HHS Public Access

Author manuscript

Anim Genet. Author manuscript; available in PMC 2020 June 01.

Published in final edited form as:

Anim Genet. 2019 June ; 50(3): 303–306. doi:10.1111/age.12778.

CMAH genotyping survey for blood types A, B and C (AB) in purpose-bred cats

Alexandra Kehl[1], **Elisabeth Mueller**[1], and **Urs Giger**[2]

[1]Laboklin, Bad Kissingen, Germany

[2]Section of Medical Genetics (PennGen), University of Pennsylvania, Philadelphia, USA

Summary

In domestic cats, the AB blood group system consists of the three types A, B and C (also called AB). Mismatches can cause acute hemolytic transfusion reactions and hemolysis of the newborn (neonatal isoerythrolysis, NI). As blood types B and C are inherited recessively to A, breeders need to know the genotype to predict blood types in offspring and avoid NI. Several *CMAH* variants have been described as being associated with the *b* and a^c alleles, and different genotyping schemes exist. Here, we genotyped 2145 cats with the original SNV panel, including SNVs c.142G>A and Δ−53, and our new scheme, with SNVs c.179G>T, c.268T>A and c.1322delT, to differentiate types A and B and added the SNV for the common a^c (c.364C>T). Based upon the new scheme, all samples were assigned the correct genotype. No discordances appeared for the *A* allele, and new breed-specific SNVs (c.179G>T, c.1322delT) for the *b* allele were discovered. Furthermore, the genotypes A/a^c (type A), a^c/a^c (C) and a^c/b (C) could be detected. We found the variant c.179G>T in additional breeds: Ragdoll, Siberian, Scottish Fold, Chartreux, Neva Masquerade, British Shorthair and Highlander. Also, the variant c.364C>T was detected in additional breeds: Bengal, British Shorthair, Maine Coon, and Scottish Fold. We conclude that our new SNV panel is superior in genotyping cats than the original SNV panel and assures correct assignments of types A, B and C to assist veterinary clinicians and breeders to recognize, confirm and avoid blood incompatibilities such as acute hemolytic transfusion reactions and NI.

Keywords

blood typing; blood group systems; cytidine monophosphate-N-acetylneuraminic acid hydroxylase; feline; single nucleotide polymorphisms

Address for correspondence: Alexandra Kehl, Steubenstraße 4, D-97688 Bad Kissingen, Germany, Tel +49 971 72020, kehl@laboklin.com.

Disclosures

Alexandra Kehl and Elisabeth Müller are employed by Laboklin, which offers blood typing and blood compatibility testing. A patent ‘Verfahren und Vorrichtung zur Bestimmung der Blutgruppe einer Katze im AB-Blutgruppensystem’ (no. 10 2017 124 998.2) on the molecular genetic markers and panel testing was submitted on February 21, 2018.

Urs Giger is the director of PennGen at the University of Pennsylvania which is a not-for-profit laboratory offering blood typing and compatibility testing and is supported by the National Institutes of Health (OD 010939). He has been a scientific advisor to Laboklin and Alvedia.

For this study only data from routine diagnostic testing requested by breeders and veterinary clinicians were used.

The feline AB blood group system is composed of type A, B and C (also called AB). It is of great clinical importance as blood incompatibilities can result in acute hemolytic transfusion reactions and hemolysis of the newborn (neonatal isoerythrolysis [NI]) due to presence of naturally occurring anti-A alloantibodies (Auer & Bell 1981; Auer *et al.* 1982; Giger *et al.* 1991; Giger 2014). The enzyme cytidine monophosphate-N-acetylneuraminic acid hydroxylase (CMAH; EC 1.14.18.2) converts sialic acid N-acetylneuraminic acid (NeuAc; type B antigen) to N-glycolylneuraminic acid (NeuGc; type A antigen) (Bighignoli *et al.* 2007).

Although immunohematological (serological) blood typing is clinically useful, genotyping cats is important in breeding purpose-bred cats to predict blood types in offspring and avoid NI. The *CMAH* locus is proposed to have three alleles with several haplotypes in the allelic series of $A>a^c>b$ (Giger *et al.* 1991; Griot-Wenk & Giger 1995). Thus, genotypes *A/A*, *A/b* and A/a^c can be observed in type A cats, *b/b* in type B and a^c/a^c and a^c/b in type C cats.

