# Deafness and vestibular dysfunction in a Doberman Pinscher puppy associated with a mutation in the *PTPRQ* gene

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**Background:** A congenital syndrome of hearing loss and vestibular dysfunction affects Doberman Pinschers. Its inheritance pattern is suspected to be autosomal recessive and it potentially represents a spontaneous animal model of an autosomal recessive syndromic hearing loss.

**Hypothesis/Objectives:** The objectives of this study were to use whole genome sequencing (WGS) to identify deleterious genetic variants in candidate genes associated with the syndrome and to study the prevalence of candidate variants among a population of unaffected Doberman Pinschers.

**Animals:** One affected Doberman Pinscher and 202 unaffected Doberman Pinschers.

**Methods:** WGS of the affected dog with filtering of variants against a database of 154 unaffected dogs of diverse breeds was performed. Confirmation of candidate variants was achieved by Sanger sequencing followed by genotyping of the control population of unaffected Doberman Pinschers.

**Results:** WGS and variant filtering identified an alteration in a gene associated with both deafness and vestibular disease in humans: protein tyrosine phosphatase, receptor type Q (PTPRQ). There was a homozygous A insertion at CFA15: 22 989 894, causing a frameshift mutation in exon 39 of the gene. This insertion is predicted to cause a protein truncation with a premature stop codon occurring after position 2054 of the protein sequence that causes 279 C-terminal amino acids to be eliminated. Prevalence of the variant was 1.5% in a cohort of 202 unaffected Doberman Pinschers; all unaffected Doberman Pinschers were heterozygous or heterozygous for the reference allele.

**Conclusion and Clinical Importance:** We report the identification of a genetic alteration on the PTPRQ gene that is associated with congenital hearing and vestibular disorder in a young Doberman Pinscher dog.

**KEYWORDS**
deafness, Doberman Pinscher, genetics, PTPRQ, vestibular disease

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# 1 | INTRODUCTION

The clinical and pathological features of Doberman Pinscher dogs suffering from congenital deafness and dysfunction of the vestibular system was first described in 1992.[1] Clinically, the pups were deaf by 3 weeks of age and displayed signs of vestibular disease (head tilt, circling, and ataxia). Pathological findings in the inner ear included a progressive neuroepithelial type of cochlear degeneration characterized by a loss of the auditory sensory cells. Findings in the vestibular system included abnormal or absent otoconia in some of the affected dogs without sensory cell loss.[1] In this breed, it is believed to be inherited in an autosomal recessive fashion.[1] The disease is seen sporadically in clinical practice and can affect various breeds of dogs and cats.

Canine deafness is common and might have either a sensorineural or conductive cause. Most hereditary deafness in dogs is pigment associated, of the cochlea-saccular pathology type, and is specifically associated with the dominant allele of the merle gene (M) or the recessive piebald gene (S). Congenital hereditary sensorineural deafness associated with white pigmentation is the most frequent cause. Although over 90 breeds of dogs are reported with congenital deafness alone, the Doberman Pinscher breed is one of the rare breeds with deafness associated with signs of vestibular disease.[2] This has been commonly referred to by the Doberman breed community as "DINGS" for the past decades. However, its cause is currently unknown.

Hearing loss in children is the most common birth defect in industrialized countries and the most prevalent sensorineural disorder.[3] Its most common environmental (non-genetic) cause is congenital cytomegaloviral infection.[4] Inherited hearing loss is monogenic in most cases. In about 70% of the cases, it is not accompanied by other physical findings (nonsyndromic hearing loss) but in the remaining cases, it can be (syndromic hearing loss).[4] Its inheritance can be autosomal recessive nonsyndromic hearing loss (ARNSHL) (80%), autosomal dominant nonsyndromic hearing loss (ADNSHL) (20%), or occur through the X-chromosome (1%) or mitochondria (2%).[5] It is a heterogeneous trait with over 100 mapped loci and 46 causally implicated genes.[3]

