

Identification of the uncommon allele HLA-A*7403 in a Caucasian renal transplant cadaveric donor: extension of the exon 4 sequence

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This report describes the unknown exon 4 sequence of the rare A*7403 allele, identified in a Caucasian renal transplant cadaveric donor from Italy. The human leukocyte antigen (HLA)-A74 alleles are subtypes of the HLA-A19 allele family,

This report describes the unknown exon 4 sequence of the rare A*7403 allele, identified in a Caucasian renal transplant cadaveric donor from Italy. This sequence is identical to that of the only known A*7401 exon 4, and this result allowed us to confirm the hypothesis of the generation of A*7403 allele from the ancestor A*7402 by point mutation in exon 2.

and they are absent or very uncommon in Italian populations (percentage of allelic frequency (af%) range: 0–0.02), while they show higher frequencies in South African and African American populations (af%: 6.5–5.0). In particular, the A*7403 variant was only identified in some African populations (Kenya and Uganda), Argentinean, Iran Balochi, Madeiran and USA Caucasian Bethesda groups (www.allelefreqencies.net).

After a preliminary serological typing that was able to assign the A2 and A74 antigens, an analysis of exons 2, 3 and 4 of the HLA-A polymorphism was performed on genomic DNA with locus-specific polymerase chain reaction primers (AlleleSEQR HLA-A kit; Atria Genetics, South San Francisco, CA), followed by direct sequencing in both directions using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Allele assignment was performed using the HLA MATCHTOOLS and MT NAVIGATOR software from the MatchTools Allele Identification Packet (Applied Biosystems), and most recently by ASSIGN 350 software (Atria Genetics), which identify the heterozygous positions within each electropherogram and assess the typing based on the alignment of the processed sequence with the updated HLA sequence library. To verify the presence of the HLA-A*7403 variant, the amplified DNA was cloned to separate the alleles using the pGEM-T Easy Vector System II cloning kit (Promega Italia, Milan, Italy). DNA inserts from the cloned allele were directly amplified from a lysate containing the bacterial clone and sequenced for exons 2, 3 and 4. Both strands of DNA were sequenced for each allele.

The sample showed the following serological typing: HLA-A2;74, B18;37, DR11 and the genotypes A*020101;7403, B*1801;3701. The new A*7403 exon 4 sequence was submitted to GeneBank (DQ870546), and the information was included in

