

The Genetics of Epilepsy in the Belgian Tervuren and Sheepdog

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Abstract

Idiopathic epilepsy is characterized by recurrent seizure activity without an identifiable underlying anatomic defect. Dogs experiencing repeated bouts of severe seizures are given therapeutic medication to control their frequency and severity. Idiopathic epilepsy has been reported in many dog breeds and was identified as the predominant health issue facing dog breeds in a recent survey by the American Kennel Club. A growing body of evidence supports a hereditary basis for idiopathic epilepsy, with a variety of genetic inheritance models proposed. In the Belgian tervuren and sheepdog, epilepsy is highly heritable with a polygenic mode of inheritance, though apparently influenced by a single autosomal recessive locus of large effect. In an effort to establish molecular linkage between the epileptic phenotype and the locus of large effect, we have screened genomic DNA from families of affected tervuren and sheepdogs with 100 widely dispersed, polymorphic canine microsatellite markers (0.595 average PIC value). Although not significant (LOD scores <3.0), three genomic regions have shown nominal linkage between markers and the epileptic phenotype. Additional related dogs are being screened with these and additional markers to increase the power to detect the presence of a linked locus.

A seizure can be defined as “a transitory disturbance of brain function” (DeLahunta 1977) reflecting hypersynchronous neuronal discharge. Recurrent seizure activity defines epilepsy (LeCouteur and Child 1989), with the epilepsy categorized by its causality. In idiopathic or primary epilepsy, there is no obvious underlying neurologic deficit causing the seizures; this is in contrast to “symptomatic” or secondary epilepsy, in which a specific initiator of the seizure is identifiable (e.g., illness, trauma, hepatic insufficiency) (Thomas 2000). Seizures have been reported in nearly all breeds of dogs. When compared with other domesticated species, the dog has the highest incidence of epilepsy, with estimates of canine epilepsy incidence ranging from 0.5% to 4.1% across all dogs (Jaggy et al. 1998; Löscher 1997). Podell and Fenner (1993) report that epilepsy accounts for 0.55% to 2.3% of all ill dogs referred to veterinary teaching hospitals. Similar to humans, idiopathic epilepsy accounts for the majority of epilepsy diagnoses in the dog (Löscher 1997). The average age of seizure onset in dogs diagnosed with idiopathic epilepsy is 1–3 years (Jaggy et al. 1998; Srenk et al. 1994; Thomas 2000).

The seizures themselves are categorized as either generalized, tonic-clonic seizures (so-called “grand mal” seizures), or focal seizures (Thomas 2000). Generalized seizure activity typifies idiopathic epilepsy in the dog. During a generalized seizure, a dog may lose consciousness and bowel or urinary control, experience spastic and/or rhythmic muscle contractions, and excessively salivate (Knowles 1994, 1998; Thomas 2000). It is considerably more rare for a dog to have a focal seizure, in which only part of the body is involved in the seizure (Jaggy et al. 1998; Thomas 2000).

Treatment of epilepsy involves a variety of medications and regimens, including daily administration of anticonvulsants, typically phenobarbital or its structural analog (Chauvet et al. 1995; LeCouteur and Child 1989). However, of dogs suffering from repeated seizures, 30% to 40% are refractory to medical intervention (Lane and Bunch 1990; Podell and Fenner 1993), and many dogs experience adverse side effects from the medications (Thomas 2000). Thus, while medication to control idiopathic epilepsy is a viable option for some dogs, prevention of the disorder is highly desirable.

A growing body of evidence supports a hereditary basis for idiopathic epilepsy in the dog (reviewed in LeCouteur and Child [1989] and Thomas [2000]) with a variety of genetic models proposed that draw primarily on human and mouse models of epilepsy. In humans, many of the characterized epileptic syndromes involve single locus models of inheritance (Greenberg et al. 1995; Zara et al. 1995); in mice, many seizure models are single locus while others are considered polygenic (Frankel 1999; Legare et al. 2000). Most canine models of epilepsy inheritance have been consistent with multilocus modes (Famula et al. 1997; Jaggy et al. 1998; Srenk et al. 1994), although a single locus model has been proposed for idiopathic epilepsy in keeshonds (Hall and Wallace 1996) and vizlas (Patterson 2001).