Some of the syndromic hearing loss syndromes include dysfunction of the vestibular system.[3] The latter is estimated to occur in 30%-90% of children with hearing impairment of various causes and in 34% of children with hereditary cause.[6] Not all genetic mutations produce both cochlear and vestibular abnormalities. Indeed, depending upon the gene and the protein encoded by the gene, along with other factors, dysfunction of the vestibular system might coexist with hearing loss or not.[6] Usher's syndrome commonly features sensorineural hearing loss and retinitis pigmentosa with symptoms of vestibular dysfunction which vary from considerably affected (Usher type 1), not affected (Usher type 2) to variable dysfunction (Usher type 3). Five genes have been identified to cause Usher syndromes with vestibular signs (*MYO7A, USH1C, PCDH15, USH2A, CLRN1*). DFNA9, a form of ADNSHL, and DFNB84, a form of ARNSHL have hearing loss and have both been associated with dysfunction of the vestibular system; the former with mutations in the *COCH* gene, the latter with mutations in the gene protein tyrosine phosphatase, receptor type Q (*PTPRQ*).[6,7]

The objectives of this study were to identify deleterious genetic variants in candidate genes associated with congenital hearing loss and dysfunction of the vestibular system in an affected Doberman Pinscher puppy using whole genome sequencing (WGS), and to determine the prevalence of any such variant in a population of Doberman Pinschers not clinically affected by the condition.

# 2 | MATERIALS AND METHODS

## 2.1 | Animal selection/phenotyping

This study was conducted in accordance with the guidelines of the Animal Care and Use Committee at The North Carolina State University (protocol number: 17-021-O). Written consent authorizing study participation was obtained from each client.

We selected 1 affected and 202 unaffected Doberman Pinschers for inclusion in this study. The proband was a 12-week-old female Doberman who had been diagnosed with congenital deafness and vestibular disorder at the College of Veterinary Medicine at North Carolina State University. A head tilt had been evident from 8 weeks of age, no other signs of vestibular disease were reported at the time and no progression was observed. Both parents were reportedly normal neurologically, and among the litter of 6 puppies, this was the only affected dog. No prior ear treatments were reported. On presentation, the dog was healthy other than a left-sided head tilt with normal gait and postural reactions. Circling to the left side was noticed as well as abnormal physiological nystagmus when the head was moved to the left and positional ventral strabismus of the left eye. A neuroanatomic diagnosis of dysfunction of the peripheral vestibular system was made by a board-certified veterinary neurologist (Supporting Information Video 1). Unilateral deafness was confirmed by an abnormal brainstem auditory evoked response study (absent on the left side); No caloric testing was performed. Routine blood cell count and serum biochemistry panel were normal. A magnetic resonance imaging study of the brain and bullae was normal, as was cerebrospinal fluid analysis. No etiology could be found for the hearing loss. This phenotype is similar to the one reported by Wilkes and Palmer.[1]

Unaffected dogs were obtained from a pool of Doberman Pinschers that had been included in a previous genetics study of dilated cardiomyopathy[8] affecting the same breed. All dogs had been presented for a cardiology work-up for the aforementioned disorder and none had dysfunction of the vestibular system nor deafness reported by the owners or noted by the attending veterinarian. The relatedness between these Dobermans was unknown. One of the proband's parents (mother) was also included in the unaffected Doberman group. Deoxyribonucleic acid (DNA) was extracted from ethylenediaminetetraacetic acid blood samples or cheek swabs obtained from each dog using the standard protocol of the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California). DNA from the other parent and siblings was unavailable for evaluation.

## 2.2 | Next-generation sequencing

Approximately 3 μg of DNA from the proband was submitted for library preparation (Illumina TruSeq) and WGS at Genewiz (South Plainfield, New Jersey). Sequencing experiments were designed as 150 bp paired-end reads, and the sample was run in 1 lane of an Illumina HiSeq 4000 high-throughput sequencing system. These reads have been made publicly available at NCBI's Short Read Archive at http://www.ncbi.nlm.nih.gov/biosample/8032746.