Many DNA polymorphisms have been described in the cat CMAH gene: Δ−53, c.139C>T, c.142G>A, c.179G>T, c.187A>G, c.268T>A, c.327A>C, c.364C>T, c.374C>T, c.376G>A, c.593A>C, c.868A>C, c.898A>G, c.933delA, c.1322delT, c.1342G>A and c.1603G>A. Several of these variants are presumed to lead to loss or reduction of the regular CMAH activity needed for the type A antigen and thereby lead to blood type B and C respectively (Bighignoli *et al.* 2007; Tasker *et al*, 2014; Gandolfi *et al.* 2016; Omi *et al.* 2016; Kehl *et al.* 2018). Originally SNVs c.142G>A and Δ−53 were used in diagnostic genotype differentiation between type A (genotype *A/A* and *A/b*) and type B (genotype *b/b)* (Bighignoli *et al.* 2007), but it was recognized that there were many genotype–phenotype discordances (Kehl *et al.* 2018). We recently showed that the *CMAH* SNV c.268T>A (p.Tyr90Asn) instead of the above was most likely to cause enzyme dysfunction. Indeed, this SNV exhibited perfect genotype–phenotype correlation in a limited number ($n = 421$) of cats (Kehl *et al.* 2018). Additionally, the SNVs c.179G>T (p.Gly60Val) and c.1322delT (p.Leu441*) are detrimental to *CMAH* function and were shown to be associated with the *b* allele in our initial genotyping–phenotyping survey (Kehl *et al.* 2018). Also, we and others have recently associated the SNV c.364C>T (p.Pro122Ser) with type C in Ragdolls (Gandolfi *et al.* 2016, Kehl *et al.* 2018). We therefore proposed a new genotyping scheme including these four SNVs (Kehl *et al.* 2018) (Table 1). Here we compare the genotyping results from the original to the new scheme in a large group of cats to further elucidate the relevance of the different SNVs in routine blood typing.

All ethylenediaminetetraacetic blood and cheek swab samples from purpose-bred cats were submitted to Laboklin by veterinary clinicians and breeders for routine diagnostic genetic testing from November 2017 to October 2018. Most samples originated from Germany, but samples were also received from other countries throughout Europe. Genomic DNA was isolated (MagNAPure 96; Hoffmann-La Roche), and genotyping for the *CMAH* SNVs Δ−53, c.142G>A, c.179G>T, c.268T>A, c.364C>T and c.1322delT was performed (TaqMan SNP Assays; ThermoFisher), using FastStart Essential DNA Probes Master and LightCycler 480 II (Hoffmann-La Roche), as previously described (Kehl *et al.* 2018). Routine immunological blood typing was performed for 37 cats using an immunochromatographic strip method, according to the manufacturer's instructions [Alvedia rapid-test (LabTest *A*

+B)]. Results and correlation between SNVs, genotypes and predicted phenotypes (with a few also regularly typed cats) were evaluated.

Our survey included 2145 pedigreed cats from 31 breeds, among which there were five breeds with more than 100 cats genotyped and a few breeds with fewer than 10 cats genotyped (Table 2). Compared to prior phenotypic blood typing surveys of different breeds (Giger *et al*. 1991; Jensen *et al*. 1994; Knottenbelt *et al*. 1999; Weingart *et al*. 2006; Forcada *et al*. 2007; Spada *et al*. 2014; Vieira *et al*. 2017), equal or more purpose-bred cats had type A blood, which may be due to breeders' selection for type A cats and the breeders' interest in determining if their type A cats are carriers for the *b* or a^c allele.

Overall 162 (7.6%) cats had the genotype *b/b* (type B blood) and were homozygous for the *A* allele at position 268 ($n = 145$) or for the deletion at position 1322 ($n = 7$) or compound heterozygous for the SNVs c.268T>A/c.179G>T ($n = 4$) or c.268T>A/c.1322delT ($n = 6$). Thus, genotyping for this additional *b* allele is important.

