## **NEW ALLELE** Alerts

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

A. Canossi<sup>1</sup>, T. Del Beato<sup>1</sup>, A. Piazza<sup>2</sup>, G. Liberatore<sup>1</sup>, G. Ozzella<sup>2</sup>, A. Tessitore<sup>3</sup> & D. Adorno<sup>1</sup>
1 CNR Institute 'Organ Transplants and Immunocytology', L'Aquila, Italy
2 CNR Institute 'Organ Transplants and Immunocytology', Rome Sections, Italy
3 Department of Experimental Medicine, University of L'Aquila, L'Aquila, Italy

Key words: HLA-A; renal transplant; SBT

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.allelefrequencies.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

NEW A	LLE	ELE	ΞA	ler	ts																			
Exon 2																								
	1									10										20				
A*01010101		S	Н	S	М	R	Υ	F	F	Т	S	V	S	R	Р	G	R	G	Е	Р	R	F	ı	Α
A*7403	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*7402/01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*01010101	GC	TCC	CAC	TCC	ATG	AGG	TAT	TTC	TTC	ACA	TCC	GTG	TCC	CGG	CCC	GGC	CGC	GGG	GAG	CCC	CGC	TTC	ATC	GC
A*7401																								
A*7402 <b>A*7403</b>																								
A 7403															42									
	1				12																			
A*01010101						30										40								
	V	G	Υ	V	D	D	Т	Q	F	V	R	F	D	S	D	Α	Α	S	Q	K	М	Е	Р	R
A*7403	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R R	-	-	-	-
A*7402/01	-							-		-		-	-			-	-							
A*01010101	GTG	GGC	TAC	GTG	GAC	GAC	ACG	CAG	TTC	GTG	CGG	TTC	GAC	AGC	GAC	GCC	GCG	AGC	CAG	AAG	ATG	GAG	CCG	CGG
A*7401 A*7402												I								- G -				
A*7402 A*7403												T								- G -				
A 1405	72										102									Ū	132			
		50										60										70		
A*01010101	Α	Р	w		Е	Q	_	0	Р	_	Y	w	D	0	Е	-	Б	N.		12	^	н	0	_
A*7403	A	Ρ.	VV	'	_	Q	E	G	-	E	T -	VV	_	Q	_	Т	R	N	M V	K	Α	п	S	Q
A*7402/01		_	-		-	_	-		-		-			-	-		-	-	V		-		-	-
A*01010101	GCG	CCG	TGG	ATA	GAG	CAG	GAG	GGG	CCG	GAG	TAT	TGG	GAC	CAG	GAG	ACA	CGG	AAT	ATG	AAG	GCC	CAC	TCA	CAG
A*7401	GCG	CCG	166	AIA	GAG	CAG	GAG	GGG	CCG	GAG	IAI	166	GAC	CAG	GAG	ACA	CGG	AAT	G	AAG	GCC	CAC	ICA	CAG
A*7401 A*7402																			G					
A*7403																			G					
							162										192							
																				F.	on 3	ì.		
							79	80										90			<u> </u>	<b>-</b>		
A*01010101	Т	D	R	Α	N	L	G	Т	L	R	G	Υ	Υ	Ν	Q	S	Е	D		G	S	Н	Т	- 1
A*7403	-	-	-	V	D	-	Α	-	-	-	-	-	-	-	-	-	-	Α	-	-	-	-	-	-
A*7402/01	-	-	-	V	D	-	-	-	-	-	-	-	-	-	-	-	-	Α	-	-	-	-	-	-
A*01010101	ACT	GAC	CGA	GCG	AAC	CTG	GGG	ACC	CTG	CGC	GGC	TAC	TAC	AAC	CAG	AGC	GAG	GAC	G	GT	TCT	CAC	ACC	ATC
							1	1												1				