Variant calling from WGS data was performed using a standardized bioinformatics pipeline for as described previously.[9] Briefly, sequence reads were trimmed using Trimmomatic 0.32[10] to a minimum phred-scaled base quality score of 30 at the start and end of each read with a minimum read length of 70 bp, and aligned to the canFam3 reference sequence[11] using BWA 0.7.13.[12] Aligned reads were prepared for analysis using Picard Tools 2.8 (http://broadinstitute.github.io/picard) and GATK 3.7[13] following best practices for base quality score recalibration and indel realignment specified by the Broad Institute, Cambridge, Massachusetts.[14,15] Variant calls were made using GATK's HaplotyeCaller walker, and variant quality score recalibration (VQSR) was performed using sites from dbSNP 146 and the Illumina 174K CanineHD BeadChip as training resources. We applied a VQSR tranche sensitivity cutoff of 99.9% to SNPs and 99% to indels for use in downstream analyses; genotype calls with a phred-scaled quality score <20 were flagged but not removed from the variant callset.

## 2.3 | Variant filtering and annotation

Variants (either heterozygous or homozygous) present in the proband were filtered against a database of whole genome sequences derived from 154 dogs that have been collected as part of ongoing research in our laboratories. These included 22 Boxers, 20 Standard Poodles, 13 Great Danes, 13 Yorkshire Terriers, 11 Cavalier King Charles Spaniels, 9 Dachshunds, 8 Scottish Deerhounds, 6 Scottish Terriers, 6 Miniature Poodles, 4 Doberman Pinschers, and 42 dogs of 18 additional breeds. Sequence data from all of these dogs was processed using the same bioinformatics pipeline described above. None of the animals in this database were known to be deaf or have a similar disease phenotype as the proband. Variants that passed our filtering step were annotated using SnpEff 4.3.[16]

## 2.4 | Sequencing methods for Chr15:22989894

Genomic DNA was amplified via polymerase chain reaction using Thermo Scientific DreamTaq Green PCR Master Mix, forward primer 5′-ACTTGAAAGGGTTGTGTGGC, and reverse primer 5′-AGGTCTGGCAATGTTGTGAC. PCR was performed on a Bio-Rad S1000 Thermal Cycler with the following cycling conditions: samples held at 95°C for 5 minutes, denatured at 95°C for 30 seconds, annealed at 66°C for 30 seconds, and extended at 72°C for 30 seconds. Cycling was repeated 45 times with the last extension step lasting 10 minutes. PCR products were visualized by gel electrophoresis to determine fragment length and the resulting amplicons were evaluated by Sanger sequencing.

Sequence files were then aligned to the canine reference sequence using Geneious software Version 9 (http://www.geneious.com).

## 3 | RESULTS

### 3.1 | Whole genome sequencing

We found 6 571 489 variants (biallellic and multiallelic) in the proband. Of these variants, 23 125 were unique to the proband. These variants had 29 065 distinct variant effects contained within 4824 annotated genes. Among the variant effects, 23 were predicted by SnpEff to have a high impact on gene function, 100 a moderate effect, 126 a low effect, and 28 816 a modifier effect. A complete list of these variants and their effects is provided in Supporting Information Table 1.

We hypothesized that given the clinical presentation of the proband, the causative variant for congenital deafness and dysfunction of the vestibular system in the proband would likely importantly alter protein function and would therefore have a high or moderate impact on gene function based upon SnpEff's variant categorization algorithm. Among the 123 high or moderate impact variant effects we identified that were unique to the proband, only 1 was in a gene known to be associated with both deafness and disease of the vestibular system in humans: *PTPRQ*.[7] This variant, which was homozygous in the proband and classified by SnpEff to be a high-impact variant, results in an A insertion at CFA15: 22 989 894, causing in a frameshift mutation (p.Asn2032LysfsTer24) in exon 39 of the gene (Ensembl transcript ENSCAFT00000009346.4) (Figure 1). This insertion is predicted to cause a protein truncation with a premature stop codon occurring after position 2054 of the protein sequence that causes 279 C-terminal amino acids to be eliminated.

