Canine tricuspid valve malformation, a model of human Ebstein anomaly, maps to dog chromosome 9

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Background: Ebstein anomaly of the tricuspid valve is a congenital cardiac malformation characterised by downward displacement of the attachment of the septal and posterior leaflets of the tricuspid valve. Canine tricuspid valve malformation (CTVM) is morphologically similar to Ebstein anomaly; familial occurrence of CTVM has been described. Several observations suggest a genetic cause but most cases appear to be sporadic.

Methods: Three purebred Labrador Retriever kindreds enriched for CTVM underwent clinical examination and echocardiography. DNA was extracted from whole blood. Genotyping was carried out using polymorphic repeat markers with an average spacing of 15 cM and polymorphic information content of 0.74.

Results: Pedigree analysis identified CTVM segregating as an autosomal dominant trait with reduced penetrance. Genome wide linkage analysis in one kindred identified a CTVM susceptibility locus on dog chromosome 9 (CFA9) with a maximum multipoint lod score of 3.33. The two additional kindreds showed a conserved disease haplotype.

Conclusions: This study identifies a CTVM susceptibility locus on CFA9 and a founder effect in apparently unrelated Labrador Retriever kindreds. These results provide the basis for a positional candidate cloning effort to identify the CTVM disease gene. Identification of the CTVM gene will permit mutation screening of patients with Ebstein anomaly, which should provide additional insights into the genetic programmes of valve development.

Ebstein anomaly of the tricuspid valve (MIM 224700) is a congenital cardiac malformation characterised by downward displacement of the attachment of the septal and posterior leaflets of the tricuspid valve. Tricuspid valve leaflets are dysplastic and the septal leaflet adheres to the interventricular septum. The papillary apparatus and chordal attachments to the valve leaflets are frequently abnormal in Ebstein anomaly and contribute to tricuspid regurgitation. The portion of the right ventricle between the anatomical valve annulus and the functional annulus created by the downwardly displaced leaflets is termed atrialised right ventricle. Associated cardiac anomalies are common.

The equivalent of Ebstein anomaly in dogs, canine tricuspid valve malformation (CTVM), is characterised by similar hallmarks: tricuspid valve leaflets are thickened and dysplastic with adherence of the septal leaflet to the interventricular septum. The papillary muscles may be fused and display shortened or absent chordae tendineae. Apical displacement of the valve apparatus is a characteristic feature. As in the human, associated intracardiac malformations have been observed. The clinical spectrum may vary from overt signs of heart failure to complete absence of symptoms; this variation is presumably dictated by the degree of anatomical deformity and the ensuing valve insufficiency.

Little is known about the pathogenesis of tricuspid valve malformation in humans or dogs. CTVM has been noted in several breeds of dogs, most commonly in Labrador Retrievers. As in Ebstein anomaly, most cases of CTVM seem to be sporadic. Both CTVM and Ebstein anomaly have been recognised in the context of familial clustering of congenital heart disease. Twin pregnancies, a family history of cardiovascular malformations, and previous miscarriages have been proposed as genetic risk factors, but the precise genetic mechanisms remain undefined. Further evidence for heterogeneous genetic factors in Ebstein anomaly comes from cytogenetic abnormalities as well as mutation of the transcription factor NKX2.5 in some cases. Taken together, these findings suggest that genetic factors may play an important role in both CTVM and Ebstein anomaly.

METHODS

Recruitment and clinical evaluation

From an index case in an unselected referral population, we identified a large kindred (CTVM1) of purebred Labrador Retrievers and sequentially evaluated first and second degree relatives. Clinical examination was carried out in accordance with institutional guidelines by board certified veterinary cardiologists (KW, LS) and consisted of clinical evaluation and a complete echocardiographic study. Genealogical records were obtained for all recruited dogs with a minimum of four successive generations, in order to search for common ancestors. CTVM1 is a single large kindred of 21 dogs originating from a geographically confined area within Ohio, Kentucky, Indiana, and Michigan (fig 1A). We hypothesised that CTVM in this kindred is a single gene disorder amenable to a genome wide linkage study. A 20 ml sample of whole blood was obtained from each participant and aliquoted for subsequent DNA extraction.

