BRIEF COMMUNICATION

Complementary DNA sequences encoding the multimammate rat MHC class II DQ α and β chains and cross-species sequence comparison in rodents

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Abstract

Sequences of the complete open reading frame (ORF) for rodents major histocompatibility complex (MHC) class II genes are rare. Multimammate rat (*Mastomys natalensis*) complementary DNA (cDNA) encoding the α and β chains of MHC class II DQ gene was cloned from a rapid amplifications of cDNA Ends (RACE) cDNA library. The ORFs consist of 801 and 771 bp encoding 266 and 256 amino acid residues for DQB and DQA, respectively. The genomic structure of *Mana-DQ* genes is globally analogous to that described for other rodents except for the insertion of a serine residue in the signal peptide of *Mana-DQB*, which is unique among known rodents.

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The Muridae is the largest family of mammals with >730 recognized species (1), including two classical laboratory models, the mouse (*Mus musculus*) and rat (*Rattus norvegicus*), and many reservoirs of human diseases [e.g. reservoirs of old world arenaviruses causing haemorrhagic fevers (2)]. To date, the genetic structure of the major histocompatibility complex (MHC) of Muridae has been intensively investigated in the two laboratory models – labelled as the H2 system in the mouse and the RT1 complex in the rat (3–5).

Mastomys natalensis, the multimammate rat, is a widely distributed African murid (6), a pest causing agricultural damage and/or acting as a natural reservoir of human pathogens (7). In West Africa, *M. natalensis* is the reservoir of Lassa virus, an arenavirus causing haemorrhagic fever (8). In southern Africa, *M. natalensis* plays a role in the transmission of the bacteria *Yersinia pestis* – the aethiologic agent of plague – between wild and commensal rodents

(9). *M. natalensis* is used as laboratory model of human diseases such as papillomavirus infections (10) and for the investigation of bilharziasis, filariasis, relapsing fever and plague (11, 12). It is surprising, then, that the multimammate rat MHC has never been characterized.

The primary aim of our work was to clone transcripts of *M. natalensis* MHC class II DQ and thereby provide data for further studies on pathogen resistance in natural populations. As sequences encoding the complete open reading frame (ORF) of DQ genes are available for a few other rodent species, we were able to compare the complementary DNA (cDNA) sequences of the multimammate rat with those of other rodents and relate them to those of other mammal species.

The individuals were obtained from a laboratory colony established in 2006 in the Danish Pest Infestation Laboratory. Progenitors of the colony were captured in Morogoro,

Tanzania, in 2006. Four individuals were randomly chosen from the F1 generation. Total RNA was isolated from liver preserved in RNAlater using RNeasy mini kit with on-column RNase-free DNase set (Qiagen Vertriebs GmbH, Wien, Austria). RNA from the four individuals was pooled two by two into two samples and used as a template for full-length cDNA synthesis using an RNA ligase-mediated RACE with the GeneRacer kit (Invitrogen GmbH, Lofer, Austria). Polymerase chain reaction (PCR) amplifications of both the 5' and the 3' ends of the cDNA were performed in two separate reactions using primers provided with the RACE kit and primers designed from mouse and rat sequences retrieved from GenBank: GeneRacer 5' Primer and MasDQA-Ex3R-Race or MusDQB-R2 for the DQA and DQB, respectively, for the amplification of the 5' ends; MasDQA-Ex2F-Race or BetaUniv-F and GeneRacer 3' Primer for the DQA and DQB, respectively, for the amplification of the 3' ends. PCRs were performed using a Multiplex PCR kit (Qiagen) in a final reaction volume of 35 µl. Primers provided with the RACE kit were used at the final concentration of 0.5 µM and primers were designed for *Mastomys* at 0.2 µM. The thermal profile started with an initial HotStarTaq DNA Polymerase activation at 95°C (15 min), followed by 5 cycles at 94°C (30 s) and 72°C (75 s), followed by 5 cycles at 94°C (30 s) and 70°C (75 s), followed by 25 cycles at 94°C (30 s), 66.5°C (30 s) and 72°C (75 s) and ending with an extension step at 72°C (10 min). The PCR products were electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining. Bands within the expected size range were purified using S.N.A.P. columns. The PCR products were cloned using TOPO TA cloning kit (Invitrogen). Plasmid DNA of positive transformants was extracted by QIAprep Spin Miniprep Kit (Qiagen) and sequenced (Macrogen, Seoul, Korea) in both directions using 5' M13 Reverse and 3' T7 promoter universal primers. For the DQA gene, we sequenced four plasmid inserts by sample for the 5' end and five plasmid inserts for only one of the samples for the 3' end. For the DQB gene, we sequenced six plasmid inserts by sample for both 5' and 3' ends. To assign the transcribed sequences to the mammal DQ gene, the cDNA sequences were compared with transcribed sequences from other Muridae and rodents. Nomenclature of the *M. natalensis* DQ (*Mana-DQ*) allelic sequences follows Klein et al. (13). *M. natalensis* MHC class II DQ exon layout is relative to the exon structure of the rat, mouse and human DQ cDNAs described in Ettinger et al. (14), which is in turn based on a structure-based study (15). The cleavage site of the signal peptide was predicted using SIGNALP 3.0 (16).

