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The first genome-wide association study concerning idiopathic epilepsy in Petit Basset Griffon Vendeen

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Summary

The dog breed Petit Basset Griffon Vendeen has a relatively high prevalence of idiopathic epilepsy compared to other dog breeds and previous studies have suggested a genetic cause of the disease in this breed. Based on these observations, a genome-wide association study was performed to identify possible epilepsy-causing loci. The study included 30 unaffected and 23 affected dogs, genotyping of 170K SNPs, and data analysis using PLINK and EMMAX. Suggestive associations at CFA13, CFA24 and CFA35 were identified with markers close to three strong candidate genes. However, subsequent sequencing of exons of the three genes did not reveal sequence variations, which could explain development of the disease. This is, to our knowledge, the first report on loci and genes with a possible connection to idiopathic epilepsy in Petit Basset Griffon Vendeen. However, further studies are needed to conclusively identify the genetic cause of idiopathic epilepsy in this dog breed.

Keywords dog, epilepsy, genome-wide association study, Petit Basset Griffon Vendeen

Epilepsy is the most common neurological disorder in dogs. The prevalence in dogs in general has been estimated to around 0.76% (Heske *et al.* 2014). However, certain dog breeds suffer from genetic epilepsy causing a much higher prevalence (Hülsmeyer *et al.* 2015). Among these breeds is the Petit Basset Griffon Vendeen (PBGV), a dog breed originating from the Vendéen region in France and originally bred for rabbit hunting. An epidemiological study previously reported that the Danish population of PBGV suffer from idiopathic epilepsy with a high prevalence (8.9%) and a significant effect on litter prevalence indicating a strong genetic influence (Gulløv *et al.* 2011). Epilepsy in the PBGV is characterized by a relatively early onset around age 2 years dominated by focal seizures alone and focal seizures evolving into generalized seizures (Gulløv *et al.* 2011). Unfortunately, a high number of dogs experiencing seizures (13.3%) are euthanized due to reasons related to their epileptic condition.

Here we report the results of a genome-wide association study (GWAS) to identify epilepsy-associated genes in PBGV. The GWAS was followed by sequencing of putative candidate genes in the most likely associated regions.

This study was performed using 30 unaffected and 23 affected PBGV dogs identified in a cohort of PBGVs at the University Hospital for Companion Animals, University of Copenhagen (Table S1). All samples were collected and used for research with the informed consent from the dog owners and the study procedures were approved by the local ethical and administrative committee at the Department of Veterinary Clinical Sciences, University of Copenhagen.

The procedures and criteria for classification of dogs as either cases or controls are described in detail in Gulløv *et al.* (2011). In brief, an extensive anamnesis was obtained for all dogs based on the dog owners' response to an elaborate questionnaire. This was followed up by telephone interviews with dog owners and finally, clinical examinations of all dogs were performed. This included physical and neurologic examinations, hematology, and blood biochemistry. The diagnosis of epilepsy in the individual dog was based on detailed information collected on seizure history, seizure phenomenology and development, seizure duration, and other characteristics of the disorder following the diagnostic guidelines, which are recommended for humans and dogs with epilepsy (De Risio *et al.* 2015). Idiopathic epilepsy in the PBGV is known to start at an early age. The average debut is around 26 months (Gulløv *et al.* 2011). Therefore, the PBGV dogs that served as controls in the present study were >3 years old at the time of inclusion. They had not

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shown any signs of epilepsy prior to or at the time of inclusion and clinical and neurological status was normal.

DNA was isolated from EDTA stabilized blood samples from all dogs with a confirmed case/control status and SNP genotypes were established using the Illumina 170K SNP-chip. Genotype data was cleaned using the PLINK software (Purcell *et al.* 2007) with parameters --maf 0.05, --geno 0.1, --hwe 0.05, --mind 0.1. All dogs passed the quality check, and after filtering, 104 700 markers remained. A multidimensional scaling plot was created using PLINK and the options --mds-plot together with --cluster. Two GWAS analyses were performed. First, a classical analysis modelling an autosomal recessive inheritance pattern was performed using PLINK. One thousand permutations were performed using the --mperm option to set a genome wide significance threshold. Secondly, a mixed linear model association analysis was performed using the EMMAX software (Kang *et al.* 2010). The later included the genetic relationship to counter effects of hidden population structures and hereby avoid possible false positive results caused by population stratification. Manhattan plots and QQ-plots were created using qqman (Turner 2014).

