Mol Biol Rep (2012) 39:8533–8538
DOI 10.1007/s11033-012-1708-7

Detection of new biallelic polymorphisms in the human *MxA* gene

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Received: 15 February 2012 / Accepted: 6 June 2012 / Published online: 20 June 2012

Abstract The interferon-inducible human MxA protein plays an important role in innate defense against an array of viruses. One might expect allelic diversity at the *MxA* locus to influence the timing and magnitude of its expression or even the range of viruses whose biological cycle is inhibited by the encoded product. Here we have collected 267 samples of genomic DNA from three distinct populations (European, Asian, and African) and have systematically sequenced the promoter of the *MxA* gene and its 17 exons in order to inventory its allelic variants. Eighteen single-nucleotide polymorphisms were detected, four of which had never been identified before. Two of these, located in the promoter (at positions −309 and −101 respectively), might affect the MxA expression pattern. The other two result in substitutions (Gly255Glu and Val268Met) in the protein's N-terminal region that might directly affect its antiviral function.

Keywords Interferon · Mx · Virus · Innate immunity

Introduction

The Mx proteins of vertebrates are high-molecular-weight dynamin-like proteins encoded, according to the species, by two or three distinct genes whose expression depends strictly on type-1 and type-3 interferons [1]. They consist of three domains: an N-terminal GTPase domain that binds and hydrolyzes GTP (G domain), a middle domain that mediates self-assembly and oligomerization (MD), and a C-terminal GTPase effector domain involved in self-assembly (GED) [2]. Some isoforms are able to inhibit the life cycle of one or several viruses and are thus components of innate immune response. The ability of an Mx protein to act against a virus depends on the nature of its GED domain, probably involved in recognizing viral targets [3]. Among the Mx proteins known to exert antiviral activity, the human MxA protein displays the broadest antiviral spectrum, inhibiting the life cycles of viruses belonging to a diverse range of families: *Orthomyxoviridae*, *Paramyxoviridae*, *Rhabdoviridae*, *Bunyaviridae*, *Picornaviridae*, and *Togaviridae* [4]. These features of MxA, demonstrated in vitro, make it appear as a key antiviral effector of innate immunity in the human species. Accordingly, its allelic polymorphism can be expected to affect the severity of viral diseases in humans [4].

The *MxA* gene (GenBank accession no. AC005612) is located on the long arm of chromosome 21 (21q22.3) and contains 17 exons over a 33-kb stretch [5, 6]. Its promoter contains three interferon-stimulated response elements (ISREs), a class II IL-6 response element, an Sp1 binding site, and several NF-kappaB binding sites [7]. To date, two single-nucleotide polymorphisms (SNPs) have been characterized. They are located in the promoter region, at positions −123 (C/A) and −88 (G/T), and affect the *MxA* expression level of [8–10]. A series of population-based studies have linked these SNPs to specific risks or clinical outcomes [11–17]. The flowering of such studies since the discovery of these first two SNPs highlights the crucial medical importance of MxA allelic variants as biomarkers of susceptibility or resistance to viral infections in humans. It is

Electronic supplementary material The online version of this article (doi:10.1007/s11033-012-1708-7) contains supplementary material, which is available to authorized users.

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thus highly desirable to screen the MxA promoter and coding sequence systematically so as to inventory its allelic variants. Here our aim was to contribute to this inventory and to examine whether genotype frequencies vary among ethnic groups. We have detected many SNPs, four of which, including two non-synonymous SNPs in the coding sequence, have never been detected before (http://snp.cshl.org/).

Materials and methods

Population samples

Two hundred and sixty-seven mouth swabs (Isohelix Swab, cat. no. SK-2S) were collected from three distinct populations between 2008 and 2011. Thirty-six swabs were obtained from Asian (Chinese and Vietnamese) university students, 131 from Belgian and French students (Western Europeans), and 100 from African students (from Algeria, Libya, Cameroun, Niger, Congo, or Rwanda). Within each ethnic group, the subjects tested were randomly selected. Each swab was rubbed for 1 min against the inside of the cheek or behind the lower or upper lip and stored at 4 °C until processed. The study was approved by the local Bioethics Committee (File #PolyMxA/AC/2010).