Determining the heritability and mode of inheritance of epilepsy is useful in three ways. First, characterization of the genetic contribution within a breed permits breeders to employ concerted selection programs designed to minimize the incidence of epilepsy should the heritability be of sufficient magnitude. Second, evidence for the presence of a single, major locus opens the possibility for the development of a molecular marker linked to the epileptic phenotype that can be used in breeding schemes. Third, it is likely that identification of the molecular determinants of canine epilepsy will shed light on the genetic mechanisms controlling human epilepsy.

Inheritance of Epilepsy in the Belgian Tervuren and Sheepdog

Belgian tervuren and sheepdog breeders worldwide have long recognized the need to determine the mode of inheritance for epilepsy in the breed (Van der Velden 1968). In tervuren and sheepdogs, the incidence of the disorder was of such a high frequency that a genetic basis was presumed, though the mode of inheritance remained elusive. A confidential survey with a 91.8% return rate provided the necessary data to assess inheritance of epilepsy in the tervuren. Data from the survey included pedigree information, birthdate, sex, and various seizure parameters for each dog. Data from tervuren older than 5 years of age were analyzed; younger animals were excluded to prevent censoring those animals that may have had their first seizure later in life. The analyses revealed an incidence of idiopathic epilepsy of 17%, no significant difference in the incidence of seizures across genders, and led to an estimate of idiopathic epilepsy heritability in this breed of 0.77 to 0.83 (Famula and Oberbauer 1998; Famula et al. 1997). This value suggests, congruent with previous reports of idiopathic epilepsy in other breeds (Schwartz-Porsche 1994; Srenk et al. 1994), that idiopathic epilepsy is a highly heritable disorder in the tervuren. The heritability estimate for Belgian sheepdogs was 0.76, reflecting the common ancestry between the Belgian tervuren and sheepdogs.

A heritability estimate in excess of 0.5 is indicative of the presence of a major gene (Morton and MacLean 1974). Although the evidence in the tervuren suggests that a single-locus model is not plausible, complex segregation analyses

were consistent with a mode of inheritance involving a single autosomal gene of large effect that influences the expression of seizures in the Belgian tervuren (Famula and Oberbauer 2000). Polygenic inheritance with a single gene of large effect is consistent with canine epileptic seizure activity in that seizure potential has been characterized as a continuum of susceptibility (Farnbach 1984). Such a continuum could represent many genes contributing to the seizure threshold with a single locus exerting great influence on the threshold of susceptibility. This would also explain how environmental factors, including physiological stressors, can potentiate seizures (Heynold et al. 1997).

The major gene appears to be inherited as an autosomal recessive locus, a finding similar to the analysis reported by Jaggy et al. (1998) for Labrador retrievers. In a tervuren, the frequency of the putative recessive allele associated with having a seizure was estimated to be 0.14 and two recessive alleles at the major locus seem to assure expression of the epileptic phenotype (Famula and Oberbauer 2000). The evidence that a single locus of large effect exists in the idiopathic epilepsy experienced by Belgian tervuren and sheepdogs indicates that a search for a genetic marker associated with that locus has a high likelihood of success (Flint and Mott 2001).

Utilizing the Emerging Canine Genome Map

Statistical evidence of a single locus of large effect in a polygenic trait has been exploited in other species to develop genetic markers to assist in the selection of particular breeding stock (Georges et al. 1995; Spelman et al. 1996). The detection of such quantitative trait loci (QTL) has also been used to identify aberrant genes in model species—for example, the mouse—that may be useful in identifying human disease gene counterparts (Flint and Mott 2001). We are implementing a similar strategy of QTL detection for the development of a genetic marker useful in identifying carriers of alleles that would predispose offspring to idiopathic epilepsy. The high estimate of heritability (0.77–0.83), coupled with the existence of appropriate family pedigrees, argues for a high likelihood of success in this endeavor.