The multidimensional scaling plot (Fig. 1) does not show signs of stratification in the dataset. Furthermore, the QQ-plots (Fig. 2) do not indicate any inflation of *P*-values, which could indicate false positive associations.

The PLINK analysis identified one SNP (BICF2G 630770657) on CFA35 position 6 342 532 (CanFam3) with a *P*-value of 0.03 after correction for multiple testing (Fig. 3a; Table S2). This marker is located close to the gene *NRN1* (CFA35: 6 070 379–6 078 965). The EMMAX analysis did not recognize any genome-wide significant associations. However, the markers with lowest *P*-values were located on CFA24 close to the *DOK5* gene and on CFA13 (Fig. 3b) close to the gene *FAM135b* (Table S3). Despite the lack of statistical significance for association, the three candidate genes were further examined.

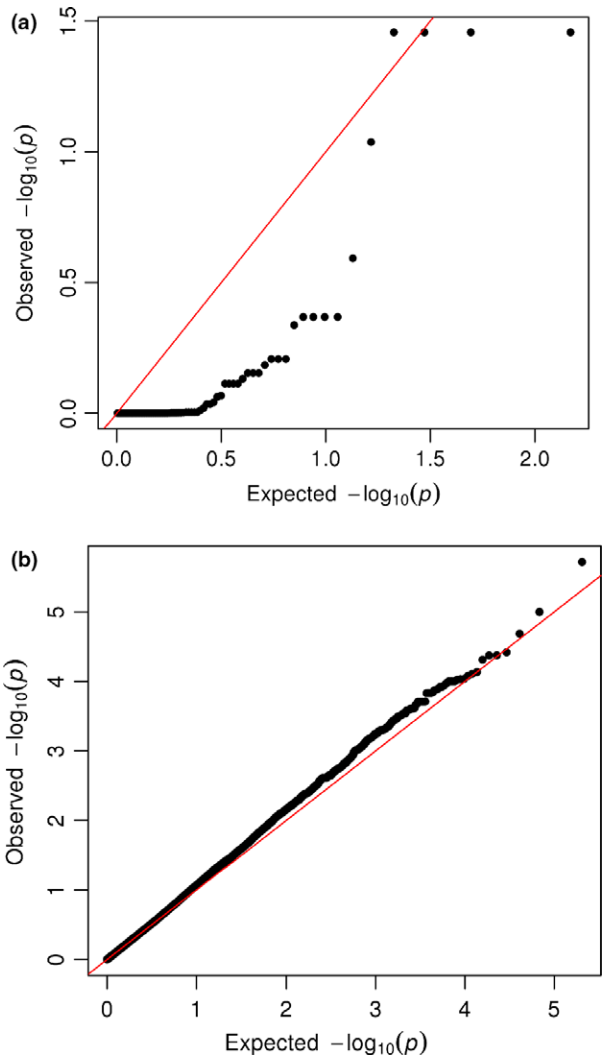

Figure 2 (a) QQ-plot for the PLINK analysis: genome-wide corrected *P*-values. (b) QQ-plot for the EMMAX analysis.

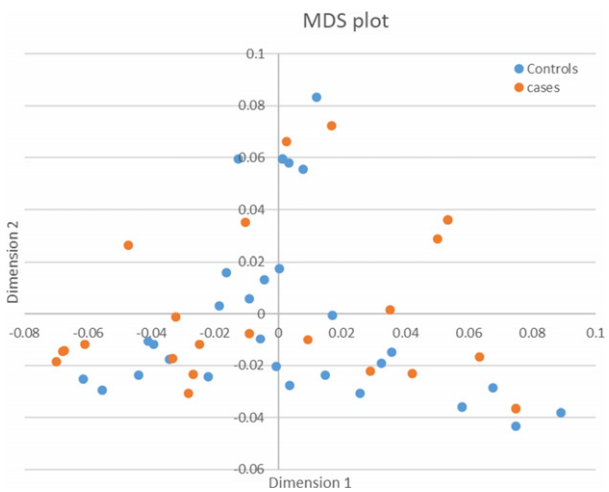

Figure 1 Multidimensional scaling (MDS) plot.

