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Thirteen novel canine DLA-88 alleles identified by sequence-based typing

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Abstract

Major histocompatibility complex genes in mammals include highly polymorphic class I and class II genes that are critical for donor-recipient matching for transplantation. Dogs have served as an effective, directly translatable model for stem/progenitor cell transplantation. Previous analyses of major histocompatibility complex class I genes in dogs point to a single highly polymorphic gene, dog leukocyte antigen (DLA)-88, as an important factor in the success or failure of hematopoietic stem cell transplants. Fifty-nine DLA-88 alleles have been identified and reported so far. Here, we extend this list by presenting 13 novel DLA-88 alleles found in domestic dogs.

Keywords

allele; canine; class I; dog; dog leukocyte antigen; DLA-88; major histocompatibility complex

Introduction

Advancement of allogeneic hematopoietic stem cell transplantation (HSCT) is due largely to preclinical progress in the canine model (1–3). The typing of highly polymorphic canine major histocompatibility complex (MHC) genes, termed dog leukocyte antigen (DLA), was fundamental in the successful development of the canine model for transplantation (4,5). As in humans, the dog MHC class I and class II genes exhibit an exceptionally high degree of

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Conflicts of Interest (None)

The authors have no financial relationships that can present a potential conflict of interest in these studies.

Authors' contributions:

Gopalakrishnan M Venkataraman: Designed the study, performed the experiments, analyzed data and co-authored the manuscript.

Lorna J. Kennedy: Provided valuable canine samples and edited the manuscript.

Marie-Térèse E. Little: Analyzed data and co-authored the manuscript.

Scott S. Graves: Supervised dog leukocyte typing core.

Beverly Torok-Storb: Provided scientific and laboratory support and edited the manuscript.

Michael A. Harkey: Co-authored and edited the manuscript.

Rainer Storb: Designed the study and edited the manuscript.

polymorphism (6–9). Histocompatibility matching of donor-recipient pairs for HSCT has traditionally focused on the most polymorphic DLA genes. The canine class I and class II genes have been localized to chromosome 12, with the exception of DLA-79, a non-classical class I gene located on chromosome 18 (6–9). Four canine class II genes have been identified; DLA-DQA1, DQB1 and DRB1 are polymorphic while DRA is not. Historically, DRB1 has been most commonly utilized for DLA-matching for HSCT among sibling donor-recipient pairs, due to this gene's high level of polymorphism, close association with other DLA genes on chromosome 12, and ease of allele typing. Of the three classical class I genes, DLA-88, DLA-12 and DLA-64, DLA-88 is the most pertinent for HSCT, as it is highly polymorphic with 59 alleles reported so far (8,10–14). DLA-88 typing was added to the DLA matching protocol, when methods for routine typing of the alleles were worked out (12). DLA-79 is the second most polymorphic class I gene, but a specific association in graft rejection could not be demonstrated, as about 80 percent of the transplanted dogs were homozygous for the most common allele (15). Alleles of DLA-12 and DLA-64 are less well studied as the polymorphism was low in the initial set of dogs typed (8). In this report, we extend the list of DLA-88 alleles by presenting 13 novel alleles.

Materials and methods

Source of DNA

A total of 427 dogs were tested in this study. Random-bred litters of beagles and mini-mongrel crossbreeds including Basenji, Golden Retriever, hounds and harriers (15), were raised at the Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA. Archived samples of genomic DNA were obtained from 200 of these animals. An additional 200 blood samples included in this study came from breeders and their pups in our colony and dogs from Marshall Bioresources, USA, Ridglan Farms Inc, USA and from Phoenix Laboratories, Edmonds, WA. In addition, DNA from 27 dogs that were typed by reference strand-mediated conformation analysis and chosen to likely contain new DLA-88 alleles were included in this screen. All experiments were approved by Institutional Animal Care and Use Committee of the FHCRC. Standard care for dogs was provided as described previously (16, 17).