To identify a marker linked to the deleterious allele, genomic DNA from canine families with many generations expressing the epileptic phenotype was obtained by soliciting owners to submit DNA samples from affected and unaffected tervuren and sheepdogs. Along with the DNA, owners submitted a three-generation pedigree and a questionnaire providing specific seizure information including frequency, onset, and duration of seizures. We elected to use buccal epithelial cells as a source of genomic DNA to ensure adequate participation by dog owners worldwide; three swabs were collected from each dog to guarantee useful and abundant DNA. The buccal DNA was isolated by immersing in 400 μ l 50 mM NaOH and incubating at 95°C for 15 min. Following removal of the cytology brush, the DNA was neutralized in 100 μ l 1 M Tris, pH 8.0, and then the DNA

Table 1. Characterization of all submitted Belgian tervuren and sheepdogs with usable DNA samples

Breed	Sex ^a		Age (years)			Seizure status		
	Male	Female	<3	3–5	>5	Never	Once	> Once
Tervuren	370	482	68	203	584	744	20	91
Sheepdogs	302	362	66	172	426	577	14	73
Totals	672	844	134	375	1010	1321	34	164

^a The sex was not reported for three tervuren.

from each of the three swabs was pooled and stored at -20°C until analyzed.

To date, we have collected usable DNA from 855 tervuren and 664 sheepdogs (Table 1). Of the collected dogs, excluding dogs that have seized only once, 12.4% are reported to have epilepsy. This percentage of affected dogs is equivalent across the two breeds (12.2% and 12.7%, for tervuren and sheepdogs respectively) and yet is lower than the original estimate (17%) derived from dogs bred in the late 1970s and early 1980s (Famula et al. 1997). This may reflect the increased awareness of Belgian breeders that epilepsy is an inherited disorder. Alternatively, it may reflect that the submitted data are from young dogs that have not yet seized. To avoid this sampling bias, only DNA from dogs over the age of 3 years was included in the genome scan.

Uncertainty of diagnosis and the late onset of the disorder contribute to the difficulties of studying idiopathic epilepsy. The accuracy of the data is essential for QTL analysis, and this depends upon the veracity of the diagnosis. Thus, this genome study is focused upon dogs exhibiting generalized epileptic seizures. While most dogs included in the study are diagnosed by their owners or breeders, who tend to be experienced in identifying generalized seizures, many dogs are clinically diagnosed by veterinarians. This focus of the study on generalized seizures permits more accurate diagnosis and, subsequently, more accurate data, although errors in classification are certainly possible given that diagnosis of idiopathic epilepsy is one of exclusion. In addition, dog owners update the phenotypic data of their dogs when health status changes.

Within the cohort of tervuren and sheepdog samples, we assembled DNA from one multigeneration (> three generations) family of tervuren that had a high incidence of having seizures and a second tervuren family with a relatively low incidence of seizing. The same was done for the sheepdog (Table 2). We specifically chose these families to represent the extremes of seizure expression. This genomic DNA was amplified using 100 polymorphic canine, di-, tri-, and tetra-nucleotide repeat-based markers (Table 3) selected from the 1999 canine genetic linkage map (Neff et al. 1999). These markers span the genome with approximately three markers per chromosome and an average coverage of 22 cM. The particular markers were chosen due to their reported polymorphism across breeds and their ability to be combined into multiplexed PCR reactions, which allows for genotyping with fewer amplification reactions. The 100 markers were multiplexed into 15 reactions and the specific amplification conditions were as reported (available at http://www.vgl.ucdavis.edu/research/canine/paper_data/index.jsp).

Aliquots of the polymerase chain reactions (PCRs) were mixed with fluorescent ladder or internal standard and loading buffer (Promega 60–400 fluorescent ladder CRX or Internal Lane Standard 600, Madison, WI) and run on a 6% denaturing acrylamide gel in an ABI 377 automated sequencer (Foster City, CA) in 1X TBE buffer (90 mM Tris, 90 mM boric acid, 2 mM EDTA, pH 8.4) at 1.1 kV and 60 mA current. DNA fragments were analyzed by the STRand genotyping software (designed by the Veterinary Genetics Laboratory at the University of California, Davis,

Table 2. Description of the tervuren and sheepdog families with low and high seizure incidence selected for the genome scan

	Sex		Age (years)		Seizure status		
	Male	Female	3–5	>5	Never	Once	> Once
Tervuren families							
High incidence	19	27	3	43	31	3	12
Low incidence	13	33	7	39	43	1	2
Sheepdog families							
High incidence	31	15	13	33	38	1	7
Low incidence	13	33	6	40	45	0	1
Additional family ^a							
High incidence	17	29	14	32	31	1	14
Totals	93	137	43	187	188	6	36

^a This family is composed of both tervuren and sheepdogs due to the recessive nature of the tervuren phenotype.