NRN1 and *DOK5* are genes that are involved in neurite outgrowth and pruning and such genes, which are involved in normal positioning of neurons and cytoarchitectual aspects of brain development may cause epilepsy (Greenberg & Pal 2007; Cowell 2014). *DOK5* (docking protein 5) encodes a cell membrane protein, which interacts with phosphorylated receptor tyrosine kinases to mediate neurite outgrowth (Shi *et al.* 2006). *NRN1* (neuritin 1) encodes a member of the neuritin family, which is expressed in differentiating neurons in the developing nervous system and in structures associated with plasticity in the adult brain. It promotes neurite outgrowth and branching and has a role in promoting neuritogenesis (Naeve *et al.* 1997). Furthermore, it has also been shown that expression of this protein has an indirect effect on neuronal excitability (Yao *et al.* 2016).

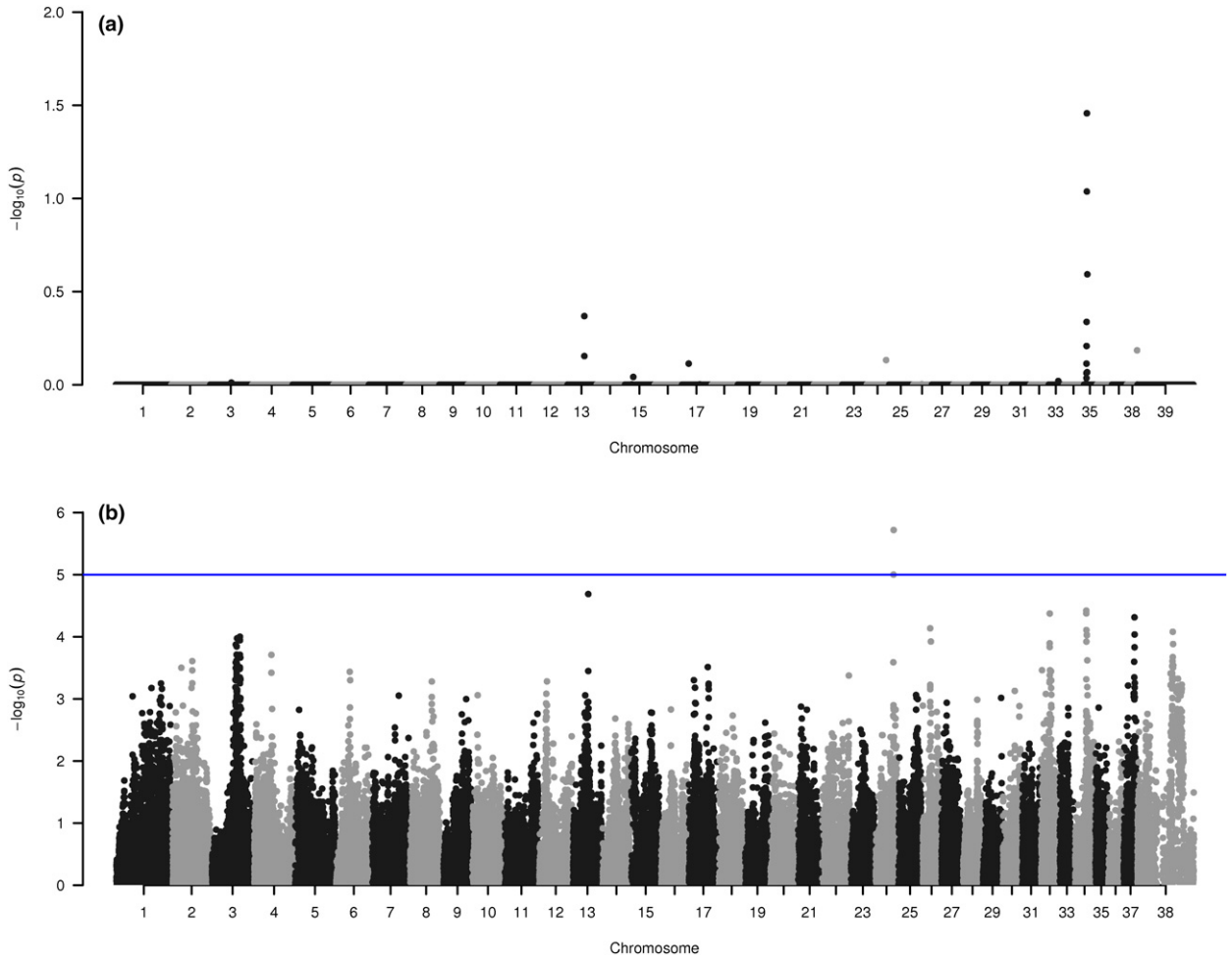

Figure 3 (a) Manhattan plot for the PLINK analysis: genome-wide corrected *P*-values. (b) Manhattan for the EMMAX analysis: point-wise *P*-values.