					100										110									
A*01010101	Q	1	M	Υ	G	С	D	V	G	Р	D	G	R	F	L	R	G	Υ	R	Q	D	Α	Υ	D
A*7403	-	М	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	Q	-	-	-	-	-
A*7402/01	-	М	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	Q	-	-	-	-	-
A*01010101	CAG	ATA	ATG	TAT	GGC	TGC	GAC	GTG	GGG	CCG	GAC	GGG	CGC	TTC	СТС	CGC	GGG	TAC	CGG	CAG	GAC	GCC	TAC	GAC
A*7401		G												C					- A -					
A*7402		G												C					- A -					
A*7403		G												C					- A -					
										312										342				
	120										130										140			
A*01010101	G	K	D	Υ	1	Α	L	N	Е	D	L	R	s	W	Т	Α	Α	D	М	Α	Α	Q	1	Т
A*7403	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*7402/01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A*01010101	GGC	AAG	GAT	TAC	ATC	GCC	CTG	AAC	GAG	GAC	CTG	CGC	TCT	TGG	ACC	GCG	GCG	GAC	ATG	GCA	GCT	CAG	ATC	ACC
A*7401							T													G				
A*7402							T													G				
A*7403							T													G				
						372										402								

--- --- --- --- --- --- --- -C-

252

--- --- -C-

270

Figure 1 Nucleotide and predicted amino acid sequences of exons 2–4 of the A\*7403 allele (amino acids 1–90) are compared with those of the A\*01010101 (reference sequence) and A\*7401/A\*7402 alleles. The numbers above indicate the corresponding amino acid and the numbers below refer to the nucleotide position, with exon 2 starting at nucleotide 1. The single nucleotide difference in codon 79 is marked with a box. Dashes indicate the identity for amino acid and nucleotide position in comparison with the reference sequence (A\*01010101) and asterisks represent the lack of information for nucleotide sequence (i.e. exon 4).