For the DQB gene, the entire coding sequence and some 5' and 3' untranslated regions (UTR) were amplified by RACE. The 5' cDNA ends amplified using GeneRacer 5' and MusDQB-R2 primers were ~400 bp long. Three different sequences were obtained from the 12 sequenced plasmid inserts: one sequence was identified by BLAST as

a DRB allele and the remaining sequences as two different DQB alleles. The 3' cDNA ends amplified using the GeneRacer 3' and BetaUniv-F primers were ~900 bp long. Four different sequences were obtained from the 12 sequenced plasmid inserts: 3 were identified as coding for the DQB gene and the last one for the haemopexin gene (BLAST 95% identity with haemopexin of *M. musculus*). Comparing the 168 shared nucleotides between the 5' and the 3' cDNA ends, two of the 3' end sequences matched the two 5' end sequences. Sequences were named *Mana-DQB*01* and *Mana-DQB*02* for the two complete coding sequences and *Mana-DQB*03* for the incomplete 3' end coding sequence (GenBank AN: FJ968784–FJ968786). The nucleotide sequences showed high similarity with rodent sequences (BLAST: identity with *M. musculus*: 90%, *R. norvegicus*: 89% and *Peromyscus eremicus*: 87%). The ~1200 bp contain an 801 bp ORF coding 266 residues (Figure 1).

For the DQA gene, the entire coding sequence with some 5' and 3' UTRs was also successfully amplified by RACE. The 5' cDNA ends amplified using GeneRacer 5' and MasDQA-Ex3R-Race primers were ~400 bp long. Three different sequences were obtained from the eight sequenced plasmid inserts. The sequences were identified by BLAST as encoding the DQA gene. The 3' cDNA ends amplified using the GeneRacer 3' and MasDQA-Ex2F-Race primers were ~850 bp long. Three different sequences were obtained from the five sequenced plasmid inserts and were identified as coding for DQA gene. Comparing the 168 shared nucleotides between the 5' and the 3' cDNA ends, one of the 3' end sequence matched one of the 5' end sequence. DQA sequences were named *Mana-DQA*01* for the complete coding sequence, *Mana-DQA*02* and *Mana-DQA*03* for the incomplete 5' end coding sequences and *Mana-DQA*04* and *Mana-DQA*05* for the incomplete 3' end coding sequences (AN: FJ968787–FJ968791). The nucleotide sequences showed high homology with rodent sequences (BLAST: identity with *M. musculus*: 91%, *R. norvegicus*: 90% and *Sigmodon hispidus*: 88%). The ~1100 bp contain a 771 bp ORF coding 256 residues (Figure 2).

