nationwide cooperative study. *Dig Dis Sci* 1992; **37:** 764-768

3 **Bahram S,** Bresnahan M, Geraghty DE, Spies T. A second lineage of mammalian major histocompatibility complex class I genes. *Proc Natl Acad Sci USA* 1994; **91:** 6259-6263

4 **Mizuki N,** Ota M, Kimura M, Ohno S, Ando H, Katsuyama Y, Yamazaki 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 Behcet's disease. *Proc Natl Acad Sci USA* 1997; **94:** 1298-1303

5 **Buffone GJ,** Darlington GJ. Isolation of DNA from biological specimens without extraction with phenol. *Clin Chem* 1985; **31:** 164-165

6 **Lee HH,** Chao HT, Ng HT, Choo KB. Direct molecular diagnosis of CYP21 mutations in congenital adrenal hyperplasia. *J Med Genet* 1996; **33:** 371-375

7 **Goto K,** Ota M, Ohno S, Mizuki N, Ando H, Katsuyama Y, Maksymowych WP, Kimura M, Bahram S, Inoko H. MICA gene and ankylosing spondylitis: linkage analysis via a transmembrane-encoded triplet repeat polymorphism. *Tissue Antigens* 1997; **49:** 503-507

8 **Goto K,** Ota M, Maksymowych WP, Mizuki N, Yabuki K, Katsuyama Y, Kimura M, Inoko H, Ohno S. Association between MICA gene A4 allele and acute anterior uveitis in white patients with and without HLA-B27. *Am J Ophthalmol* 1998; **126:** 436-441

9 **Perez-Rodriguez M,** Corell A, Arguello JR, Cox ST, McWhinnie A, Marsh SG, Madrigal JA. A new MICA allele with ten alanine residues in the exon 5 microsatellite. *Tissue Antigens* 2000; **55:** 162-165

10 **Hedrick PW.** Genetics of Populations. 2nd ed. Sudbury, Massachusetts: *Jones and Bartlett Publishers* 2000: 1-553

11 **Lee YJ,** Chen MR, Chang WC, Lo FS, Huang FY. A freely available statistical program for testing association. *MD Comput* 1998; **15:** 327-330

12 **Svejgaard A,** Ryder LP. HLA and disease associations: detecting the strongest association. *Tissue Antigens* 1994; **43:** 18-27

13 **Todd JA,** Bell JI, McDevitt HO. A molecular basis for genetic susceptibility to insulin-dependent diabetes mellitus. *Trends Genet* 1988; **4:** 129-134

14 **Walker RH.** The HLA system. In: Walker RH, eds. Technical Manual. Bethesda: *American Association of Blood Banks* 1993: 287-307

15 **Singal DP,** Blajchman MA. Histocompatibility (HL-A) antigens, lymphocytotoxic antibodies and tissue antibodies in patients with diabetes mellitus. *Diabetes* 1973; **22:** 429-432

16 **Nerup J,** Platz P, Anderson OO, Christiy M, Lyngsoe J, Poulsen JE, Ryder LP, Nielsen LS, Thomsen M, Svejgaard A. HL-A antigens and diabetes mellitus. *Lancet* 1974; **2:** 864-866

17 **Papasteriades C,** Al-Mahmoud I, Papageorgakis N, Romania ST, Katsas A, Ollier W, Economidou I. HLA antigen in Greek patients with cholelithiasis. *Dis Markers* 1990; **8:** 17-21

18 **Pokorny CS,** McCaughan GW, Gallagher ND, Selby WS. Sclerosing cholangitis and biliary tract calculi-primary or secondary. *Gut* 1992; **33:** 1376-1380

19 **Solow H,** Hidalgo R, Singal DP. Juvenile-onset diabetes HLA-A, -B, -C, and-DR alloantigens. *Diabetes* 1979; **28:** 1-4

20 **Farid NR,** Sampson L, Noel P, Barnard JM, Davis AJ, Hillman DA. HLA-D-related (DRw) antigens in juvenile diabetes mellitus. *Diabetes* 1979; **28:** 552-557

21 **Raum D,** Awdeh Z, Yunis EJ, Alper CA, Gabbay KH. Extended major histocompatibility complex haplotypes in type 1 diabetes mellitus. *J Clin Invest* 1984; **74:** 449-454

22 **Robinson WP,** Barbosa J, Rich SS, Thomson G. Homozygous parent affected sib pair method for detecting disease predisposing variants: Application to insulin dependent diabetes mellitus. *Genet Epidemiol* 1993; **10:** 273-288

23 **Ota M,** Katsuyama Y, Mizuki N, Ando H, Furihata K, Ono S, Pivetti-Pezzi P, Tabbara KF, Palimeris GD, Nikbin B, Davatchi F, Chams H, Geng Z, Bahram S, Inoko H. Trinucleotide repeat polymorphism within exon 5 of the MICA gene (MHC class I chain-related gene A): allele frequency data in the nine population groups Japanese, Northern Han, Uygur, Kazakhstan, Iranian, Saudi Arabian, Greek and Italian. *Tissue Antigens* 1997; **49:** 448-454

24 **Goto K,** Ota M, Ando H, Mizuki N, Nakamura S, Inoue K, Yabuki K, Kotake S, Katsuyama Y, Kimura M, Inoko H, Ohno S. MICA gene polymorphisms and HLA-B27 subtypes in Japanese patients with HLA-B27-associated acute anterior uveitis. *Invest Ophthalmol Vis Sci* 1998; **39:** 634-637

25 **Fukuda K,** Sugawa K, Wakisaka A, Moriuchi J, Matsuura N, Sato Y. Statistical detection of HLA and disease association. *Tissue Antigens* 1985; **26:** 81-86

26 **Thomson G.** HLA disease associations: Models for the study of complex human genetic disorders. *Crit Rev Clin Lab Sci* 1995; **32:** 183-219

27 **Hawkins BR.** Human leukocyte antigens in chinese. Hong-Kong: *Hong-Kong University Press* 1987: 1-122

28 **Kobayashi T,** Tamemoto K, Nakanishi K, Kato N, Okubo M. Immunogenetic and clinical characterization of slowly progressive IDDM. *Diabetes Care* 1993; **16:** 780-788

29 **Zwirner NW,** Fernandez-Vina MA, Stastny P. MICA, a new polymorphic HLA-related antigen, is expressed mainly by keratinocytes, endothelial cells, and monocytes. *Immunogenetics* 1998; **47:** 139-148

30 **Groh V,** Bahram S, Bauer S, Herman A, Beauchamp M, Spies T. Cell stress-regulated human major histocompatibility complex class I gene expressed in gastrointestinal epithelium. *Proc Natl Acad Sci USA* 1996; **93:** 12445-12450

31 **Bahram S,** Mizuki N, Inoko H, Spies T. Nucleotide sequence of the human MHC class I MICA gene. *Immunogenetics* 1996; **44:** 80-81

32 **Ho KJ,** Lin XZ, Yu SC, Chen JS, Wu CZ. Cholelithiasis in Taiwan. Gallstones characteristics, surgical incidence, bile lipid composition and role of β-glucuronidase. *Dig Dis Sci* 1995; **40:** 1963-1973

Edited by Xu XQ and Wang XL