--- --- ---

282

A\*7401

A\*7402

A\*7403

--- --- -T- G-- ---

--- --- -T- G-- ---

- C -

																		ΝE	W	ΑL			A1	erts
																						•		
A*01010101	К	R	K	W	E	Α	150 V	н	Α	Α	E	Q	R	R	V	Υ	160 L	Е	G	R	С	V	D	G
<b>A*7403</b> A*7402/01	Q Q	-	-	-	-	-	A A	R R	V V	-	-	-	L L	-	A A	-	-	-	-	T T	-	-	E E	W W
A*01010101	AAG		AAG	TGG	GAG	GCG			GCG	GCG	GAG	CAG	_	AGA	GTC	TAC	стс	GAG	GGC		TGC	GTG	GAC (	
A*7401 A*7402	C C							- G - - G -					TT - TT -		- C -					AC - AC -			G <sup>-</sup>	Γ Γ
A*7403	C	432					-	- G -				462			- C -					AC -			G	
		432	170									18	.0				Exon -	4				492	190	
A*01010101	L	R	R	Υ	L	Е	N	G	К	Е	Т			R -	Г	[		_	K	т	Н	М	Т	Н
<b>A*7403</b> A*7402	-	-	-	-	-	-	-	-	-	-	-	-	-	- -	-	·   -	A .		-	-	-	-	-	-
A*7401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	.   -	А	_	-	-	-	-	-	-
A*01010101	СТС	CGC	AGA	TAC	CTG (	GAG A	AAC (	GGG .	AAG (	SAG A	ACG C	тс с	AG C	GC AC							A CAT			
A*7401 A*7402																	_		* * * *	·(		* * *	T	* * *
A*7403																.   -	- G-			(	3		T	
A*04010101									200										210					
A*01010101 <b>A*7403</b>	H	P A	I V	S	D -	H	E	Α -	T -	L	R -	C	W	Α -	L -	G S	F -	Υ -	P -	Α -	E -	1	T -	L
A*7402	*	*	,	*		*		*	*	*		*	*	*	*	*	*		*		*		*	*
A*7401	-	Α	V	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-
A*01010101 A*7401	CAC	CCC G-T		TCT	GAC	CAT	GAG	GCC	ACC	CTG	AGG	TGC	TGG	GCC	CTG	GGC A	TTC	TAC	CCT	GCG	GAG	ATC	ACA	CTG 
A*7402	* * *	* * *	* * * G	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * * A	* * *	* * *	* * *	* * *	***	* * *	* * *	* * *
A*7403		G-1	G	582										612		A								642
A*01010101					220									2	30									
A*7403	T -	W -	Q -	R -	D -	G -	E -	D -	Q -	T -	Q -	D -	T -	E -	L '	∨ I -	=	Г R 	P -	_		<b>}</b>		
A*7402			*	٠	٠			*	*	•	•	*	•	*	*	•	*		*		,			
A*7401	-	-	-	-	-	-	-	-	-	-	-	-	-	-	- 	-			-	- -			 	
A*01010101 A*7401	ACC				GAT		GAG		·					SAG C	-T -					T GC	CA GC		AT G0	эA · -
A*7402 <b>A*7403</b>	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	***		* * * *	** *	** *	** *	** * -T -	* * *	* * * * *	· *   * * · -	* **	* **	* **	* *	* * * * *	*
A 1400										672									70	2				
	240										250										260			
A*01010101 <b>A*7403</b>	Т	F	Q	K	W	A	A S	٧	V	٧	P	S	G	E Q	E	Q	R	Y	Т	С	Н	٧	Q	H
A*7402																								
A*7401	-	-	-	-	-	-	S	-	-	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	-
A*01010101 A*7401	ACC	TTC	CAG	AAG	TGG	GCG	GCT	GTG	GTG	GTG	CCT	TCT	GGA	GAG C	GAG	CAG	AGA	TAC	ACC	TGC	CAT	GTG	CAG	CAT
A*7402	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *
A*7403						732	Т							C		762								
A*01010101	_	_		_		_	270	_		_														
A*7403	E -	G -	L -	P -	K -	P -	L -	T -	L -	R -	- W													
A*7402	*	٠	*	*	٠	٠	٠	*	*	*	*													
A*7401	- GAG	- GGT	- CTC	-	- : AAG	-	-	-	- CTC	-	- TGG	G												
A*01010101 A*7401												-												
A*7402 <b>A*7403</b>	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	* * *	*												
A 1700		792										822												

Figure 1 Continued.

## **NEW ALLELE Alerts**

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  $(C \rightarrow G)$  (amino acid position 79, Ala  $\rightarrow$  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 alloreactive 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.

## Correspondence

Dr Angelica Canossi P.le Collemaggio L'Aquila 67100 Italy Tel: +39 0862 414275 Fax: +39 0862 410758

e-mail: a.canossi@itoi.cnr.it

Received 6 February 2007; accepted 13 February 2007

doi: 10.1111/j.1399-0039.2007.00829.x

## References

- Marsh SGE, Steven GE. Nomenclature for factors of the HLA system, update August 2006. *Tissue Antigens* 2006: 68: 537–9.
- Robinson J, Malik A, Parham P, Bodmer JG, Marsh SGE. IMGT/HLA – a sequence database for the human major histocompatibility complex. *Tissue* Antigens 2000: 55: 280–7.
- Blasczyk R, Wehling J, Onaldi-Mohr D et al. Structural definition of the A\*74 group: implications for matching in bone marrow transplantation with alternative donors. *Tissue Antigens* 1996: 48: 205–9.
- Clayberger C, Rosen M, Parham P et al. Recognition of an HLA public determinant (Bw4) by human allogeneic cytotoxic T lymphocytes. *J Immunol* 1990: 144: 4172–6.
- Marsh SGE, Albert ED, Bodmer WF et al. Nomenclature for factors of the HLA System, 2004. *Tissue Antigens* 2005: 65: 301–69.