



## A 16-bp deletion in the canine *PDK4* gene is not associated with dilated cardiomyopathy in a European cohort of Doberman Pinschers

Marta Owczarek-Lipska\*, Theresa-Bernadette Mausberg\*, Hannah Stephenson†, Joanna Dukes-McEwan†, Gerhard Wess‡ and Tosso Leeb\*

\*Vetsuisse Faculty, Institute of Genetics, University of Bern, Bremgartenstrasse 109a, 3001, Bern, Switzerland; †Small Animal Teaching Hospital, University of Liverpool, Leahurst, Chester High Road, Neston, CH64 7TE, UK; ‡Clinic of Small Animal Medicine, LMU University, Veterinärstrasse 13, 80539, Munich, Germany

Accepted for publication 17 June 2012

**Source/description:** Following a genome-wide association study (GWAS), a 16-bp deletion in the 5' splice site of intron 10 of the *pyruvate dehydrogenase kinase, isozyme 4* (*PDK4*) gene on CFA 14 has been reported to be associated with dilated cardiomyopathy (DCM) in Doberman Pinschers.<sup>1</sup> This *PDK4* variant was further reported to be absent from 100 dogs of other breeds.<sup>1</sup> We have previously performed a GWAS in a European cohort of Doberman Pinschers and found a significant genome-wide association for DCM on CFA 5, but not on CFA 14.<sup>2</sup> The objective of this study was to evaluate the association of the *PDK4* variant on CFA 14 in our cohort of Doberman Pinschers.

**Animals:** We analyzed the 180 Doberman Pinschers from our previous study.<sup>2</sup> These dogs consisted of a discovery cohort collected in Germany (71 DCM cases and 70 controls) and a validation cohort collected in UK (15 DCM cases and 24 controls). Additionally, we analyzed 490 dogs from 89 other breeds for the presence of the variant allele (Table S1).

**Genotyping:** The reported variant in the *PDK4* gene is CFA14:g.20,829,667\_20,829,682del (genome build CanFam 3.1). We used a FAM-labeled forward primer, 5'-TGCCAAGTACTTTCAAGGAGA-3', and an unlabeled reverse primer, 5'-CCCACCTTGCATGGACTCTCT-3', which amplify a 185- or 169-bp product from the wild-type (ins) or variant (del) allele respectively. We performed fragment size analysis on an ABI 3730 capillary sequencer (Applied Biosystems) as described.<sup>3</sup> We used Fisher's exact tests to calculate significance of differences between allele and genotype frequencies in DCM cases and controls.

**Association with DCM in Doberman Pinschers:** We observed the variant *PDK4* allele with the 16-bp deletion in both DCM cases and controls with an overall allele frequency of 16%. The deletion allele was slightly more frequent in DCM cases than in controls, but the difference was not significant in any of our cohorts (Table 1). We also did not observe a significant genotypic association in our cohorts (Table S2).

**Analysis of other breeds:** We analyzed 490 dogs from 89 other breeds and found the homozygous ins/ins genotype in 469 dogs. We detected the heterozygous del/ins genotype in 21

**Table 1** Allele frequencies and association with DCM.

Cohort	n	DCM cases		Controls		P-value
		Del	Ins	Del	Ins	
Discovery	141	0.20	0.80	0.16	0.84	0.54
Validation	39	0.07	0.93	0.08	0.92	1.00
Combined	180	0.17	0.83	0.14	0.86	0.47

dogs (Table S1). We did not observe any homozygous del/del individuals in the other breeds.

**Conclusions:** The reported association of the *PDK4* variant with DCM<sup>1</sup> that was obtained in a cohort of 132 Doberman Pinschers of presumably American origin could not be replicated in a larger cohort of European origin. This *PDK4* variant is not specific to Doberman Pinschers but is also present at low frequency in diverse other dog breeds. We found no evidence for an involvement of the *PDK4* gene in the etiology of DCM.

**Acknowledgments:** This work was mainly supported by the LUPA Project ([www.eurolupa.org](http://www.eurolupa.org)), which was funded by the European Commission (FP7, GA: 201370). The authors acknowledge all dog owners who actively participated in sample collection.

### References

- 1 Meurs K.M. *et al.* (2012) *Human Genetics* **131**, 1319–25.
- 2 Mausberg T.-B. *et al.* (2011) *PLoS ONE* **6**, e20042.
- 3 Owczarek-Lipska M. *et al.* (2009) *Mammalian Genome* **20**, 187–92.

[Correction added on 3 December 2012, after first online publication: References 1 and 2 were previously listed with the wrong reference numbers and are now referenced to the correct publications]

**Correspondence:** T. Leeb ([tosso.leebe@vetsuisse.unibe.ch](mailto:tosso.leebe@vetsuisse.unibe.ch))

### Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1** Genotype frequencies of the 16-bp deletion in the *PDK4* gene in 490 dogs from 89 various dog breeds.

**Table S2** Genotype frequencies and association with DCM.

As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.