DNA extraction and SNP detection

Genomic DNA was extracted from mouth epithelial cells with a commercially available kit (Isohelix cat. no. BEK-50). Each specimen was characterized quantitatively (Nanodrop) and qualitatively (Isohelix cat. no. DQC-50) and stored at −20 °C until used. SNPs were detected by PCR amplification of 12 successive segments of the *MxA* gene, followed by sequencing of the PCR products. A first step was to validate combined long-range and nested PCR amplification of the 9.8-kb gene segment comprising the promoter and exons 1–5. Then, 11 exon-specific PCR protocols were developed. Primer sequences are listed in Table 1 and the detailed characteristics of each PCR

Table 1 Sequences of primers used to amplify *MxA* gene promoter and exons

Primer name	Primer sequence 5′ → 3′	Annealing temperature (°C)	Exon	PCR product size
Long-range PCR F	ATCTCCCACTCACAGCCAGTTAGCC	55.9	Promoteur—Exon 5	9,631
Long-range PCR R	GCAGCTGGGTGCAATTTTCCTAAAG	52.6		
Nested PCR F	CATGGGTCTGCTTGACTCAGCCCTC	57.5	Promoteur—Exon 5	9,044
Nested PCR R	CTCCCTGGGTCAACTCCTTCACCTC	57.5		
MxAex6F	TTAGACTTATTGAAGTTGGCCGT	64	6	193
MxAex6R	TCTTCCTACCACACCTGATCTGT	68		
MxAex7F	GCTTTCAAGCGTTAGTAAGCAAA	64	7	138
MxAex7R	AAAAGAAGTAGAAGGAACCGTGG	66		
MxAex8F	TCCCAATCACAGAAAATTGAATC	62	8	155
MxAex8R	GCTTCCATTCAACTTGACAGAAC	66		
MxAex9F	CTGAGCCTATAGCTCTGCTTCTG	70	9	139
MxAex9R	CTGCTCCCTTAGCAGGTCTTAGT	70		
MxAex10F	GAGATGGGATGTCCATAACTCAA	66	10	199
MxAex10R	GGCAGTATTTTGTAATCCCCTTC	66		
MxAex11F	GAGCTCATTCCATATCATTGTGG	66	11	79
MxAex11R	ATAGTCTATCAGCATTCCCCCTG	68		
MxAex12 + 13F	GTTGTTGAATCTCAAATTGCTCC	64	12 + 13	265
MxAex12 + 13R	CCTTTAGGAAGGATTGGGTTATG	66		
MxAex14F	ACTCCCCTTCCTGAGATGACTAA	68	14	159
MxAex14R	GAGGAAAGTGCTGAAGTTTGGT	64		
MxAex15F	GGGGTCATTTGCTTTTAAGATTC	64	15	77
MxAex15R	CGTTTAAGCACTCCCTCCTTTAT	66		
MxAex16F	GGGCTAGAAACCTGCCTTAGATA	68	16	249
MxAex16R	AGGCTAACAAGGAGGTAAACGAC	68		
MxAex17F	TGCCTGCTTACCTTTATAGAGCA	66	17	682
MxAex17R	TATTAAGTTAGCACCGTTGGCTT	64		

reaction are available on request. The PCR products were purified with the innuPREP DOUBLEpure kit (Analytik Jena) and sequenced on an ABI-3100 Genetic Analyzer using Big Dye terminator chemistry (Applied Biosystems). The primers listed in Online Resource #2 were used to sequence the 9.8-kb gene segment comprising the promoter and exons 1–5. Primers were designed with Netprimer [http://www.premierbiosoft.com/netprimer]. Nucleotide sequence and identity analyses were carried out, respectively, with the Chromas v.2.21 (http://www.technelysium.com.au) and BLAST programs. Sequences were aligned with GeneDoc v.2.7.000 (http://www.psc.edu/biomed/genedoc).

