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SHORT COMMUNICATION

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Linkage confirms canine *pkd1* orthologue as a candidate for bull terrier polycystic kidney disease

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Summary

Bull terrier polycystic kidney disease (BTPKD) is a Mendelian disorder with many features reminiscent of human autosomal dominant polycystic disease, the latter disease being due to mutations at *PKD1* and *PKD2* loci. We investigated the role of the canine *pkd1* orthologue in BTPKD via linkage analysis of a large kindred in which the disorder is segregating. Twelve microsatellite markers around the canine *pkd1* locus (CFA6) were amplified from the genomic DNA of 20 affected and 16 unaffected bull terriers. An additional 28 affected dogs were genotyped at five key microsatellites. A highly significant multi-point LOD score that peaked over the canine *pkd1* locus was observed (LOD = 6.59, best two-point LOD score LOD = 6.02), implicating this as the BTPKD locus.

Keywords dog, linkage analysis, *PKD1*, polycystic kidney disease.

Bull terrier polycystic kidney disease (BTPKD) is an autosomal dominant disease characterized by bilateral renal cortical and medullary cysts and interstitial inflammation and fibrosis (O'Leary *et al.* 1999). BTPKD is clinically and pathologically similar to autosomal dominant polycystic kidney disease (ADPKD) in humans (Dalgaard 1957; Hossack *et al.* 1988). For ADPKD in humans, 85–90% of cases are caused by mutations in *PKD1*, with most of the remaining cases caused by mutations in *PKD2* (Peters & Sandkuijl 1992). Inherited diseases in dogs that are similar to those in humans are likely to be caused by mutations in orthologous genes (Mellersh *et al.* 2000), making these loci candidates in BTPKD.

Microsatellite linkage has excluded the canine *pkd2* locus from involvement in BTPKD (O'Leary *et al.* 2006). While no disease-associated mutations were found in the canine *pkd1* orthologue when mRNA from affected dogs was sequenced (O'Leary *et al.* 2003), these findings do not entirely exclude a *pkd1* mutation from being associated with BTPKD. Mutations which lead to cryptic splicing, a deletion in a part of *pkd1*, and mutations involving chromosomal duplications and rearrangements may be disease-associated and not have been detected. Mutations may also be undetected if they are present in alternate, rare, aberrantly spliced, unstable, or rapidly degraded transcripts, or in an intragenic non-coding sequence such as 5′ and 3′ untranslated regions, non-exonic promoters, enhancers and other regulatory sequences. Silent sequence variants may also cause disease by affecting gene expression via influence on transcription, processing or translation (Strachan & Read 1996). Thus, the *pkd1* canine locus has not been entirely excluded as being disease-associated in BTPKD.

Indeed for ADPKD, the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac) records 435 mutations in *PKD1*; in non-coding regions there are 35 splicing mutations, three small deletions, eight gross deletions and six likely rare polymorphisms in the 3′ UTR. The ADPKD mutation database http://pkdb.mayo.edu/ reports 314 likely pathogenic mutations in *PKD1* of which 35 are splicing mutations in intronic regions.

The canine *pkd1* orthologue is localized approximately 41.9 Mbp from the telomere on the p arm of canine chromosome CFA06 (Jonasdottir *et al.* 2000). Here, a genetic linkage study has been performed to definitively exclude or confirm the canine *pkd1* locus as a candidate locus for BTPKD.

Bull terrier polycystic kidney disease is diagnosed routinely (O'Leary *et al.* 1999). The age of onset of BTPKD is not definitively known, although puppies 8 weeks of age have been diagnosed using renal ultrasonography. No known cases of late onset disease or new disease occurring after initial examination are currently recorded. Animals in this

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study varied between 8 weeks and 14 years. All animals with BTPKD in this study were descended from one affected dog. Initially, gDNA from 20 bull terriers with BTPKD and 16 bull terriers without this disease was extracted from peripheral blood collected in EDTA using a salting-out extraction method (Miller *et al.* 1988). Subsequently, a further 28 DNA samples from animals with BTPKD were also extracted [three of these 28 dogs had their *pkd1* mRNA sequenced in a previous study (O'Leary *et al.* 2003)].

Eleven microsatellite markers (Table 1) were identified in the region of the canine *pkd1* orthologue on canine chromosome CFA06 (Jonasdottir *et al.* 2000; http://research.nhgri.nih.gov/dog_genome/). One microsatellite, *PKD1mic1* (GenBank accession no. NC_006588.2), a $(GAAA)_{44}$ repeat, was identified using RepeatMasker (http://www.repeatmasker.org/). The forward primer was CCCCAGAACCTTCTCCA and the reverse primer was GGTCCCGGCTACTAATG. Primers were designed using PRIMER3 (http://frodo.wi.mit.edu/primer3/input.htm). Twelve markers were optimized in simplex PCR and 11 were then amplified in multiplex PCR. M13 tailing (Oetting *et al.* 1995; O'Leary *et al.* 2006) was used to fluorescently label the PCR product for genotyping. *PKD1mic1* was only amplified in singleplex and was amplified in 64 dogs. All 11 markers were amplified in the initial 36 dogs, with markers *FH3445*, *FH3282*, *PKDmic1*, *FH3246* and *FH2370* subsequently amplified in an additional 28 affected dogs (amplification conditions available upon request).

