Idiopathic epilepsy in the Border Collie: using genome-wide association study and whole genome sequencing approaches to identify genetic risk factors

Christopher A. Jenkins[1,2], Luisa De Risio[3], Tiina Heinonen[4], Jim Johnson[1], Lorna J. Kennedy[2], Andrea D. Short[2], Sofie F. M. Bhatti[5], Donna Foster[1], Peter Leegwater[6], Tarja S. Jokinen[4], Rowena Packer[7], Koen Santifort[8], Paul Mandigers[6], Holger Volk[9], Cathryn S. Mellersh[1], Marjo Hytönen[4], Hannes Lohi[4*], Sally L. Ricketts[1,2*]

[1] Kennel Club Genetics Centre, Department of Veterinary Medicine, University of Cambridge, UK; [2] Division of Population Health, Health Services Research & Primary Care, University of Manchester, Manchester, UK; [3] Linnaeus Veterinary Ltd, UK; [4] University of Helsinki, Finland; [5] Ghent University, Faculty of Veterinary Medicine, Belgium; [6] University of Utrecht, The Netherlands; [7] Royal Veterinary College, UK; [8] Evidensia Small Animal Hospital Arnhem, Arnhem, The Netherlands; [9] Department of Small Animal Medicine and Surgery, University of Veterinary Medicine, Hannover, Germany *Contributed equally

Background and Aims

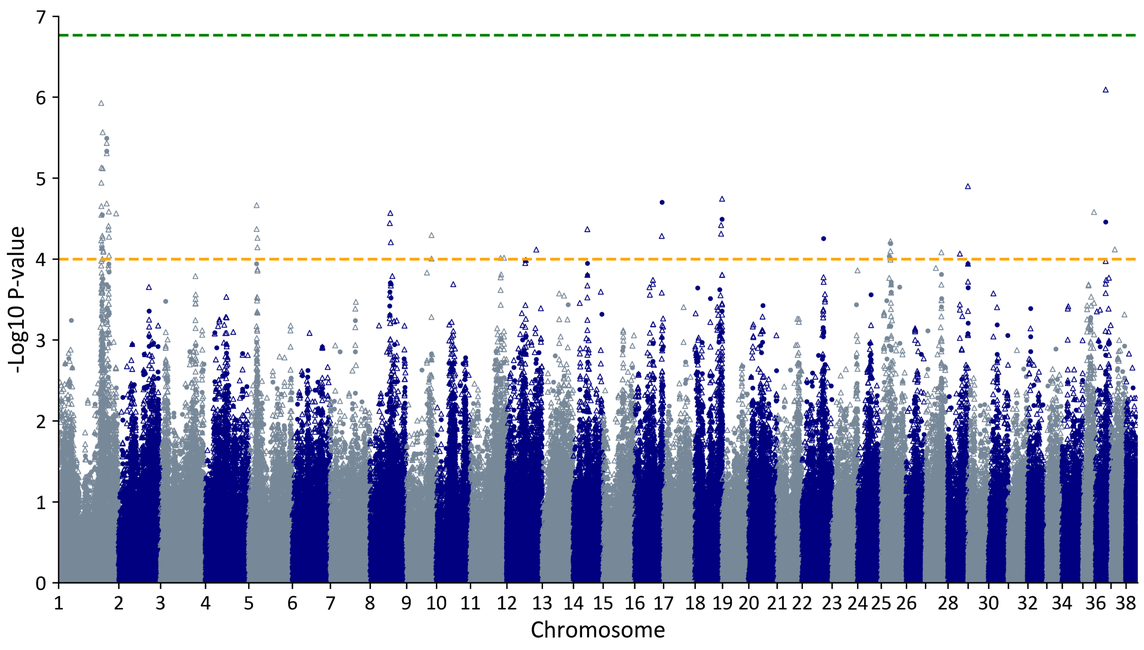
Idiopathic epilepsy (IE) is a chronic and life-limiting neurological disorder characterised by recurrent seizures. The Border Collie (BC) is one of the most commonly and severely affected breeds, though little is known regarding the genetic factors that increase this breed's susceptibility to IE. It is likely that IE in most breeds, as shown by previous studies[1-4], is a complex disease, with a polygenic set of risk factors predisposing certain breeds to greater risk of IE. Improved understanding of the genetic architecture and biological pathways underlying IE in the BC could lead to better treatments and prevention of the disease.