Statistical methods

Allele and genotype frequencies were estimated by direct counting. The heterogeneity of population samples and conformity with the Hardy–Weinberg equilibrium were

Table 2 Synopsis of reported single nucleotide polymorphisms in human *MxA* gene

Genomic segment	Nucleotide change	Alleles	SNP ID	Amino acid change	Ensembl	NCBI SNP	This study
Promoter	−309	C/G	Not listed yet	–	ND	–	+
	−159	C/A	rs35745218	–	ND	+	–
	−139	C/T	rs34188463	–	ND	+	–
	−123	C/A	rs17000900	–	ND	+	+
	−101	G/A		–	ND	–	+
	−88	G/T	rs2071430	–	ND	+	+
Exon 1	+20	A/C	rs464138	–	+	+	+
Exon 5	+5824	C/T	rs114131127	–	+	+	–
Exon 7	+10892	G/A	rs17000915	–	+	+	–
Exon 9	+14738	C/T	rs467960	–	+	+	+
Exon 10	+15523	G/A	Not listed yet	G/E	–	–	+
	+15527	T/G	rs55748803	–	+	+	–
	+15561	G/A	Not listed yet	V/M	–	–	+
Exon 11	+17578	G/A	rs62623435	G/R	+	+	–
	+17599	C/T	rs116233935	–	+	+	–
Exon 12	+19278	G/A	rs115187240	–	+	+	–
Exon 13	+19777	G/A	rs469390	V/I	+	+	+
	+19784	C/T	rs34717738	A/V	+	+	–
	+19827	T/C	rs75157475	–	+	+	–
	+19897	G/T	rs35132725	E/X	+	+	–
Exon 14	+22960	T/C	rs2070229	–	+	+	+
	+23046	T/G	rs78721041	V/G	+	+	–
Exon 15	+24998	A/C	rs75718352	N/T	+	+	–
Exon 16	+26476	G/A	rs1804113	V/I	+	+	–
	+26508	A/G	rs1050008	–	+	+	+
	+26577	C/T	rs117998041	–	+	+	+
	+26580	G/A	rs469304	–	+	+	+
Exon 17	+32352	C/T	rs111867117	–	+	+	–
	+32376	G/T	rs2230454	Q/H	+	+	–
	+32398	C/A	COSM71731	L/I	+	–	–
	+32537	A/G	rs1557370	–	+	+	+
	+32605	C/T	rs2230455	–	+	+	–
	+32639	C/T	rs116752870	–	+	+	–
	+32640	G/A	rs2230456	–	+	+	+
	+32685	A/G	rs1804112	–	+	+	–
	+32748	A/G	rs60687893	–	+	+	+
	+32749	T/A	rs55992492	–	+	+	+
	+32925	G/A	rs73372143	–	+	+	+