The variant c.364C>T was described as being the cause of type C in Ragdolls and a few random-bred cats (Gandolfi *et al.* 2016; Kehl *et al.* 2018). Of the 204 Ragdolls tested, indeed 24 (12%) showed the genotype A/a^c (type A), 12 (6%) had the genotype a^c/b (type C) and two (1%) were homozygous a^c/a^c (type C). Among the 1941 genotyped non-Ragdoll cats, the variant c.364C>T was rarely found: in only nine heterozygous and one homozygous Bengals, two British Shorthairs, two Maine Coons and one Scottish Fold cat (and one Thai-Siam-hybrid) in the heterozygous state (expressing type A blood). The homozygous a^c/a^c Bengal cat was confirmed to possess type C blood by phenotyping. Thus, the variant c.364C>T seems to be more widespread and exist in more breeds than originally described (Gandolfi *et al*. 2016; Kehl *et al*. 2018). Additionally, more than one genetic cause for type C can be suspected in other breeds, as one British Shorthair cat with type C (and without the c.364 variant) has been described (Gandolfi *et al*. 2016).

The variant c.179G>T was recently described as causative for type B in Turkish Angora, Neva Masquerade as well as Domestic Shorthair cats (Kehl *et al*. 2018). In this study, this variant was also found in five Ragdolls, four Siberian cats and one Scottish Fold cat in the heterozygous state together with an *A* allele and thus type A blood. Additionally, the variant was found in one British Shorthair, Highlander, Chartreux and Neva Masquerade cats in the heterozygous state together with the variant c.268T>A, suggesting a compound heterozygous status causing type B blood. However, their blood types were not confirmed by immunohematological testing.

The variant c.1322delT was recently described as being causative for type B in Ragdoll and Scottish Fold cats (Kehl *et al*. 2018). In the present study, this variant was found exclusively in Ragdolls: 54 cats (23%) were heterozygous for the c.1322delT variant; six of these 54 cats were also heterozygous for c.268T>A, suggesting the genotype *b/b* (type B); and eight of these 54 cats showed the genotype a^c/b (type C). If these two variants c.179G>T and c.1322delT would not have been included in the genotyping scheme, 77 (3.6%) cats would have been genotyped incorrectly.

Furthermore, due to contradicting diplotypes for c.142G>A and Δ−53, 105 (5%) of genotyped cats could not be assigned to one blood type with the original SNV panel. Noteworthy, these discordances were found in different breeds, but especially in Bengals (10.3% with genotype *A/A*) and Maine Coons (8.8% with genotype *A/A*). However, based upon our new SNV panel, these 105 cats could be readily assigned as having type A or B blood (Table 1).

Additionally, we assigned different genotypes to 54 cats compared to the original SNV panel: 39 type A, A/a^c; three type C, a^c/a^c; and 12 type C, a^c/b. Of the 39 A/a^c cats, 37 would have been genotyped as *A/A* with the original scheme and the other two cats already had discordant genotyping results with the original two SNVs. All three a^c/a^c cats and eight of the 12 a^c/b cats would have been genotyped as *A/A* and four as *A/b* with the original SNV panel. The original scheme also missed 10 type B cats that were *b/b* compound heterozygotes and seven type B cats homozygous for the SNV c.1322delT.

All *A/A* genotyping results of the new scheme were typed *A/A* in the original genotyping scheme, whereas 95 cats with discordant diplotypes for the SNVs c.142G>A and Δ−53 were assigned *A/A*. Based upon our new SNV panel all cats with undetermined blood type could be assigned a specific blood type.

The 37 cats that we also blood typed by an immunohematological method showed perfect genotype–phenotype correlation, further supporting the excellent genotype–phenotype correlation shown in our prior study of 421 cats (Kehl *et al.* 2018).

This survey shows the superiority of the new genotyping scheme (SNVs c.179G>T, c. 268T>A, c.364C>T and c.1322delT) over the old scheme (SNVs c.142G>A and Δ−53). Type C cats with the genotypes a^c/a^c and a^c/b can now also be detected. Additionally, the B type caused by either SNVs c.179G>T or c.1322delT alone or as a compound heterozygote with the SNV c.268T>A can be found. Moreover, no genotyping–phenotyping discordances were observed. The new SNV panel also demonstrates its strength in detecting the ‘breed-related’ SNVs, such as c.179G>T, c.364C>T and c.1322delT, in additional breeds.