The deduced amino acid sequences were aligned and numbered according to the structure of RT1-B of the rat (14) and H2-IA of the mouse (17) (Figures 1 and 2). SIGNALP predicted the signal peptide cleavage site between Gly and Arg at -1 and 1 and between Gly and Gln/Glu at -1 and 1a for the mature *Mana-DQB* and *Mana-DQA*, respectively. The predicted signal peptide cleavage sites were similar to those for other rodents and mammals, although the human, cattle and dog signal peptides of DQB are four amino acids longer than that of *Mastomys* (14). The β65–66 deletion, very common in rat and mouse haplotypes (14, 17), was not found in the three *Mastomys* sequences (Figure 1). In rodents, this deletion has only been described in mouse and rat so far, appearing absent from the two other rodent genera, *Peromyscus* and *Ctenomys* (18, 19). The β84a

Arg that form a hydrogen bonds with the backbone of the peptide were identified at amino acids β 82 and α 76, respectively (15, 20).

In summary, we have characterized cDNA encoding the entire mature proteins of the *Mana-DQA* and *DQB* class II molecules. Comparison with other rodent DQ proteins identifies a major discrepancy in the leader peptide of the *Mana-DQB* protein, which is insertion of a Ser residue. There has been no study investigating the functional role of polymorphism in the signal peptide in MHC class II molecules. However, this region shows some polymorphism in humans [e.g. DQB1 (21)], and in addition to the insertion of the Ser residue in the signal peptide of *Mana-DQB* compared with DQB sequences in other rodents, the signal peptide of the *Mana-DQA* shows one polymorphic site (Figure 2). In