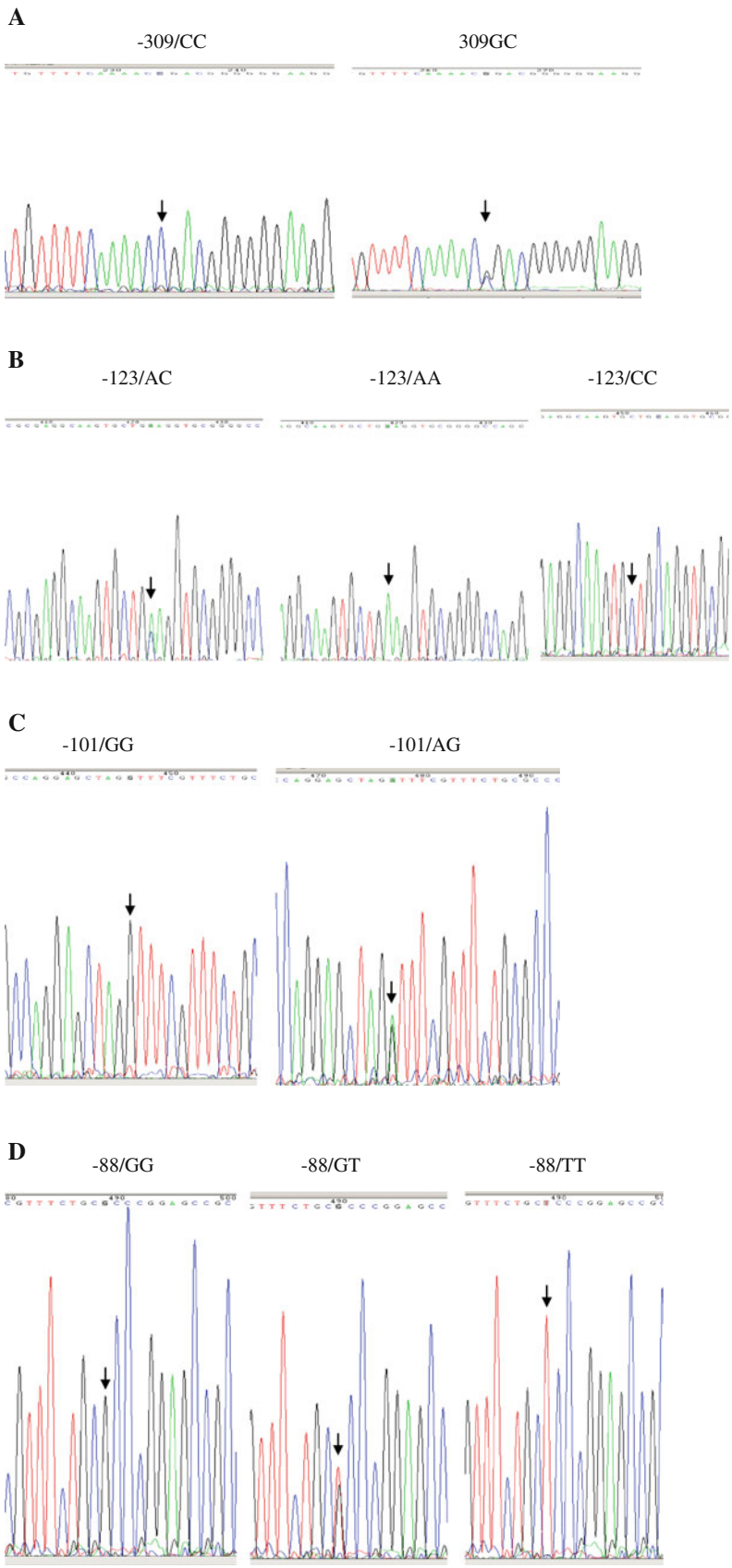

Fig. 1 Examples of chromatograms revealing the polymorphisms discovered in the *MxA* promoter region. A → D : positions −309, −123, −101 and −88, respectively

evaluated with Fisher's exact test and the chi^2 test. The criterion for statistical significance was $P < 0.05$.

Results

The promoter and the 17 exons of the *MxA* gene were sequenced from the genomic DNA of 267 subjects from Belgium and France ($n = 131$), Africa ($n = 100$), or Asia ($n = 36$). Eighteen SNPs were identified in the cohort, four of which are not listed in international databases (Table 2). The first two (−309 C/G and −101 G/A), whose chromatograms are presented (Fig. 1), are located in the promoter. They were detected in the African population, although quite rarely, since only two individuals heterozygotic at −309 and a single individual heterozygotic at −101 were identified. Two new SNPs were identified in exon 10: +15523 G/A and +15561 G/A. At position +15523, the A allele appeared very rare and exclusively in the Asian population (two GA heterozygotic individuals were detected). This rare allele encodes a protein with a Gly255Glu substitution. At position +15561, the A allele appeared very rare, having been found in only one (African) individual (who was homozygous for this allele). This rare variant encodes a protein with a Val268Met substitution.

Genotype and allele frequencies are displayed in Online Resources #4–8. A significant departure from Hardy–Weinberg equilibrium was observed in 4 out of 45 tests ($P < 0.0001$ in the African population for the −123 and −88 polymorphisms, $P < 0.005$ in the African population for the +15561 polymorphism, and $P < 0.001$ in the Western European population for the +32537 polymorphism). This number of significant results is no larger than might be expected to occur through chance alone. Seventeen tests showed significant differences between the three populations ($P < 0.05$). The C/G, A/C, A/G, and G/T allele distributions at positions −309, −123, −101, and −88, respectively, within the promoter, were similar in the three groups, with a highly dominant allele at each location (C, C, G, and G respectively). The most noticeable features detected were (i) the total absence of the −309G and −101A alleles in the Asian and Western European populations and (ii) the low prevalence of the high-MxA-producer genotypes AA (−123) and TT (−88). In the UTR-5′ region, the distribution of the C/A (+20) allele appeared significantly different among groups, the C allele being dominant in Asia and the A allele elsewhere. Within the coding region, the three most noticeable between-population differences were the absence of allele A in one (32925) or two (15523 and 32640) of the populations studied.