### 3.2 | Prevalence

Out of the 202 Doberman Pinschers tested for the mutation, 6 dogs were heterozygous for the *PTPRQ* gene mutation, resulting in an allele frequency of 1.5% in the population of Doberman Pinschers evaluated. The dam of the proband was heterozygous for the mutation.

## 4 | DISCUSSION

In our study, we identified an A insertion at CFA15:22 989 894 causing a frameshift mutation in exon 39 of the gene *PTPRQ* that is associated with the congenital deafness and vestibular disorder syndrome that has been described in Doberman Pinscher. Regrettably, in this study, only 1 parent could be tested and only 1 affected dog was studied.

Autosomal recessive nonsyndromic hearing loss (ARNSHL) and signs of vestibular dysfunction occur in humans with mutations in the gene *PTPRQ* and also in autosomal dominant progressive hearing loss.[17] This gene encodes a member of the type III receptor-like protein-tyrosine phosphatase family (PTPs).[7,18] The encoded protein catalyzes the dephosphorylation of phosphotyrosine and phosphatidylinositol. By removing phosphate groups from tyrosine residues, they propagate or inhibit signal transduction, and play roles in cellular processes such as
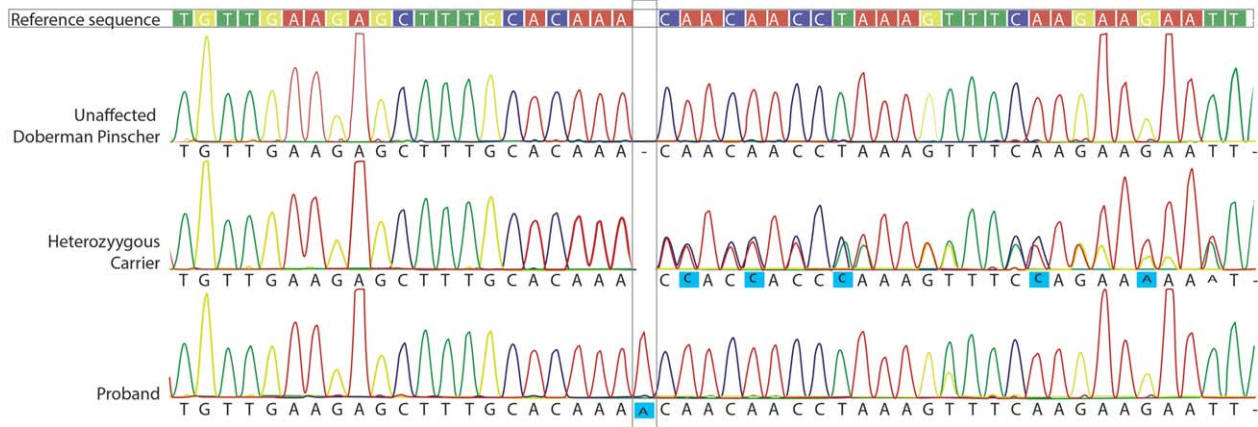
**FIGURE 1** Results of sequencing analysis on PTPRQ gene of an unaffected Doberman Pinscher, a heterozygous carrier and the proband, a Doberman Pinscher puppy with hearing loss and vestibular dysfunction