		Signal peptide and start of the mature protein		Exon 2 (first external domain)												Exon 3 (second external domain)											
				-20	-10	label1	9a10	20	30	40	50	60	70	80	90	100											
MURU AODR MEI MEND LTA IIIE AA		Mana-DQA*01	MPKSRALILGVLALTTMLR	PCGGQDDIE	ADHIGNYGINVYQSYGNSGQPTTFEPDGDGEFYVLDLKKETVWRLLPEFGQLTSFDPQGGGLQNIATAKHNDILIKRNSNPATN												RAPAATVFPKSPVLLGQPN										
		Mana-DQA*02	G.....E.N.....	V.S..TT.....PS..Y.....	ELL..RR.....A..L.....													KI.ESS.....									
		Mana-DQA*03	G.....E.....	V.MF.GDF.....Y.A.....	ELL..RR.....I..A..LN.....													K.V.....									
		Mana-DQA*04	G.....E.....	V.MF.GDF.....Y.A.....	ELL..RR.....I..A..LN.....													K.V.....									
		Mana-DQA*05	G.....E.....	V.MF.GDF.....Y.A.....	ELL..RR.....I..A..LN.....													K.V.....									
		H2-IIa_u	V.S.....V.....P.DI..Y.....	EL.....I.M.....A..R.....	G...GV.T.....													Q.....									
		H2-IIa_q	V.....V.....P.DI..Y.H.....	EW.....M.....	G...GGWT.....F.....													Q.....									
		H2-IIa_f	E.....V.F.....S.....P.DI..Y.....	EW.....E.....G...G.T.....F.....														Q.....									
		H2-IIa_d	C.....N.....SL..E.....	V.F..TT.....P.DI..Y.H.....	EL.....K.....IL.E.....AE.....G.T.....F.....													Q.....									
		H2-IIa_b	V.T.....S.....P.DI..Y.....	EL.....M.....A.....VV.....GV.T.....														Q.....									
		H2-IIa_s	V.V..TT.....P.DI..Y.H.....	EW.....I.M.....	G.YT.G.T.....													Q.....									
		H2-IIa_k	V.S.....T.....P.DI..Y.....	EL.....M.....A..R.R.E.....	G...E.T.....													Q.....									
		H2-IIa_r	V.V..TT.....P.DI..H.....	EW.....M.....	VV.....E.T.....F.....A.....													Q.....									
		RT1-Ba_X14879	L.....S.....	VAA.....M.Y.E.R.....H.....	E.....I..I.....	II.....E.M.....Q.V.....													KV.E.....S.....								
		RT1-Ba (BN)	L.....S.....	VAA.....M.Y.E.R.....Y.H.....	E.....I..I.....	II.....E.M.....Q.V.....													KV.E.....S.....								
		RT1-Ba (ACI)	L.....S.....	V.S.....EM..Y.K.R.Y.....	EK.....I..I.....	II.....E.M.....Q.V.....													V.E.....S.....								
		RT1-Ba (BUF)	L.....S.....	L.S.....T.....Y.E.K.Y.....	ET.S.....NI..I.....	II.....E.M.....Q.V.....													V.E.....S.....								
		RT1-Ba (BDIX)	L.....S.....	V.S.....EM..Y.K.R.Y.....	E.....I..I.....	II.....E.M.....Q.V.....													V.E.....S.....								
		RT1-Ba (SHR)	L.....S.....	L.S.....T.....Y.E.K.Y.....	ET.S.....NI..I.....	II.....E.M.....Q.V.....													V.E.....S.....								
		RT1-Ba (LEW)	L.....S.....	VAA.....M.Y.E.R.....H.....	E.....I..I.....	II.....E.M.....Q.V.....													KV.E.....S.....								
		RT1-Ba (BB/DR)	L.....S.....	V.S.....T.....Y.E.K.Y.H.....	ER.....I..I.....	II.....E.M.....Q.V.....													V.E.....S.....								
		Pele-DQA	V.A..L.F.....PK.Y.H.....	EL.....I..I.....	II.....E.M.....Q.V.....														V.EV.....Y.M.....								
		Sih1-DQA	MP..T..W.I.....A.T.S.....E..K.....	V.I..MTM.....HKP..YI.....	EL.....S.....	I..E.....D..V.....E.T.....													KV.EV.....L.....								
		BoLA-DQA*2801	VLN.....T.....T.....MS.S.....E..V.....	V.V.I..SI.....PS..Y.H.....EQ.....E.....A.Q..L.SRM.....LA.R.....IM.LHV.F.T.F.....A.....															KV.EV.....S.....M.....								
		DLA-DQA1	ILN.V.....T..I..I..MS.S.....EE..V.....	V.V.A.....S.....PS..Y.H.....E.....E.....V.STFR.....A.R.L.II.Q..N.MT.....Q.A.....															V.EV.....S.....M.....								
		HLA-DQA*01011	ILNK..L..A.....VMS.....E..V.....	V.VASC.V.L.F..PS..Y.H.....E.....ER.....A.W.....SKFGG.....A.R.M.V.....N.M.....Y.....A.....															V.EV.....S.....T.....								
		external domain)												Connecting peptide, transmembrane domain, cyto-plasmic tail													
				110	120	130	140	150	160	170	180	190	200	210	220												
MURU AODR MEI MEND LTA IIIE AA		Mana-DQA*01	TLICFVDNIIFPPVISITWLRNSKPVTEGVYETSP	FLNSPDHSPHKMSYLTFFVSDYDI	YDCKVEHGWGLDFVLKHN	EPETIPAPMSSELTETVCALGLSVGLIGIVGVTI	FLIKLRSGGTSRHPGPL																				
		Mana-DQA*02																									
		Mana-DQA*03																									
		Mana-DQA*04																									
		Mana-DQA*05																									
		H2-IIa_u	N.....S.AD.....	FV.R.Y.....L.....I..D.....	E.....V.....I.Q.....																						
		H2-IIa_q	N.....S.D.....	V.R.....L.....I..D.....	E.....V.....I.Q.....P.....																						
		H2-IIa_f	N.....S.D.....	V.R.....L.....I..D.....	E.....V.....I.Q.....																						
		H2-IIa_d	N.....S.D.....	V.R.....L.....I..D.....	E.....V.....I.Q.....																						
		H2-IIa_b	N.....S.AD.....	FV.R.Y.....L.....I..D.....	E.....V.....I.Q.....																						
		H2-IIa_s	N.....S.D.....	V.R.....L.....I..D.....	E.....V.....I.Q.....																						
		H2-IIa_k	N.....S.D.....	FV.R.Y.....L.....I..D.....	E.....V.....I.Q.....																						
		H2-IIa_r	N.....S.D.....	V.R.....L.....I..D.....	E.....V.....I.Q.....																						
		RT1-Ba_X14879	N.....LVE.....	A.....I.FND.....	V.....V.....I.Q.....D.P.....																						
		RT1-Ba (BN)	A.N.....LVE.....	TP.....A.....I.FND.....	V.....V.....I.Q.....D.P.....																						
		RT1-Ba (ACI)	N.....S.....	I.S.Y.....L.A.I..ND.....	R.....V.....I.Q.....D.S.....																						
		RT1-Ba (BUF)	N.....S.....	I.S.Y.....L.A.I..ND.....	R.....V.....I.Q.....D.S.....																						
		RT1-Ba (BDIX)	N.....S.....	I.S.Y.....L.A.I..ND.....	R.....V.....I.Q.....D.S.....																						
		RT1-Ba (SHR)	N.....S.....	I.S.Y.....L.A.I..ND.....	R.....V.....I.Q.....D.S.....																						
		RT1-Ba (LEW)	N.....S.....	I.S.Y.....L.A.I..ND.....	R.....V.....I.Q.....D.S.....																						
		RT1-Ba (BB/DR)	N.....S.....	I.S.Y.....L.A.I..ND.....	R.....V.....I.Q.....D.S.....																						
		Pele-DQA	I.....VN.....S.....	A.....I..D.V.....E.M.....	V.....IV..M.....I.Q.....P.....																						
		Sih1-DQA	N.....K.....SI.D.....	S.....V.....I..D.V.....LDVT.....P.....	IV.....I.Q.....																						
		BoLA-DQA*2801	H.....N.....GHS.....S.....	IRS.Y..L.IK..A.L.L.....L.....	T.....TM..V..T.....I.Q.....DP.....Q.....																						
		DLA-DQA1	L.....NV.....K.RHS.....S.....	FAKG..S.I..L.AE.....L.....	V.T.....A..V..M..V.I.Q.....V.A.....Q.....																						
		HLA-DQA*01011	L.....VN.....S.GQS.....S.....	KS.....F.I..L.ADE.....Q.L.....	V.....V..I.Q.....V.A.....Q.....																						