Discussion

In this study of polymorphisms in the *MxA* gene and of the corresponding allele frequencies within geographically diverse populations, we have highlighted genetic variation linked to ethnic origin. The variation of some allele frequencies was sufficient to distinguish at least one population as « different » from the others. For example, the Asian group shows lower +20A and +26580A allele frequencies than the other populations. Only in the case of the −123 and −88 polymorphisms were the allele distributions about the same throughout the three populations. The findings of this study corroborate the scant population data available. The low prevalence of the high-MxA-producer genotypes AA (−123) and TT (−88) has been noted previously [8], as has the high occurrence of the +20C allele in Asian populations [14].

We have detected four new polymorphisms, two in the coding region of *MxA* (one leading to a Gly255Glu substitution and the other to a Val268Met substitution in the N-terminal domain) and two in the promoter region. The available structural data are not sufficient for predicting which specific MxA functions might be altered by these variations [18]. Nevertheless, BLOSUM 62 matrix scoring of the two substitutions according to their putative impacts on protein structure [19] revealed that Gly255Glu might significantly affect MxA structure (score: -2), while Val268Met should not (score: +1). On the other hand, both new polymorphisms revealed in the *MxA* promoter region might significantly alter the gene's expression pattern, since the first two polymorphisms ever identified in the *MxA* promoter appear to be linked to the *MxA* expression level, the specific risk of infection, and the clinical outcome of disease [8–10]. A highly significant difference in the distribution of −88 G/T has been observed between patients with persistent and self-limiting HBV infections [15], and the presence of the AG haplotype (−123A and −88G) in an individual appears to correlate significantly with susceptibility to HBV infection [16]. Furthermore, a lower frequency of the −88 GG genotype has been observed in self-limiting HCV infection and in nonresponders to therapy [11]. In Japanese individuals, the −88 GT genotype may confer to the host genetic susceptibility to subacute sclerosing panencephalitis [12]. Also, a significantly higher frequency of the −123A, −88T haplotype, correlating with overexpression of MxA, has been observed in multiple sclerosis [14]. As regards susceptibility to the SARS coronavirus, −123A allele carriers appear to be significantly less at risk of infection [17], a GT genotype at position −88 appears associated with increased susceptibility [20], and the GG genotype at position −88 appears to be more frequent in hypoxemic than in non-hypoxemic patients [13]. All these observations make it

a priority to conduct functional testing of the new MxA promoter haplotypes revealed here and to include them in future population-based case–control studies. Of particular interest is the fact that the new −101A allele restores a near-canonical ISRE module in the promoter, which theoretically should increase MxA expression in response to interferons α, β, and λ.

Acknowledgments The authors are grateful to Michael Sarlet for efficient and skilled technical assistance.