In conclusion, the new genotyping scheme for the feline AB blood group system applied in this large survey provides detailed typing results that are important for breeders and veterinarians confronted with inconsistent immunohematological typing results. Although genotyping is not yet a replacement for immunohematological testing in feline clinical practice, this new genotyping scheme is an additional step forward to assuring blood compatibility in cats, as already used in human blood banking. There may well be additional new SNVs discovered in some breeds and geographical regions in the future that may cause type B and C and may have to be added to the current genotyping scheme to be inclusive.

Acknowledgements

The authors would like to thank the staff at Labogen, Laboklin, Bad Kissingen, Germany for their routine testing and commenting on our results.

References

Auer L & Bell K (1981) The AB blood group system of cats. Animal Blood Groups Biochemistry and Genetics 12, 287–97.

Auer L, Bell K & Coates S (1982) Blood transfusion reactions in the cat. Journal of American Veterinary Medicine Association 180, 729–730.

Bighignoli B, Niini T, Grahn RA, Pedersen NC, Millon LV, Polli M, Longeri M & Lyons LA (2007) Cytidine monophospho-N-acetylneuraminic acid hydroxylase (CMAH) mutations associated with the domestic cat AB blood group. BMC Genetics 8, 27. [PubMed: 17553163]

Forcada Y, Guitian J & Gibson G (2007) Frequencies of feline blood types at a referral hospital in the south east of England. Journal of Small Animal Practice 48, 570–3. [PubMed: 17608664]

Gandolfi B, Grahn RA, Gustafson NA, Proverbio D, Spada E, Adhikari B, Cheng J, Andrews G, Lyons LA & Helps CR (2016) A novel variant in CMAH is associated with blood type AB in Ragdoll cats. PLoS ONE 11, e0154973. [PubMed: 27171395]

Giger U (2014) Blood typing and crossmatching to ensure blood compatibility In: Kirk's Current Veterinary Therapy XV (Ed. by Bonagura JD & Twedt DC), web-based version Chapter 26. Elsevier Saunders, St. Louis, MO.

Giger U, Bucheler J & Patterson DF (1991) Frequency and inheritance of A and B blood types in feline breeds of the United States. Journal of Heredity 82, 15–20. [PubMed: 1997588]

Griot-Wenk ME & Giger U (1995) Feline transfusion medicine. Blood types and their clinical importance. Veterinary Clinical North American Small Animal Practice 25, 1305–22.

Jensen AL, Olesen AB & Arnbjerg J (1994) Distribution of feline blood types detected in the Copenhagen area of Denmark. Acta Veterinary Scandinavia 35, 121–4.

Kehl A, Heimberger K, Langbein-Detsch I, Boehmer S, Raj K, Mueller E & Giger U (2018) Molecular characterization of blood type A, B, and C (AB) in domestic cats and a CMAH genotyping scheme. PLoS ONE 13, e0204287. [PubMed: 30235335]

Knottenbelt CM, Addie DD, Day MJ & Mackin AJ (1999) Determination of the prevalence of feline blood types in the UK. Journal of Small Animal Practice 40, 115–8. [PubMed: 10200921]

Omi T, Nakazawa S, Udagawa C, Tada N, Ochiai K, Chong YH, Kato Y, Mitsui H, Gin A, Oda H, Azakami D, Tamura K, Sako T, Inagaki T, Sakamoto A, Tsutsui T, Bonkobara M, Tsuchida S and Ikemoto S (2016) Molecular Characterization of the cytidine monophosphate-n-acetylneuraminic acid hydroxylase (CMAH) gene associated with the feline AB blood group system. PLoS ONE 11, e0165000. [PubMed: 27755584]

Spada E, Miglio A, Proverbio D, Antognoni MT, Bagnagatti De Giorgi G, Ferro E & Mangili V (2014) Signalment and blood types in cats being evaluated as blood donors at two Italian university blood banks. Veterinary Medicine International 2014, 704836. [PubMed: 24757577]

Tasker S, Barker EN, Day MJ & Helps CR (2014) Feline blood genotyping versus phenotyping, and detection of non-AB blood type incompatibilies in UK cats. Journal of Small Animal Practice 55, 185–9. [PubMed: 24697343]

Vieira SM, Ferreira RRF, de Matos AJ, Cardoso IM, Graça RMC, Soares AR, Blasi-Brugué C, Sánchez IM & Gopegui RR (2017) Distribution of feline AB blood types: a review of frequencies and its implications in the Iberian Peninsula. JFMS Open Report 3, 2055116917727693.