Echocardiographic assessment of tricuspid valve morphology

Definitive assignment of affected status was made according to 2D echocardiogram criteria: thickening of the valve leaflets, redundancy of the parietal leaflet, apical displacement of the basal attachment of the valve leaflets (particularly the septal leaflet), abnormal adherence of the septal leaflet to the interventricular septum, and presence of a large, fused right ventricular papillary muscle rather than normal, small, discrete muscles. For morphometric analysis of the tricuspid valve apparatus, the offset between the mitral valve annulus and functional tricuspid valve annulus was measured in accordance with the approach used in humans.
Genotyping and linkage analysis

Genotyping with polymorphic markers was carried out as described elsewhere using the canine minimal screening set, which contains 172 markers with an average spacing of 15 cM and polymorphic information content of 0.77. Additional markers for fine mapping not included in the minimal screening set were identified using the most recent version of the integrated dog map and purchased from Research Genetics (Huntsville, AL) or custom synthesised (Integrated DNA Technologies, Coralville, IA). Alleles were scored by two independent observers. Two point linkage analysis was performed using MLINK 5.1. Multipoint lod scores were calculated with the LINKMAP program implemented in FASTLINK software version 4.1. For all markers, allele frequencies were determined from kindred members. The phenocopy rate was estimated at 0.001, gene frequency at 0.001 with no sex difference, and penetrance at 70%. The Haldane map function was used to convert recombination fractions into genetic distances. Mating loops were broken at dogs with the least ambiguity in genotype (dogs II.4 and III.10 of CTVM1).

RESULTS

Recruitment and clinical evaluation

The two diagnostic criteria of downward displacement of the attachment of the tricuspid valve leaflets and adherence of the septal leaflet to the interventricular septum were fulfilled in all 11 affected dogs in the CTVM1 kindred (fig 2). Varying degrees of tricuspid regurgitation were observed in all affected dogs. Leaflet dysplasia, parietal leaflet redundancy, and papillary muscle abnormalities were variable. The offset of the functional tricuspid valve versus the mitral valve annulus was

Figure 1  Pedigree structures with the highlighted disease haplotype. (A) Kindred CTVM1. Please note, dog I.2 has been bred in both generation I (mating with dog I.3) and generation III (mating with dog III.8), and dog III.1 is identical to dog II.5 in kindred CTVM2. (B) Kindred CTVM2. (C) Kindred CTVM3. (D) Summary of genotyped markers, disease associated alleles, and map distances in cR.
significantly different between dogs with and without valve dysplasia (13.04 (SD 6.57) mm vs 6.14 (SD 0.84) mm, p<0.05). No other intracardiac defects were observed.

Genetic analysis
Pedigree evaluation of CTVM1 indicated that the disease segregates as an autosomal dominant trait with reduced penetrance (fig 1A). Two loops of inbreeding were identified: (1) the mating between dogs III.8 and I.2 and (2) the mating between dogs III.9 and III.10. The diagnosis of CTVM in dog IV.1 suggested incomplete penetrance, making dog II.2 an obligate mutation carrier.

In kindred CTVM1, a total of 140 tested polymorphic markers excluded ~60% of the genome before a suggestive linkage to dog chromosome 9 (CFA9) was detected at REN75M10 with a two point lod score of 1.56. Subsequently, fine mapping with adjacent markers showed that the “1-1-2-2-3-2-2” haplotype for the markers C03304 to REN126A15 segregates with disease (fig 1A). Lod score analysis for the combined disease haplotype yielded a maximum two point lod score of 3.02 at θ=0, and a maximum multipoint lod score for the three most informative markers within the critical interval of 3.33 at REN75M10, indicating odds of greater than 2000:1 that CTVM is linked to a locus on CFA9. The disease haplotype defines the centromeric (marker C03304) and telomeric (marker REN126A15) boundaries of the critical region with a size of 11 cM.

Evidence for a founder effect
We identified two smaller kindreds (fig 1B, CTVM2 and fig 1C, CTVM3). Members of the CTVM3 kindred are from a geographically distinct region (California) with three affected offspring; CTVM2 dogs were recruited in Ohio. All affected dogs in kindreds CTVM2 and CTVM3 also fulfilled the diagnostic criteria for CTVM. None of the dogs in the series reported here showed any other intra- or extracardiac malformation. Dog III.1 of kindred CTVM1 was also bred in kindred CTVM2, appearing there as dog II.5 (fig 1A, B).

In kindred CTVM2, the disease haplotype was conserved for five out of seven alleles associated with disease in kindred CTVM1. Kindred CTVM3 also showed conservation of the disease haplotype for six out of seven alleles linked to disease in kindred CTVM1. These findings in CTVM2 and CTVM3 are consistent with a founder effect. Based on identification of a founder effect, all three kindreds were integrated into one comprehensive linkage analysis assuming an affected founder four generations ago; this yielded a maximum multipoint lod score of 3.53 at REN206J15 and 3.66 at REN124K12. Subsequent review of breeding records confirmed a common ancestor for dogs I.2 (CTVM1) and I.1 (CTVM3) four generations ago.

Overall, low polymorphism information content is evident in several typed markers. For example, the two markers REN206J15 and REN124K12 exhibit homozygosity of alleles in most carriers of the disease haplotype. Further analysis in kindred CTVM1 showed that one haplotype of II.4 and II.5 is identical for all tested markers from GALK1 (data not shown) to C09250 (data not shown), spanning a ~40 cM interval that includes the entire critical region (fig 1). This haplotype, however, is not disease related and leads to homozygosity of alleles in this interval for the unaffected dogs III.3 to III.6. For dogs II.4 and II.5, breeding records subsequently showed a common ancestor three generations ago. These findings illustrate the decrease in allele variability in selected offspring.