This is not the first time that genes with functions like *DOK5* and *NRN1* have been linked to epilepsy in dogs, humans or model organisms (Table S4). Most notably, Seppälä *et al.* (2011) identified a mutation in the *LGI2* gene causing epilepsy in the Lagotto Romagnolo dog breed. Similarly, several epilepsy causing mutations have been described in *LGI1* (which is very similar to *LGI2*) in humans, and the involvement of this gene in epilepsy has been intensely investigated in human, mouse, rat and zebra fish (Cowell 2014). *LGI1* and *LGI2* have very important functions in neurite outgrowth and pruning just like *DOK5* and *NRN1*. Hence, we consider those two genes potential candidate genes that might contain mutations, which could cause epilepsy.

The third candidate gene, *FAM135b*, qualifies as a candidate gene due to its importance for neurite integrity and survival (Sheila *et al.* 2019) and due to its interaction with *ZDHHC17* (also known as *HIP14*) and *KAT5* (also known as TIP60; Stelzl *et al.* 2005; Butland *et al.* 2014; Huttlin *et al.* 2015; Huttlin *et al.* 2017). These genes play roles in neuronal signaling and neural growth, respectively (Huang *et al.* 2004; Pirooznia *et al.* 2012).

All exons in the three candidate genes were amplified by PCR and sequenced using Sanger sequencing. The following transcript sequences were used as reference: ENSCAFT00000049974.2 (*DOK5*), ENSCAFT00000015082.4 (*NRN1*) and ENSCAFT00000001815.4 (*FAM135b*). Primers for PCR and sequencing are listed in Table S5. Two cases and two controls were used for PCR and sequencing. Sequences from cases and controls were compared with reference sequences using Seqscape® Software v. 3.0 (Life Technologies) and/or Clustal Omega (Sievers *et al.* 2011). No sequence variation was found in the coding parts of the three genes. Furthermore, all splice-donor and splice-acceptor sites were intact in both cases and controls.

In conclusion, the present study identified weak evidence for an association between idiopathic epilepsy in PBGV and loci on CFA13, CFA24 and CFA35. All three loci contain genes that can be considered good candidate genes for the phenotype, namely *DOK5*, *NRN1* and *FAM135b*. However,

the present study rules out genetic variation in the coding parts of those genes as an explanation for the epilepsy in PBGV.

Further studies should be based on a larger cohort in order to increase power of the association study. If similar collections of PBGV epilepsy cases and controls are available in other countries, a joint effort to identify the genetic causes for idiopathic epilepsy in PBGV would be of great value. Confirmation of one or more of the regions identified in the present study would prompt further analyses focused on the mentioned candidate genes and potential regulatory elements in the region(s). Pursuing this further is also of interest with respect to using the PBGV (and other breeds) with genetically driven idiopathic epilepsy in translational searching for the genetic footprint of genetic epilepsies in humans. A huge advantage of studying purebred dogs with genetic epilepsy as a translational model is the possibility of genealogical monitoring of several generations within a relatively short period. The fact that dogs have a relatively short life span and that a high number of offspring coming from the same parent combination, or combinations where offspring share one parent in each generation is an attractive genetic research platform. Another advantage is that mating in such families is controlled, meaning that the true identity of the parents is very close to 100%, which is not the case in humans.

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Conflicts of interest

The authors have no conflict of interest to declare.

Data availability statement

Raw genotype and phenotype data are available in the Dryad repository. Accession https://doi.org/10.5061/dryad.2ngf1vhnf.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 Dogs, ID-numbers, diagnosis and sex.

Table S2 Result of the PLINK analysis including genotype counts in all dogs as well as in cases and controls separately.

Table S3 Result of the EMMAX analysis including genotype counts in all dogs as well as in cases and controls separately.

Table S4 Epilepsy associated genes with a known effect on neurite growth and pruning.

Table S5 Primers for PCR and sequencing.