DLA-88 typing

Dog genomic DNA was isolated from whole blood using either Puregene DNA purification kit (Gentra Systems, Minneapolis, MN) or QIAamp DNA blood mini kit (Qiagen, Valencia, CA). Amplification and direct sequencing reactions and conditions were as described (12) except for the use of polymerase chain reaction (PCR) reverse primer, DLA88R57e3. The locus specific primers used for PCR were DLA88F-8e1: 5'-GCGGCGACGGCCAGTGTCCCCGGAG-3' and DLA88R57e3 5'-GACCTGAGTCCATATTCCTTCC-3'. The primers used for sequencing were DLA88F-8e1, DLA88R57e3, DLA88I1F: 5'-CCCGGCATCTCCCCCTG-3', DLA88I2F: 5'-GAACCCGCGGAACTCCCGGAGG-3', and DLA88I2R: 5'-GTGACGCCCGACCCGACCCTC-3'. The amplification primers were chosen based on their ability to amplify the polymorphic HVR regions of interest as a single fragment that includes DLA-88 exon 1 - exon 3, and for their inability to amplify the corresponding region

from other class I genes. The forward primer maps to 8 bases upstream of the ATG start codon on exon 1 while the reverse primer maps to 57 bases downstream of exon 3. It was not possible to design a forward primer that can distinguish DLA-88 from DLA-64 in the region of interest. This handicap was remedied by designing a reverse primer, DLA88R57e3, that would anneal to DLA-88 but not DLA-64. Similarly, the same reverse primer sequence is also present in DLA-12 gene, so the specificity for DLA-88 amplification is rendered by the forward primer, DLA88F-8e1. In all cases, when possibilities of new alleles were suggested by the direct sequencing data, new amplicons were generated by high fidelity PCR as modified from methods previously described (12,13) using the same PCR primers indicated above and sequenced after cloning individual alleles. Briefly, amplification reactions (typically 30 μ l) contained 100 ng of genomic DNA, 200 nM each of the primers and 200 μ M each of dNTPs. Velocity DNA polymerase (0.5 μ l) was used with the 5 \times GC buffer provided by the manufacturer (Bioline, Tauton, MA). All reactions were run in a Biorad C1000 Cyclor (Bio-Rad, Hercules, CA) using the following amplification conditions: 96 $^{\circ}$ C for 2 min; 30 cycles of 96 $^{\circ}$ C for 30 s, 65 $^{\circ}$ C for 15 s, and 72 $^{\circ}$ C for 60 s; and 68 $^{\circ}$ C for 5 min. PCR products analyzed by electrophoresis on a 1 % agarose gel produced a sharp band of about 1.1 kb. The DNA fragments were cloned in pCR2.1-TOPO vector using the TOPO TA Cloning Kit (Invitrogen). Plasmid insert DNA from multiples clones were sequenced to identify the alleles. Plasmid sequence-derived primers (TopoF1: 5' - CAGCTATGACCATGATTACGCCAAGC -3' and TopoR1 5' - GCCAGGGTTTTCCCAGTCACGACG -3') and DLA-88-specific primers indicated above were used for sequencing the plasmid insert fragments. In all cases, whether by direct sequencing or plasmid-cloned sequencing, the polymorphic regions of interest were sequenced in both directions, reactions were run in the FHCRC sequencing core facility and a custom-built software (developed in-house) was used to resolve the combinations of alleles.

New allele discovery and designation

For all sequences, the intron-exon boundaries were identified by aligning with previously described DLA-88 genomic sequence (8,18); the derived amino acid sequence of the new alleles were aligned with those of closely related known alleles and the reference allele DLA-88*001:01 using ClustalW2 (19) [<http://www.ebi.ac.uk/clustalw2/>]. All new alleles were verified by cloning and sequencing multiple independent PCR products, using DNA derived from at least 2 dogs. Only alleles encountered in multiple animals and repeatedly confirmed by multiple independent PCR and sequencing reactions were considered for new allele designations. New alleles found in our breeder population were also confirmed in pups after breeding. The allele identification process met the published conventions of the International Society of Animal Genetics (ISAG) Comparative MHC Nomenclature Committee and the new allele designations were assigned in consultation with the curator of the canine DLA database, Dr. L. J. Kennedy (20, 21). A rooted phylogenetic tree was derived using a web based software program at phylogeny.fr in the 'One Click' mode (22). Amino acid sequences of all known DLA-88 alleles along with that of HLA-A*01:01 as an out-group were used. The 'One Click' mode by default uses a set of reliable, widely used programs: MUSCLE for multiple alignment, Gblocks for automatic alignment curation, PhyML for tree building and TreeDyn for tree drawing.