Table 3. 100 microsatellite markers, chromosomal location, type of marker, and polymorphic information content for Belgian terverein and sheepdogs as used in the genome scanning study to identify linkage with epilepsy

Marker	Location	Type	PIC
FH2199	31	tetra	0.9047
FH2138	8	tetra	0.8836
FH2165	33	tetra	0.8631
FH2233	21	tetra	0.862
FH2200	12	tetra	0.8423
FH2293	10	tetra	0.8081
FH2247		tetra	0.8068
FH2137	3	tetra	0.7949
PEZ18	27	tetra	0.7888
FH2175	16	di	0.7867
FH2201	7	di	0.7797
PEZ13	4	tetra	0.7719
FH2361	33	tetra	0.7695
FH2313	1	tetra	0.7604
AHT103	4	di	0.7599
C22.279	22	di	0.7552
FH2305	30	tetra	0.7457
PEZ10	14	tetra	0.745
C13.758	13	di	0.7387
FH2326	1	tetra	0.7378
CXX263		di	0.7375
C28.176	28	di	0.7358
CPH14	5	di	0.7311
FH2001	23	tetra	0.7284
FH2202	12	tetra	0.7281
PEZ08	17	tetra	0.7183
FH2130	26	di	0.7181
D-INRA2	29	di	0.7129
PEZ03	19	tri	0.7047
C17.402	17	di	0.6996
AHT137	11	di	0.6977
C05.771	5	di	0.697
PEZ22	7	tetra	0.697
PEZ02	7	tetra	0.6952
CPH08	19	di	0.6944
FH2054	12	tetra	0.6882
FH2004	11	tetra	0.6815
FH2140	5	tetra	0.6689
Wilms-TF	18	tetra	0.6617
FH2289	27	tetra	0.6604
FH2164	6	tetra	0.6599
C14.866	14	di	0.6588
C08.618	8	di	0.6582
FH2161	21	tetra	0.6573
C09.173	9	di	0.6531
AHT111	2	di	0.65
PEZ11	8	tetra	0.6458
C11.750	11	di	0.6411
FH2356	18	tetra	0.6411
FH2324	25	tetra	0.6388
D-INRA21	21	di	0.6362
LEI004	37	di	0.6323
FH2283	23	tetra	0.63
C31.646	31	di	0.6294
AHTk292	18	di	0.6255
VIASD10	7	tetra	0.6073
FH2079	24	tetra	0.6036
C09.250	9	di	0.596
RVC1	15	di	0.5939
AHT132	2	di	0.5907

Marker	Location	Type	PIC
PEZ05	12	tetra	0.5849
C01.424	1	di	0.582
LEI007		di	0.5744
AHT121	13	di	0.5677
C10.404	10	di	0.5616
AHTk253	23	di	0.5607
PEZ12	3	tetra	0.5561
FH2148	20	tetra	0.5551
C20.446	20	di	0.5529
AHT139	15	di	0.5485
C15.608	15	di	0.5482
AHT133	37	di	0.5478
C25.213	25	di	0.5258
C04.140	4	di	0.521
LEI002	27	di	0.5131
C29.002	29	uni	0.5045
CPH16	20	di	0.4697
AHTk211	26	di	0.4672
LEI003		di	0.4612
C27.671	27	di	0.4599
C22.763	22	di	0.4598
C06.636	6	di	0.4297
AHT130	18	di	0.4273
C13.365	13	di	0.4232
FH2328	29	tetra	0.4202
C08.410	8	di	0.4194
LEI005	22	di	0.4102
C13.391	13	di	0.3913
CPH03	6	di	0.3884
C07.620	7	di	0.3827
FH2274	2	tetra	0.3734
CPH02	32	di	0.3576
CXX161		di	0.3408
C23.123	23	di	0.2533
FH2145	3	tetra	0.2477
C03.877	3	di	0.1578
C20.253	20	di	0.1526
LEI006		di	0.1102
C16.147	16	di	0.0591
AHT136	11	di	0.0522

Type of marker may be di-, tri-, or tetranucleotide repeat; markers are listed in descending PIC values. PIC = polymorphic information content.