Figure 2 Comparison of the translated amino acid sequences of *Mastomys natalensis* MHC class II DQA gene (*Mana-DQA*) and other representative of murids, rodents and mammals. Sequences are split according to their domain structure. Numbering of *Mana-DQA* includes an insertion in exon 2, designated amino acid 9a and three extra amino acids at the start of the mature protein designated 1a, 1b and 1c (in bold). These numbers are inserted for structural consistency across MHC class II molecules (15). The symbols used indicate the following: -, indel; ., same amino acids as those in *Mana-DQA**01; ~, alignment filler. The sources of the sequences are as follows: *Mus musculus* H2-IIAa (K01922–K01926 and M11356–M11358), *Rattus norvegicus* RT1-Ba (X14879, AY626190, AY626193, AY626194, AY626196, AY626197 and AY626199), *Peromyscus leucopus* Pele-DQA (U34805), *Sigmodon hispidus* Sih1-DQA (AY169005), *Bos taurus* BoLA-DQA (AF037314), *Canis lupus familiaris* DLA-DQA1 (NM_001011726) and *Homo sapiens sapiens* HLA-DQA*01011 (IMGT/HLA AN: HLA00601). Positions of the primers used for the RACE, MasDQA-Ex2F-Race (5'-atgtggacttggaagaaggag-3') and MasDQA-Ex3R-Race (5'-tgttgggctgaccagcagcac-3') are underlined. HLA, human leucocyte antigen; MHC, major histocompatibility complex.

humans, signal sequences of MHC class I molecules have been shown to play a role in NK-cell-mediated lysis through their presentation by the class I human leucocyte antigen-E molecule (22). The interspecific and intraspecific polymorphisms of signal peptide shown here suggest that the functional role of leader peptide sequences in MHC class II molecules deserves closer inspection.

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