Results

Identification of new DLA-88 alleles

All sequences obtained were found to be specific DLA-88 sequences, and not derived from other class I gene fragments as revealed by sequence analysis of gene-specific variations within polymorphic exons, as well as intron 1 and intron 2. Fifty of the 59 previously published alleles were identified among the dogs tested. In addition, thirteen novel alleles were also discovered. The nucleotide sequences for these alleles are recorded in the GenBank database, including complete intron 2 sequences. Table I lists the new alleles along with GenBank accession numbers, and source (laboratory and breed) of the dogs carrying those alleles. Seven of the new alleles were found within the relatively small group of dogs of diverse breeds pre-screened by reference strand-mediated conformation analysis, and these alleles accounted for 26 of the 54 allele identifications in this group.

Figure 1 shows the nucleotide sequences of the 13 alleles aligned with reference allele, DLA-88*001:01. The hyper-variable regions (HVR I–III) and variations between the alleles in the polymorphic exons 2 and exon 3 are indicated. Nine of the 13 alleles had the same intron 2 sequence as the most common 200 bp intron 2 found in the reference allele 001:01 and most other alleles. However, 4 alleles varied within intron 2 from the canonical 001:01 allele, each having 1–3 base substitutions (Figure 2). Alleles 003:02 and 047:01, each also carried a single base deletion.

Figure 3 shows the alignment of the amino acid sequence of exons 2 and 3 of the new alleles with the reference allele, 001:01. Most amino acid substitutions are seen in positions known to be polymorphic and the majority of these changes are seen within the three HVR. Allele 001:03 is a minor variant of the reference allele 001:01, with a single amino acid change, Thr instead of Met at position 45 in exon 2, which is outside the HVR. A new variant amino acid position is reported here that was not previously seen: Ser153 in alleles 046:01 and 049:01. The alignment of amino acid sequences of the new DLA-88 alleles along with all the other published alleles in a clustered arrangement as well as in their numerical order, as generated by ClustalW (18) are included in the supplementary information (supplementary fig. 1). A phylogenetic tree derived from this alignment analysis is also provided (supplementary fig. 2). A summary of the known DLA-88 alleles' nomenclature and their primary references is also included (supplementary table 1). The nucleotide and amino acid sequences of all these alleles are depicted in FASTA format in supplementary figure 3.

Conclusion

In this report, we extend the list of known DLA-88 alleles by presenting 13 additional alleles discovered during retrospective analyses of archival canine donor/recipient genomic DNA samples and new canine DNA samples from many breeds earmarked as likely to contain new alleles. Since the animals tested in this study were often related, and no attempt was made to randomize the study with respect to breed or family, no estimates of allele frequency should be inferred from this data. The true extent of diversity of the DLA genes in canines, particularly of the class I genes, is still not known. Ongoing studies include a

diversity survey to genotype and sequence DLA-88 in numerous dog breeds. A detailed characterization of haplotype diversity of DLA genes among breeds will:

- Allow a donor registry for clinical transplantation in dogs.
- Help focus MHC-linked disease association studies.
- Provide a more comprehensive understanding of the MHC diversity in dogs.
- Promote further study of the CTL response in dogs.
- Enhance the preclinical canine transplantation model, which predicts for engraftment and graft-versus-host-disease after HSCT in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DLA	dog leukocyte antigen
FHCRC	Fred Hutchinson Cancer Research Center
HSCT	hematopoietic stem cell transplantation
HLA	human leukocyte antigen
HVR	Hyper-variable region
MHC	Major histocompatibility complex
PCR	polymerase chain reaction