Minimal consensus overlap of ancestral haplotypes narrows the critical region
A recombination event in animal III.2 of CTVM3 confines the centromeric boundary of the critical interval to RARA. The
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disease haplotype in kindred CTVM2 defines the telomic boundary as REN126A13. Taken together, these findings narrow the critical region to a ∼4.3 cM region.

CTVM exhibits reduced penetrance
In each kindred, haplotype analysis identified dogs exhibiting non-penetrance. When dogs in all three kindreds are considered, the number with the disease haplotype (25 dogs) compared to the number with the CTVM phenotype (17 dogs) gives an estimate of penetrance as 68%. However, not all littermates could be recruited into our study, limiting accurate estimation of penetrance.

DISCUSSION

CTVM maps to dog chromosome 9
Findings in this study show that CTVM in Labrador Retrievers, the equivalent of Ebstein anomaly in humans, is a monogenic disease mapping to CFA9. This is the first successful genome-wide mapping effort of a locus linked to congenital cardiac malformation in Canis familiaris. Haplotype analysis defined a critical interval that is homologous to a gene rich region of human chromosome 17q12 to 17q23 (fig 3). This region has been studied very extensively in humans, since one of the genes implicated in breast cancer, BRCA1, is located in this interval. However, the size of the critical region on CFA9 has to be interpreted cautiously as not all markers are integrated into the meliotic linkage map, some having been assigned positions based only on radiation hybrid mapping.

Genetic findings of a founder effect reflect breed history
Identification of a conserved disease haplotype in all three kindreds provides evidence for a founder effect. Selective mating strategies, frequently used in purebred dogs, have apparently led to a biased distribution of alleles, as evidenced by identification of a conserved haplotype unrelated to disease in dogs II.4 and II.5 of kindred CTVM1. Decreased genetic diversity in individual breeds may make mapping more difficult owing to limited allelic variability, but also may facilitate genetic studies because of the homogeneity of modifying genetic influences, leading to a more uniform phenotype. We speculate that the relative uniformity of the disease phenotype, that is, tricuspid valve dysplasia without other cardiac malformations, is a result of relative genetic homogeneity in the kindreds we studied. Further study of this possibility in the dog model may lead to new insights into the mechanisms of variation of disease severity, which is a hitherto poorly understood phenomenon.

The ancestors of modern Labrador Retrievers have been identified as two animals bred at the end of the 19th century in England. Import of Labrador Retrievers into the United States occurred during the early part of the 20th century. A population reduction, presumably creating a population bottleneck, occurred in the period between the World Wars, followed by rapid expansion in subsequent years. Selective breeding strategies have led to a strong influence of individual dogs that may have more than 100 offspring (“popular sire effect”). Given this background, a CTVM founder effect is not surprising. A founder effect has been shown in several other genetic models in dogs, even in the absence of known common ancestors. As an example, recessively inherited yellow coat colour in Labrador and Golden Retrievers cosegregates with a mutation in the MC1R gene, suggesting a common founder for this trait in both dog breeds.

Canis familiaris as model for congenital heart disease
Genetic analysis of congenital heart disease in human populations is difficult owing to small family size, long generation time, locus heterogeneity, and reduced penetrance; classical linkage analysis and positional cloning approaches to gene discovery have remained the exception. One solution is to seek isolated populations which share a common genetic background and whose disease is probably caused by segregation of a major mutant allele. Human populations with such features are rare and, moreover, difficult to ascertain. By comparison, domestic dog breeds offer more than 300 well-defined, isolated breeding populations ideally suited for genetic studies of disease. The dog population numbers 80 million in the USA and constitutes an ideal reservoir of naturally occurring congenital heart malformations, with characteristics amenable to genetic analysis and a disease spectrum reflecting many of the clinically relevant conditions in humans.

Candidate gene identification
Little is known about the genetic mechanisms leading to valvar heart disease. Valve formation is initiated early in heart morphogenesis, and cardiac valves arise when specialised cells in the AV canal and the outflow tract regions are induced by myocardial signals to undergo epithelial-mesenchymal transformation; many of the genes and signal pathways involved in this process are important both in atrioventricular and semilunar valve morphology. Previous studies have shown completely conserved synteny between the CTVM critical region and human chromosome 17q. Assuming that gene order is also conserved, several genes on chromosome 17q are interesting candidates (fig 3). For example, the transcription factor TFX2, playing a pivotal role in chamber differentiation and atrioventricular canal formation, the signalling protein TOBI, which is an important transmitter in the EDRB2 cascade involved in cardiac muscle and valve formation, or FZD2, a receptor in the WNT-Dishervised signal transduction cascade expressed during heart morphogenesis, will receive high priority in our positional cloning efforts.

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