Both simplex and multiplex PCR products were diluted with 200 μl MQ H_2O, with 1 μl of diluted PCR product transferred to 96-well PCR plate. Ten microlitres of diluted Rox-Labeled MegaBACE ET 900-R Size Standard (GE Healthcare Biosciences) was also added to the well. The size standard was diluted by adding 1.5 μl of size standard to 1000 μl of Tween 20 (Aldrich Chemical Co.). The PCR product and size standard were denatured at 95 °C for 1 min, and immediately placed on ice to prevent renaturation. Fragment separation was carried out on an ABI Prism 3700 DNA Analyzer (ABI Biosystems) according to the manufacturer's recommendation. Data analysis was carried out using GENOTYPER 3.7 software (ABI Biosystems).

Microsatellite markers from panels 1 and 2, recommended by International Society of Animal genetics (ISAG) for parentage verification, were used in 10 animals (http://www.isag.org.uk/).

Preliminary pedigree analyses were carried out using the SIB-PAIR program (Duffy 2005), including testing for Mendelian errors. A segregation analysis confirming the clinical impression of a dominant pattern of inheritance was carried out using PAP version 5.0 (Hasstedt 2002). The RELATIVE program (Goring & Ott 1997) was used to confirm the recorded pedigree information for first degree relationships from the marker data.

CRI-MAP (Washington University) was used to perform two-point and multi-point linkage analyses under a fully penetrant dominant model. We also compared the two-point results for the peak marker to those obtained using the SUPERLINK package, obtaining the same results. The frequency of the disease gene used in the LOD score calculation was 0.001. The markers were ordered using positions in the canine sequence, and genetic map positions interpolated from the map of Breen *et al.* (2001), and confirmed using SIMWALK2 (QIMR). The inbreeding coefficient was calculated (Lynch & Walsh 1998).

After including all connecting (unphenotyped) individuals and removing uninformative branches, the analysed

Table 1 Microsatellite markers from the region of the canine *pkd1* orthologue used in the current linkage analysis study.

Marker	No. of alleles	Multiplex	Label	No. of repeats[1]	Allele size range	Expected heterozygosity	Position	
							Mbp	cM
FH3933	3	MP 1	Vic	Tetra	492–500	0.61	24.792	24
FH2119	3	MP 1	Vic	Tetra	208–322	0.34	49.202	35
FH3246	6	MP 1	Ned	(Tetra)	335–351	0.4	50.315	37
FH2370	4	MP 1	6-Fam	Tetra	392–426	0.55	58.367	42
REN149M14	2	MP 2	Ned	(Di)	376–378	0.26	31.316	25
AHTh171[2]	2	ISAG	Vic	(Di)	125–141	0.19	10.203	10
FH2734	2	MP 2	Vic	Di	228–230	0.22	19.945	20
LEI.2A11	3	MP 2	Ned	(Di)	168–198	0.06	41.282	30
FH3282	5	MP 3	Ned	(Tetra)	344–374	0.36	43.596	34
REN54C11	2	MP 3	Vic	(Di)	179–183	0.08	39.797	27
FH3445	5	MP 3	6-Fam	(Tetra)	352–386	0.49	50.507	39
FH2164	3	MP 3	Vic	Tetra	300–342	0.25	37.992	26
PKD1mic1	7	SP	Vic	Tetra	425–463	0.70	41.385	31

[1]Brackets surround microsatellite repeat polymorphisms that were not reported at http://research.nhgri.nih.gov/dog_genome/ but identified bioinformatically by searching the dog genome database at http://www.ncbi.nlm.nih.gov/genome//guide/dog/ for microsatellite primer sequences using the BLASTN program.

[2]Microsatellite from ISAG parentage panels genotyped in 10 dogs.

pedigree contained 402 animals. The mean inbreeding coefficient for 55 affected animals was 0.071, and 0.106 for 20 affected genotyped animals.

The animals that were tested using the ISAG panels revealed the presence of two likely mutations in microsatellite *FH3933*. The observed intermarker recombination was consistent with the chosen genetic marker map. Multipoint linkage analysis of the markers using the sequence order using CRI-MAP was consistent with our working map (observed map *AHTh171*-12.2-*FH2734*-12.2-*REN149M14*-0.0-*FH3282*-0.1-*FH2119*-0.1-*FH3246*-8.2-*FH3445*-7.4-*FH2370*).

None of the microsatellite markers exhibited Mendelian inconsistencies that were not resolvable on regenotyping; however, three animals were flagged by the SIMWALK2 mistyping algorithm as possibly being mistyped. These genotypes were deleted for these three loci. This did not change the evidence for linkage.