References

1. Haller O, Staeheli P, Kochs G (2009) Protective role of interferon-induced Mx GTPases against influenza viruses. Rev Sci Tech 28:219–231
2. Haller O, Gao S, von der Malsburg A, Daumke O, Kochs G (2010) Dynamin-like MxA GTPase: structural insights into oligomerization and implications for antiviral activity. J Biol Chem 285:28419–28424
3. Lee SH, Vidal SM (2002) Functional diversity of the Mx proteins: variations on a theme of host resistance to infection. Genome Res 12:527–530
4. Haller O, Kochs G (2011) Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. J Interferon Cytokine Res 31:79–87
5. Aebi M, Fah J, Hurt N, Samuel CE, Thomis D, Bazzighe L, Pavlovic J, Haller O, Staeheli P (1989) cDNA structures and regulation of two interferon-induced human Mx proteins. Mol Cell Biol 9:5062–5072
6. Tazi-Ahnini R, di Giovine FS, McDonagh AJ, Messenger AG, Amadou C, Cox A, Duff GW, Cork MJ (2000) Structure and polymorphism of the human gene for the interferon-induced p78 protein (MX1): evidence of association with alopecia areata in the Down syndrome region. Hum Genet 106:639–645
7. Ronni T, Matikainen S, Lehtonen A, Palvimo J, Dellis J, Van Eylen F, Goetschy JF, Horisberger M, Content J, Julkunen I (1998) The proximal interferon-stimulated response elements are essential for interferon responsiveness: a promoter analysis of the antiviral MxA gene. J Interferon Cytokine Res 18:773–781
8. Hijikata M, Ohta Y, Mishiro S (2000) Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt −88) correlated with the response of hepatitis C patients to interferon. Intervirology 43:124–127
9. Suzuki F, Arase Y, Suzuki Y, Tsubota A, Akuta N, Hosaka T, Someya T, Kobayashi M, Saitoh S, Ikeda K, Kobayashi M, Matsuda M, Takagi K, Satoh J, Kumada H (2004) Single nucleotide polymorphism of the MxA gene promoter influences the response to interferon monotherapy in patients with hepatitis C viral infection. J Viral Hepat 11:271–276
10. Kong XF, Zhang XX, Gong QM, Gao J, Zhang SY, Wang L, Xu J, Han Y, Jin GD, Jiang JH, Zhang DH, Lu ZM (2007) MxA induction may predict sustained virologic responses of chronic hepatitis B patients with IFN-alpha treatment. J Interferon Cytokine Res 27:809–818
11. Knapp S, Yee LJ, Frodsham AJ, Hennig BJ, Hellier S, Zhang L, Wright M, Chiaramonte M, Graves M, Thomas HC, Hill AV, Thursz MR (2003) Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR. Genes Immun 4:411–419
12. Torisu H, Kusuhara K, Kira R, Bassuny WM, Sakai Y, Sanefuji M, Takemoto M, Hara T (2004) Functional MxA promoter polymorphism associated with subacute sclerosing panencephalitis. Neurology 62:457–460
13. Hamano E, Hijikata M, Itoyama S, Quy T, Phi NC, Long HT, Ha LD, Ban VV, Matsushita I, Yanai H, Kirikae F, Kirikae T, Kuratsuji T, Sasazuki T, Keicho N (2005) Polymorphisms of interferon-inducible genes OAS-1 and MxA associated with SARS in the Vietnamese population. Biochem Biophys Res Commun 329:1234–1239
14. Furuyama H, Chiba S, Okabayashi T, Yokota S, Nonaka M, Imai T, Fujii N, Matsumoto H (2006) Single nucleotide polymorphisms and functional analysis of MxA promoter region in multiple sclerosis. J Neurol Sci 249:153–157
15. Peng XM, Lei RX, Gu L, Ma HH, Xie QF, Gao ZL (2007) Influences of MxA gene −88 G/T and IFN-gamma +874 A/T on the natural history of hepatitis B virus infection in an endemic area. Int J Immunogenet 34:341–346
16. Cao B, Liu X, Hou F, Li W, Han Z, Zhang Q, Dai Y, Xu C, Qi H (2009) The haplotype of the MxA gene promoter is associated with hepatitis B virus infection in a Chinese population. Liver Int 29:1383–1388
17. Ching JC, Chan KY, Lee EH, Xu MS, Ting CK, So TM, Sham PC, Leung GM, Peiris JS, Khoo US (2010) Significance of the myxovirus resistance A (MxA) gene –123C> a single-nucleotide polymorphism in suppressed interferon beta induction of severe acute respiratory syndrome coronavirus infection. J Infect Dis 201:1899–1908
18. Gao S, von der Malsburg A, Dick A, Faelber K, Schröder GF, Haller O, Kochs G, Daumke O (2011) Structure of myxovirus resistance protein a reveals intra- and intermolecular domain interactions required for the antiviral function. Immunit 35: 514–525
19. Henikoff S, Henikoff JG (1992) Amino acid substitution matrices from protein blocks. Proc Natl Acad Sci USA 89:10915–10919
20. He J, Feng D, de Vlas SJ, Wang H, Fontanet A, Zhang P, Plancoulaine S, Tang F, Zhan L, Yang H, Wang T, Richardus JH, Habbema JD, Cao W (2006) Association of SARS susceptibility with single nucleic acid polymorphisms of OAS1 and MxA genes: a case-control study. BMC Infect Dis. doi:10.1186/1471-2334-6-106