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Exon 2

			5	10	15	20	25	30	
DLA88*001:01	1	g	GCTCCCACTCCCTGAGGTATTCTACACCTCCGTGCCGGCCGGCCGGGGACCCCGCTTCATCGCCGTGGTACGTGGACGAC						89
001:03	1							89
003:02	1	CGG.....				T..A...T.T..G.....		89
016:03	1							89
028:04	1							89
032:02	1	G...G.....				T..A...T.T..G.....		89
036:01	1							89
040:01	1							89
042:02	1							89
046:01	1	G...G.....				T..A...T.T..G.....		89
047:01	1							89
049:01	1	G...G.....				T..A...T.T..G.....		89
050:01	1							89
508:02	1							89

			35	40	45	50	55	60	
DLA88*001:01	90	A	CGCAGTTCGTGCGGTTCGACAGCAGCGCCACTGGGAGGATGGAGCCGGCCGGCCCGCTGGATGGAGCAGGAGGGCCGAGTATTG						179
001:03	90							179
003:02	90	GG.G.CA.A.G.....				G.....		179
016:03	90							179
028:04	90	G						179
032:02	90							179
036:01	90							179
040:01	90	C.....						179
042:02	90							179
046:01	90							179
047:01	90	G						179
049:01	90							179
050:01	90	C.....						179
508:02	90	C.....						179

HVR I

			65	70	75	80	85	90	
DLA88*001:01	180	G	GCACGGGAGACGGACCGCCAGGAGACCGCACAGAGGTACCGAGTGGACCTGGACACCCCTGGCGGGCTACTACACCAGACGGAGGC						269
001:03	180							269
003:02	180	T.....A.....GT.....						269
016:03	180	C.C.....AT.....AG.....						269
028:04	180	A.TT.....GT.....G.....						269
032:02	180	C.....T.T.....A.....GT.....G.....						269
036:01	180	G.....AA.T.....CT.....G.....						269
040:01	180	C.....AT.....GT.....A.....						269
042:02	180	C.....TT.....GAG.....G.....T.....						269
046:01	180	G.....AA.T.....GT.....A.....						269
047:01	180	A.TT.....GAG.....G.....T.....						269
049:01	180	G.....AA.T.....A.....GT.....A.....						269
050:01	180	G.....						269
508:02	180	G.....AA.T.....GT.....T.....						269

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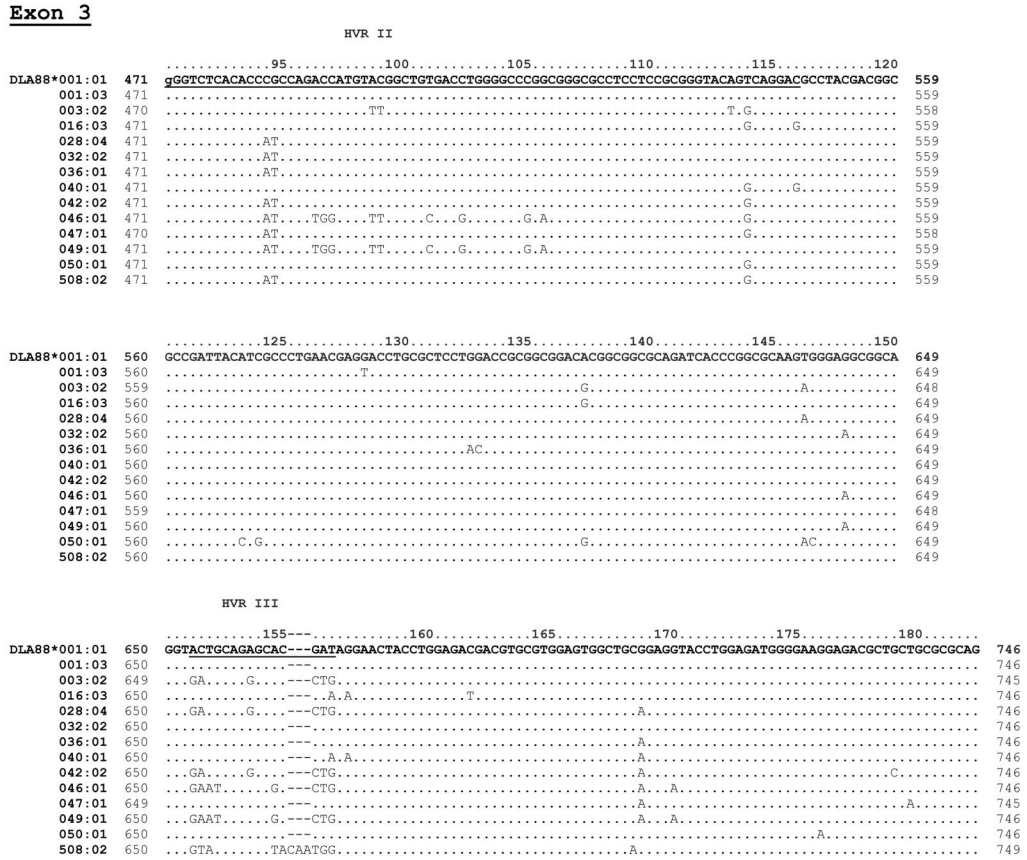