This large-scale collaborative project aims to identify genetic factors that are consistently associated with IE in the BC. We have conducted a genome-wide association meta-analysis and subsequent replication study in an independent set of cases and controls. We plan to conduct genome sequencing to identify candidate causal DNA variants underlying any regions showing reproducible association with IE; and an analysis to search for variants that cluster in specific biological pathways but that may not be detected using GWAS (i.e. common/rare in the breed).

Genome-wide association study – Axiom array

For our genome-wide association study meta-analysis we utilised three independent study sets genotyped using the Illumina canineHD array – two University of Cambridge (UC) datasets and one from the University of Helsinki. After genotyping a subset (48) of the UC sets on the higher density canine Axiom array, we utilised genotype imputation to increase the resolution of all three datasets up to this level[6].

We define cases as BC diagnosed with IE with at least a tier I level of confidence[5] (around half of dogs in the UC sets were diagnosed at a tier II level of confidence[5]). Controls are defined as BC over the age of 8 years that have been reported by owners to have never had a seizure.



The analysis included 271 Border Collies (104 cases and 167 controls) from three study sets. There were 291,450 SNPs in common between the three datasets that were available for meta-analysis. This combined analysis of IE identified 20 suggestive associations on 16 canine chromosomes using an empirical threshold of association of $P < 1 \times 10^{-4}$ (see orange line in **Figure** above). We did not find any associations reaching Bonferroni significance (green line in **Figure** above).

Genome-wide association study – WGS level

To search for additional association signals we subsequently used genotype imputation to increase the resolution of our study sets to DNA variants at whole genome sequence (WGS) level and repeated the meta-analysis. This analysis of 123 cases and 187 controls and 5,993,209 SNPs revealed nine additional suggestive association signals using an empirical statistical threshold of $P < 1 \times 10^{-5}$.

Replication study

We obtained genotype data for 27 of the SNPs in an independent replication set of BC comprising 271 IE cases and 307 controls from three study sets from the UK and Europe. Logistic regression analysis, adjusting for study, was used to analyse the association between SNPs and IE.

This analysis identified five SNPs that showed associations with IE that were directionally consistent with the discovery GWAS, although none were statistically significant after correction for multiple testing. We are currently testing their utility in a polygenic risk score.

Figure: Distribution of five-SNP risk score in cases and controls.

Acknowledgements

This study is funded by the Petplan Charitable Trust. Part of the DNA extraction costs for the replication set have been supported by a grant to SLR from the University of Cambridge Career Support Fund. SLR, CSM and CAJ are supported by the Kennel Club Charitable Trust.

References

1. Koskinen et al. Identification of a common risk haplotype for canine idiopathic epilepsy in the ADAM23 gene. BMC Genomics 2015; 16: 465.
2. Koskinen et al. ADAM23 is a common risk gene for canine idiopathic epilepsy. BMC Genetics 2017; 18: 8.
3. Belanger et al. Genome-wide association analysis of idiopathic epilepsy in the Belgian shepherd. Canine Medicine and Genetics 2020; 7:12.
4. Deschain et al. The first genome-wide association study concerning idiopathic epilepsy in Petit Basset Griffon Vendeen. Animal Genetics 2021; 52: 762-766.
5. De Risio et al. International veterinary epilepsy task force consensus proposal: diagnostic approach to epilepsy in dogs. BMC Veterinary Research 2015; 11: 148.
6. Jenkins et al. Improving the resolution of canine genome-wide association studies using genotype imputation: A study of two breeds. Animal Genetics 2021; 52: 703-713.