		150															160																													
A*01010101	K	R	K	W	E	A	V	H	A	A	E	Q	R	R	V	Y	L	E	G	R	C	V	D	G																						
A*7403	Q	-	-	-	-	-	A	R	V	-	-	-	L	-	A	-	-	-	-	T	-	-	E	W																						
A*7402/01	Q	-	-	-	-	-	A	R	V	-	-	-	L	-	A	-	-	-	-	T	-	-	E	W																						
A*01010101	AAG	CGC	AAG	TGG	GAG	GCG	GTC	CAT	GCG	GCG	GAG	CAG	CGG	AGA	GTC	TAC	CTG	GAG	GCC	CGG	TGC	GTG	GAC	GGG																						
A*7401	C--	---	---	---	---	---	-C-	-G-	-T-	---	---	---	TT-	---	-C-	---	---	---	---	AC-	---	---	--G	T--																						
A*7402	C--	---	---	---	---	---	-C-	-G-	-T-	---	---	---	TT-	---	-C-	---	---	---	---	AC-	---	---	--G	T--																						
A*7403	C--	---	---	---	---	---	-C-	-G-	-T-	---	---	---	TT-	---	-C-	---	---	---	---	AC-	---	---	--G	T--																						
	432															462															492															
		170										180										Exon 4										190														
A*01010101	L	R	R	Y	L	E	N	G	K	E	T	L	Q	R	T		D	P	P	K	T	H	M	T	H																					
A*7403	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-																					
A*7402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																					
A*7401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-																					
A*01010101	CTC	CGC	AGA	TAC	CTG	GAG	AAC	GGG	AAG	GAG	ACG	CTG	CAG	CGC	ACG	G	AC	CCC	CCC	AAG	ACA	CAT	ATG	ACC	CAC																					
A*7401	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	G--	---	---	--G	---	---	--T	---																					
A*7402	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	**	***	***	***	***	***	***	***	***																				
A*7403	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	G--	---	---	--G	---	---	--T	---																					
	582										612										642																									
		200															210																													
A*01010101	H	P	I	S	D	H	E	A	T	L	R	C	W	A	L	G	F	Y	P	A	E	I	T	L																						
A*7403	-	A	V	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-																						
A*7402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																						
A*7401	-	A	V	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-																						
A*01010101	CAC	CCC	ATC	TCT	GAC	CAT	GAG	GCC	ACC	CTG	AGG	TGC	TGG	GCC	CTG	GGC	TTC	TAC	CCT	GCG	GAG	ATC	ACA	CTG																						
A*7401	---	G-T	G--	---	---	---	---	---	---	---	---	---	---	---	---	A--	---	---	---	---	---	---	---	---																						
A*7402	---	***	***	***	***	***	***	***	***	***	***	***	***	***	***	---	---	---	---	---	---	---	---	---																						
A*7403	---	G-T	G--	---	---	---	---	---	---	---	---	---	---	---	---	A--	---	---	---	---	---	---	---	---																						
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		220															230																													
A*01010101	T	W	Q	R	D	G	E	D	Q	T	Q	D	T	E	L	V	E	T	R	P	A	G	D	G																						
A*7403	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																						
A*7402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																						
A*7401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																						
A*01010101	ACC	TTC	CAG	CGG	GAT	GGG	GAG	GAC	CAG	ACC	CAG	GAC	ACG	GAG	CTC	GTG	GAG	ACC	AGG	CCT	GCA	GGG	GAT	GGA																						
A*7401	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	--T	---	---	---	---	---	---	---	---																						
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		240															250															260														
A*01010101	T	F	Q	K	W	A	A	V	V	V	P	S	G	E	E	Q	R	Y	T	C	H	V	Q	H																						
A*7403	-	-	-	-	-	-	S	-	-	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	-																						
A*7402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																						
A*7401	-	-	-	-	-	-	S	-	-	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	-																						
A*01010101	ACC	TTC	CAG	AAG	TGG	GCG	GCT	GTG	GTG	CCT	TCT	GGA	GAG	GAG	CAG	AGA	TAC	ACC	TGC	CAT	GTG	CAG	CAT																							
A*7401	---	---	---	---	---	---	T--	---	---	---	---	---	---	C--	---	---	---	---	---	---	---	---	---	---																						
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A*7403	---	---	---	---	---	---	T--	---	---	---	---	---	---	C--	---	---	---	---	---	---	---	---	---	---																						
	732										762																																			
		270																																												
A*01010101	E	G	L	P	K	P	L	T	L	R	W																																			
A*7403	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																						
A*7402	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																						
A*7401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-																						
A*01010101	GAG	GGT	CTG	CCC	AAG	CCC	CTC	ACC	CTG	AGA	TGG	G																																		
A*7401	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---																						
A*7402	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---																						
A*7403	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---																						
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Figure 1 Continued.

the WHO Nomenclature Report as a confirmation of this sequence in August 2006 (HWS10004057)(1).

Twelve different A*74 allele variants are presently known (January 2007, the international ImMunoGeneTics project (IMGT)/HLA Database, <http://www.ebi.ac.uk/imgt/hla> (2)), most of them showing nucleotide differences in exon 2 (HLA-A*7403, *7404, *7405, *7406, *7407, *7409, *7410, *7411) and the remaining in exon 1 (HLA-A*7401) and exon 3 (HLA-A*7412N, *7408); the exon 4 sequence was known only for the A*7401 allele.

Nucleotide sequence alignments with HLA-A alleles from the IMGT/HLA Sequence Database showed that the exon 4 sequence of the A*7403 is identical to that of the A*7401 allele (Figure 1), and this result allowed us to confirm the hypothesis of the generation of A*7403 allele from the ancestor A*7402 by point mutation in the exon 2 at nucleotide position 235 (C→G) (amino acid position 79, Ala→Gly) in the $\alpha 1$ domain of the heavy chain in A*7403 (3). In fact, its sequence motif at position 235 of the second exon does not exist on other alleles of the classical or non-classical HLA genes, and no HLA class I donor allele has been found.

Seeing that the difference between A*7403 and A*7401/02 alleles may have the capability to stimulate all-or-none response (4), for the crucial position of residue 79 in the Bw4/Bw6 sequence stretch, and the extended variability of the A*74 allele group, we may suggest the importance to define the HLA-A*74 subtypes at high resolution in bone marrow transplantation with different potential donors.

The name A*7403 was officially assigned by the WHO Nomenclature committee in April 1996. This follows the agreed policy that, subject to the conditions stated in the most recent Nomenclature Report (5), names will be assigned to the new sequences as they are identified. Lists of such new

names will be published in the next WHO Nomenclature Report.

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