Figure 1. Nucleotide sequence alignment of the new DLA-88 alleles
 Nucleotide sequence of exons 2 and 3 for the 13 new alleles are shown aligned to the reference allele DLA-88*001:01. The nucleotide position numbers indicated start from the first base of exon 2. The exon 2 alignment starts with a lowercase ‘g’ from exon 1 to provide full codon context. The codon numbers are indicated above the reference allele sequence. Nucleotide position 270 from exon 2 is included as the first position (in lower case ‘g’) along with the exon 3 sequence. The exon 3 sequence numbering is also indexed from the first base of exon 2 and continues through the intron 2 sequence. The new alleles are presented in sequential order with the differences from the reference allele indicated. The HVR I, II and III are in bold and underlined.


```

001:01 1 gtgagcggcgcgggcccggggacgacgacccccatcccccgaccggcgcccgaggggtcgccccgaggggtcgggtccgggcgtcaccgccg 90
001:03 1 .....a..... 90
003:02 1 .....a.....t.g..... 90
040:01 1 .....a..... 90
047:01 1 .....a..... 90

001:01 91 agtccgagggccccgccccctccccctcgtccccgaccccggggaactccccgggaggttttactttctctttggactgattggcg 180
001:03 91 ..... 180
003:02 91 .....t..... 179
040:01 91 .....t..... 180
047:01 91 .....t..... 179

001:01 181 cctgtcggggcgggccag 200
001:03 181 ..... 200
003:02 180 ..... 199
040:01 180 ..... 200
047:01 181 ..... 199

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Figure 2. Alignment of atypical DLA-88 introns

New alleles with atypical intron 2 are shown aligned with the reference allele (DLA-88*001:01) intron 2 sequence. The numbering pertains to the full intron 2 sequence. All other alleles reported in this paper have an intron 2 sequence that is identical to the 200 bp intron 2 of the reference allele DLA-88*001:01.

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Exon 2

		HVR I																										
		10	20	30	40	50	60	70	80	90																		
88*001:01	1	*SHSLRYFYTSVSRPGRGDPFRFIAGVYDDTQFVRFSDAATGRMEPRAPWMEQEGPEYWD <u>RETAKETAQRVRLD</u> TLRGYINQSEA																		90								
88*001:03	1	*.....T.....																		90								
88*003:02	1	*.....R.....LY.S.....A.....SQKV.....V.....S.N.V.....																		90								
88*016:03	1	*.....V.....PQ...I.....S.....																		90								
88*028:04	1	*.....V.....NF.....V.G.....																		90								
88*032:02	1	*.....D.A.....LY.S.....V.....Q..IS..N.V...G.....																		90								
88*036:01	1	*.....V.....G..KV...L..G.....																		90								
88*040:01	1	*.....T.....V.....Q..I...V...G..I.....																		90								
88*042:02	1	*.....V.....Q..F...RG..G..I.....																		90								
88*046:01	1	*.....D.A.....LY.S.....V.....G..KV...V...N...G..I.....																		90								
88*047:01	1	*.....V.....NF...RG..G..I.....																		90								
88*049:01	1	*.....D.A.....LY.S.....V.....G..KV...V...N...G..I.....																		90								
88*050:01	1	*.....T.....V.....G.....V.....																		90								