In preliminary parametric two-point linkage analysis under the dominant model, suggestive results were obtained for the microsatellite marker we have denoted *PKD1mic1*, 500 kbp distal to the canine orthologue of *pkd1*. This was also one of the more polymorphic markers in our panel, so therefore we genotyped additional affected animals at this

Table 2 Two-point linkage analysis of BTPKD, modelled as a fully penetrant autosomal dominant gene, vs. microsatellite marker loci on canine chromosome 6 using the CRI-MAP linkage analysis program. All 11 markers were amplified in the initial 36 dogs, with markers *FH3445*, *FH3282*, *PKDmic1*, *FH3246* and *FH2370* subsequently amplified in an additional 28 affected dogs.

	Recombination fraction with trait locus										
Marker	0.001	0.01	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45
AHTh171[1]	0.60	0.59	0.54	0.47	0.39	0.32	0.24	0.17	0.10	0.05	0.01
FH2734	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FH3933	0.30	0.29	0.26	0.21	0.17	0.13	0.10	0.06	0.04	0.02	0.00
REN149M14	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
FH2164	0.60	0.59	0.54	0.47	0.39	0.32	0.24	0.17	0.10	0.05	0.01
REN54C11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
LEI.2A11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PKD1mic1	6.01	5.89	5.38	4.74	4.08	3.43	2.80	2.17	1.55	0.94	0.39
FH3282	1.50	1.47	1.33	1.16	0.98	0.81	0.65	0.49	0.34	0.21	0.10
FH2119	0.60	0.59	0.54	0.47	0.39	0.32	0.24	0.17	0.10	0.05	0.01
FH3246	1.20	1.18	1.07	0.93	0.79	0.64	0.49	0.34	0.21	0.10	0.03
FH3445	−0.12	0.82	1.28	1.28	1.17	1.01	0.84	0.66	0.47	0.30	0.14
FH2370	−3.29	−1.33	−0.09	0.30	0.44	0.47	0.43	0.35	0.24	0.13	0.04

[1]Microsatellite from ISAG parentage panels genotyped in 10 dogs.

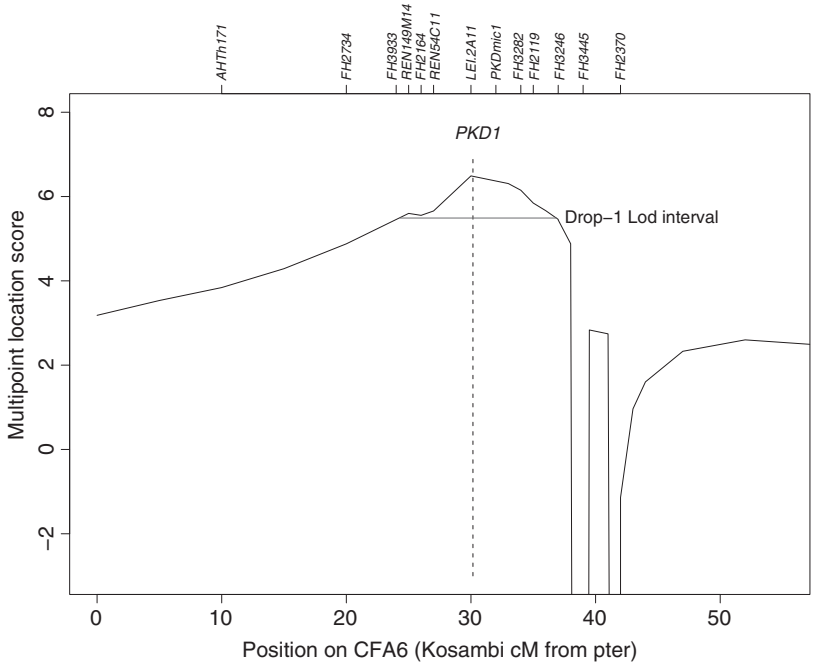

Figure 1 Results of multi-point linkage analysis of BTPKD under a fully penetrant autosomal dominant model vs. codominant markers on canine chromosome 6 using CRI-MAP. The vertical line marks the genomic location of the canine homologue of human *PKD1*, and the horizontal segment the 'drop-1-lod' location interval around the peak lod score. All eleven markers were amplified in the initial 36 dogs, with markers *FH3445*, *FH3282*, *PKDmic1*, *FH3246* and *FH2370* subsequently amplified in an additional 28 affected dogs.

marker. The final two-point results are shown in Table 2, where the peak observed LOD score was 6.02 at a recombination distance of zero for *PKD1mic1*. In the multi-point analysis under the dominant model, a multi-point location (LOD) score of 6.59 was obtained at the position of *pkd1* (Fig. 1), midway between *LEI.2A11* and *FH2734*. The 'drop-1-lod' confidence interval for the detected locus spanned from *FH3933* to near *FH3246*.

This study shows that the canine *pkd1* locus is a strong candidate gene for BTPKD. This should allow development of a genetic diagnostic test for this disease and further studies may confirm BTPKD as a new animal model of human ADPKD.

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