CA, and available at www.vgl.ucdavis.edu/STRand). Genotypes for each marker were assigned to each animal.

The polymorphic information content (PIC) values within these dogs for the markers ranged from a high of 0.905 (representing 17 distinct alleles) to a low of 0.05, with an average PIC value of 0.595. Eighty-eight percent of the markers had PIC values in excess of ~0.400. Thus, using a minimum value of 0.4 as indicating informativeness, 88 markers were informative and thereby useful for mapping within the terverein and sheepdogs. Assessment of the degree of informativeness of the microsatellite makers is particularly critical to evaluate given the lack of heterozygosity within families of purebred dogs.

The data were initially analyzed by the SIBPAL function of Statistical Analysis for Genetic Epidemiology (S.A.G.E., 1997), a sib-pair linkage computational program. The results

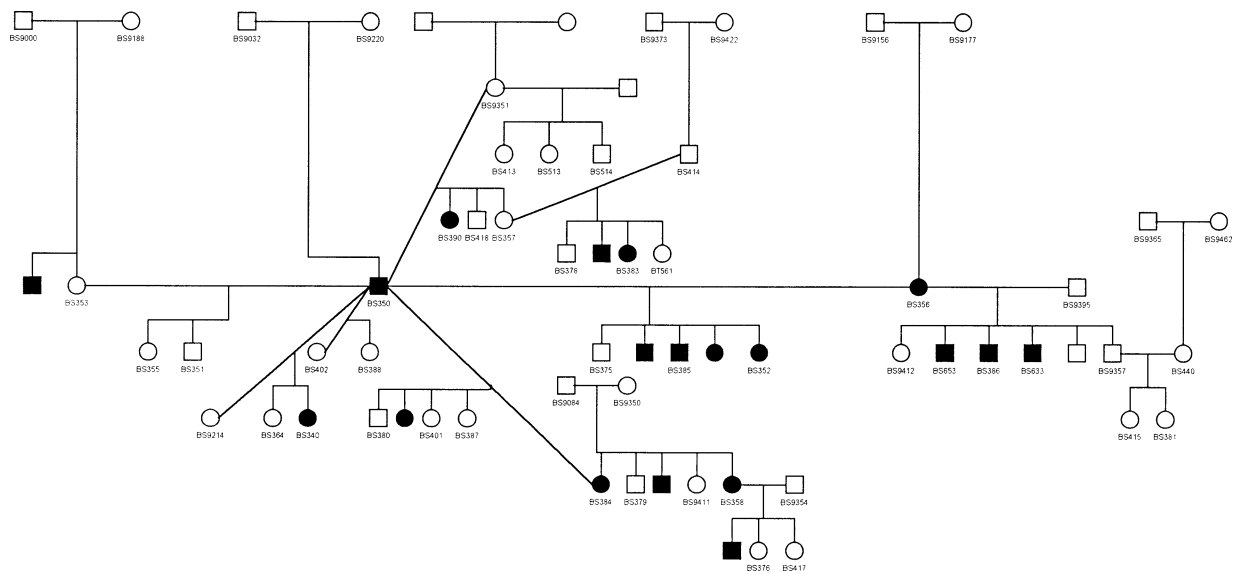


Figure 1. Pedigree of a representative family used in genome scanning studies. Filled squares and round symbols are diagnosed epileptic male and female dogs, respectively. The epileptic status of dogs in the top row is unknown.

from these initial linkage analyses suggested significant linkage between the seizing phenotype and a few microsatellite markers ($P < .01$). However, upon closer examination of the results, some significant markers were of low PIC value, while others were not determined to be significant in both the tervuren and sheepdogs. Because of the heritage of these dogs, it is expected that the linked markers should be the same in the two breeds. An additional weakness to the sib-pair analysis was that within the tervuren family data, there were only three affected/affected pairs and 15 affected/unaffected pairs. A similar proportion was seen for the sheepdogs: two affected/affected pairs and 12 affected/unaffected pairs.