88*508:02	1	*.....T.....V.....G..KV...V...I.....																		90								

Exon 3

		HVR II						HVR III								
		100	110	120	130	140	150	160	170	180						
88*001:01	91	<u>GSHTRQTFMYGCDLPGGRLLRGYSQDAYDGDYIALNEDLRSWTAADTAAQTTRKWEAAGTAEHD</u> -RNYLETTCVEWLRRLYLEMGKETLLRA												182		
88*001:03	91												182		
88*003:02	91F.....W.....A.....R.....D.G.L.....												182		
88*016:03	91R.E.....A.....E-K.....M.....												182		
88*028:04	91I.....R.....R.....D.G.L.....Q.....												182		
88*032:02	91I.....												182		
88*036:01	91I.....Y.....												182		
88*040:01	91R.E.....Y.....E-K.....Q.....												182		
88*042:02	91I.....R.....D.G.L.....Q.....												182		
88*046:01	91I.W.F...V...D.....R.....ES.RL.....Q.....												182		
88*047:01	91I.....R.....												182		
88*049:01	91I.W.F...V...D.....R.....ES.RL.....Q.....												182		
88*050:01	91R.....L.....A.....T.....												182		
88*508:02	91I.....R.....V..LQW.....Q.....												183		

Figure 3. Amino acid sequence alignment of the new DLA-88 alleles
 The amino acid sequence of 13 new alleles, in bold, are shown aligned with the reference allele DLA-88*001:01. The amino acid sequence starts with an asterisk ‘*’ at position 1 to indicate the partial glycine codon from exon 2. The three hypervariable regions (HVR) are indicated. The amino sequences of the new alleles are presented in sequential order with the amino acid substitutions from the reference allele indicated. The HVR I, II and III are in bold and underlined.

Table 1

New DLA-88 Alleles and Number of Dogs Typed by Breed

DLA-88 Allele	Accession number	No. of dogs with allele	Source of sample, Number of dogs and Breed type
001:03	KR818710	9	FH: 4 Papillion, 5 Schipperke
003:02	KF911090	6	FH: 3 Hound mix LK: 1 each of Hovawart, Newfoundland, Shetland Sheep Dog
016:03	KF939645	11	FH: 1 Beagle, 3 Beagle-Mongrel LK: 1 Doberman, 2 Great Dane, 1 Japanese Akita, 1 Newfoundland, 2 West Highland White terrier
028:04	KF939646	10	FH: 4 Beagle, 2 Beagle-Golden Retriever, 1 Beagle-Mongrel, 1 Hound mix LK: 1 Cairn Terrier, 1 Hovawart
032:02	KF911096	7	FH: 2 Hound mix, 5 Beagle-Mongrel
036:01	KF911094	7	FH: 2 Hound mix, 5 Beagle-Mongrel
040:01	KR818708	4	LK: 2 Great Dane, 2 Newfoundland
042:02	KT337312	2	FH: 2 Hound mix
046:01	KR818709	4	LK: 1 Japanese Akita, 3 West Highland White Terrier
047:01	KF939647	10	FH: 5 Beagle, 2 Beagle-Golden Retriever, 1 Hound mix, 1 Beagle-Mongrel LK: 1 Hovawart
049:01	KF911093	7	FH: 1 Beagle, 3 Hound mix, 3 Mongrel
050:01	KF911095	2	FH: 2 Beagle
508:02	KF911092	11	FH: 1 Beagle, 10 Hound mix

The number of dogs and the type of dog breed for each allele analyzed. This table does not represent allele frequencies in these different breeds. The nucleotide sequences of these new DLA-88 alleles are recorded in GenBank, and accession numbers are listed. The laboratory source of the dog blood samples is FH for the Fred Hutchinson Cancer Research Center Seattle, WA and LK for LJ Kennedy, Manchester, UK.