Weingart C, Arndt G & Kohn B (2006) Prävalenz der blutgruppen A, B und AB bei haus- und rassekatzen im raum Berlin und Brandenburg. Kleintierpraxis 51, 189–97.

Table 1

Comparison of genotyping and predicted phenotypic blood typing results according to the original and new SNV panel of 2145 purpose-bred cats.

New SNV panel					Original SNV panel				
Genotype	**c.179G>T[a]**	**c.268T>A[2]**	**c.364C>T[3]**	**c.1322delT[4]**	**c.142G>A[2]**	**Δ−53[b]**	**Genotype**	**Number of cats**	**Predicted phenotype**
	GG	TT	CC	TT	GG	NN	*A/A*	1290	
A/A	GG	TT	CC	TT	GG	NP	Discordant	93	
	GG	TT	CC	TT	GG	PP	Discordant	2	
	GG	TA	CC	TT	GA	NP	*A/b*	488	
	GT	TT	CC	TT	GG	NN	*A/A*	15	
A/b	GG	TT	CC	T*	GG	NN	*A/A*	37	A (91.7%)
	GG	TA	CC	TT	GG	NP	Discordant	3	
	GG	TA	CC	TT	AA	NP	Discordant	1	
	GG	TT	CT	TT	GG	NN	*A/A*	37	
A/a[c]	GG	TT	CT	TT	GG	NP	Discordant	1	
	GG	TT	CT	TT	GA	NN	Discordant	1	
	GG	TA	CT	TT	GA	NP	*A/b*	4	
a[c]/b	GG	TT	CT	T*	GG	NN	*A/A*	8	C (0.7%)
a[c] / a[c]	GG	TT	TT	TT	GG	NN	*A/A*	3	
	GG	AA	CC	TT	AA	PP	*b/b*	145	
	TT	TT	CC	TT				x	
	GG	TT	CC	**	GG	NN	*A/A*	7	
b/b	GT	TA	CC	TT	AA	NP	Discordant	4	B (7.6%)
	GG	TA	CC	T*	GA	NP	*A/b*	6	
	GT	TT	CC	T*				x	

N not present, P present;

*deletion, X not seen.

[1]Omi *et al.* 2016;

[2]Bighignoli *et al.* 2007; Gandolfi *et al.* 2016

[d] Kehl *et al.* 2018.

Table 2:

Distribution of *CMAH* genotypes of 2145 purpose-bred cats from 31 breeds (16 breeds with $n \geq 8$ cats per breed shown; additional 14 breeds shown in footnote)

	Genotypes							
Breed	***A/A***	***A/b***	***b/b***	***b/b* compound**[1]	***A/a*^{c}**	***a*^{c}*/b***	***a*^{c}*/a*^{c}**	***n***
Abyssinian	25	6	1					32
Bengal	136	2			9		1	148
Birman	55	49	2					106
British Shorthair	128	189	109	1	2			429
Chartreux	2	3	2	1				8
Devon Rex	6	9	3					18
Highlander	13	17	7	1				38
Maine Coon	787	159	10		2			958
Neva Masquerade	4	7		1				12
Norwegian Forest	45	3						48
Persian	11	3	1					15
Ragdoll	90	61	9	6	24	12	2	204
Savannah	9	1						10
Scottish Fold	6	6	4		1			17
Siberian	17	8	2					27
Somali	6	8	1					15

Note: Australian Mist ($n = 1$), Burma ($n = 2$), Cornish Rex ($n = 2$), Exotic Shorthair ($n = 6$), Oriental Shorthair ($n = 6$), Russian Blue ($n = 6$), Siamese ($n = 2$) and Tonkinese ($n = 2$) all had the regular *A/A* genotype (all type A blood). Egyptian Mau ($n = 6$), Ocicat ($n = 3$), Savannah ($n = 10$), Sphynx ($n = 4$), Thai ($n = 5$) and Turkish Angora ($n = 5$) were either *A/A* or *A/b* (all type A). In addition, one Thai cat was (*A/a*^{C}) which still is a type A cat.

[1] *b/b* compound cats are cats with heterozygous genotypes at two loci in *CMAH* gene (c.179G>T, c.268T>A and c.1322delT) suggesting the genotype *b/b*.