Though the sib-pair analysis is relatively simple to interpret, sib-pair analysis lacks the capability to incorporate complex relational pedigrees prevalent in dog breeding schemes. To accommodate these pedigrees, we turned to a more computationally robust strategy (Almasy and Blangero 1998) made possible through sequential oligogenic linkage analysis routines (SOLAR), the public domain package for linkage analysis. In addition to the capacity of SOLAR for large, extensive pedigrees, SOLAR also has the capability to analyze quantitative traits, characterizing the threshold theory of epilepsy expression in the dog (Cunningham and Farnbach 1988), along with gene-environmental interaction, multivariate traits, and epistasis. This program relies upon variance component methodologies to estimate identity by descent (IBD) values. Hsueh et al. (2001) empirically evaluated the above computational approach and found that the greater the effect of the QTL on the phenotypic variation, the more precise the QTL localization was in terms of identifying the gene's chromosomal location—that is, identified linkage peaks would fall within 10 cM of the true gene location. The significant effect of the

major locus for epilepsy in the tervuren and sheepdogs lends itself to this computational linkage approach.

The number of dogs screened with the microsatellite multiplexed panels was also increased by the addition of another family of highly affected dogs (Table 2), bringing the total number screened to 230. Of those dogs, 104 tervuren and 126 sheepdogs with 36 probands were defined as those dogs having more than one seizure (Figure 1 is a representative pedigree of an affected family). Two-point LOD scores were developed for each of the markers. In human studies, LOD scores in excess of 3.0 are considered “significant” for linkage (Shete and Amos 2002). While the majority of LOD scores for the markers in the present study were unremarkable using this criterion, three promising genomic regions have been suggested by LOD scores that do not surpass the 3.0 threshold but are in excess of 1.0. For example, one marker with a LOD score of 2.674 is adjacent to a nearby marker of LOD score 1.871. Two other genomic regions also bear further investigation with nominal evidence of linkage (LOD scores of 1.179 and 1.025). However, to avoid reporting misleading results, the lack of statistically significant association precludes any linkage conclusions at this time, although the moderate LOD scores support the hypothesis that linkage between epilepsy and genetic markers does exist for the tervuren and sheepdog. Identification of three regions with possible linkage to the seizing phenotype is either congruent with the polygenic nature of canine epilepsy or represents a statistical artifact; screening of additional dogs with markers targeted to these regions will resolve these possibilities.

Future Directions

We will continue to utilize the multiplexed microsatellite markers to screen additional tervuren and sheepdogs to

increase to 500 our total number of dogs screened. The rationale for doing this is to increase our ability to detect linkage and improve the LOD scores for markers that are currently identified as potentially linked to the epileptic disorder. Chase et al. (1999) report an increase in power to detect loci linkage by increasing the size of the families evaluated rather than increasing the number of markers screened. Recent evidence suggests that credible QTL detection is possible by evaluating only several hundred animals if the QTL has sufficient magnitude in its effect (Cardon and Bell 2001; Risch 2000). In addition, we are expanding the number of markers evaluated to ensure adequate coverage of the genome. As noted in Table 3, some chromosomal regions are not represented by the panel we have employed, and some of the markers were not informative. Selected markers from the minimal screening set of 172 canine microsatellite markers (Richman et al. 2001) will be added to our analyses to maximize coverage of the genome.

The potential chromosomal linkages indicated by the original screening are also being refined by targeting the chromosomal regions with additional markers and candidate genes derived from human chromosomal synteny. Should these linkages prove significant to particular markers as indicated by high LOD scores, our plan is to screen a canine bacterial artificial chromosome (BAC) library for clones containing these markers and sequence the BAC ends to generate single nucleotide polymorphisms (SNPs). The dogs will then be genotyped for these SNPs to target the region(s) with a dense set of markers. By greatly refining the linkage, the chromosomal regions needing to be evaluated for their involvement in regulating the expression of epilepsy in the terrier and sheepdog will be narrowed. The ultimate objective is to identify the mutation of the major locus that predisposes the dog to epilepsy.

Identification of a genetic marker in one breed may prove useful in other breeds afflicted with epilepsy. In addition, the similarities between human and dog seizure disorders (Löscher 1997) lend support to the concept that the identification of genomic regions involved in regulating epilepsy in one species will aid in determining genetic regulation in the other. With highly controlled and extensive dog breeding programs, it is probable that the advances may come from the canine mapping efforts first.

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