cell proliferation and differentiation.[19] More precisely, hair cell stereociliary bundles are critical for transduction and encoding of vestibular and auditory stimuli. The hair bundle is held together by many extracellular protein links connecting adjacent stereocilia such as tip links, shaft links, ankle links, lateral links, and kinocilial links. Their mechanism of action has been described elsewhere.[20] The shaft links contain the protein phosphatidylinositol phosphatase, and therefore genetic deletions produce hair cell bundle defects, loss of hair cells in the base of the cochlea, and deafness.[21] The results of investigations performed on mice lacking the receptor like inositol lipid phosphatase PTPRQ revealed a loss of high frequency auditory hair cells and deafness but also a distinct phenotype in the vestibular system and suggest similar hair bundle defects might underlie the dysfunction of the vestibular system reported in humans with mutations in PTPRQ.[22] Of note, quantitative PCR analysis identified widespread expression of PTPRQ in human fetal tissues, with highest expression in kidney, lung, and cochlea.[7] In 2 families with PTPRQ gene mutations, 1 member of the 1st family had a homozygous nucleotide substitution, c.1491T > A in exon 19, leading to a premature stop codon at position 497 of the protein (p.Tyr497X) whereas in a member of the 2nd family, a homozygous mutation at exon 19, c.1369A > G, leading to the substitution of a glycine for an arginine at position 457 of the protein (p.Arg457Gly). With the latter mutation, a large hydrophilic and positively charged amino acid is replaced by a small uncharged residue without a side chain, thereby impairing any ionic interactions.[7]

Although rapid progress has been made over the past several years in identifying genes involved in deafness (over 90 different genes affecting development or function of the inner ear) in mice,[23] and progress is being made in dogs,[2] the same cannot be said for vestibular function. Several reasons might explain why little is known about the genetic of dysfunction of the vestibular system: first, dysfunction of the peripheral vestibular system is difficult to assess directly; second, relatively few investigators are systematically looking for vestibular impairment in human or animal genetic studies; third, the ability of the central nervous system to compensate for alterations in vestibular function, when it is present at birth or progresses gradually, renders its identification difficult clinically or via case history.[6] These reasons make it difficult to ascertain that vestibular function is altered but also weakens its correlation with genetic data. In mice, several mutations affecting otoconia and associated loss of macular receptor function have none the less been reported, including several genes that have, for example, been proven to be critical for the formation of normal otoconia (Nox3, Otop1, Slc30a4, pldn, Cyba).[6] It is unclear if or how the PTPRQ mutation leads to the specific otoconia alterations previously reported in Doberman Pinschers,[1] but the absence of otoconia leads to clinical presentation similarities between dog and mice including head tilt and incoordination.[6] However, the deafness, which is described within the PTPRQ mutations publications, was bilateral, unlike in our dog.

In addition to evaluating the PTPRQ mutation we identified in the proband, we also screened a population of 202 unaffected Doberman Pinschers for the same mutation. In this population, we found the allele frequency to be low at 1.5%, and all unaffected dogs were heterozygous or homozygous for the reference allele. This low allele frequency, coupled with a likely autosomal recessive inheritance pattern, would explain the relatively low prevalence of clinical disease across the breed.

Universal screening programs of newborns for hearing defects has allowed for a better understanding and identification of etiologies, including genetic, in children suffering with hearing loss or impairment.[4] Routine screening for the reported phenotype in the Doberman Pinscher breed accompanied by judicious breeding decisions based on the identification of the genetic mutation reported in this study could allow rapid elimination of the problem from the breed.

The main limitation of this study is the examination of only 1 affected dog and the inability to test both parents (only 1 could be assessed) or littermates. The established importance of this gene as a cause of hearing loss and dysfunction of the vestibular system in 2 other species, the deleterious nature of the mutation, and the low allele frequency in the breed makes this mutation likely pathogenic for this syndrome. However, we underscore the unilateral character of the deafness in this dog, unlike the dogs reported by Wilkes and Palmer.[1] Therefore, further genetic evaluation of Doberman Pinschers presenting with the aforementioned phenotype are required to confirm our

findings. This might not only allow for a better evaluation of the prevalence of this mutation in this breed but also for the development of a genetic test and eradication of the disorder.

## CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

## OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

## INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Institutional animal care and use committee (IACUC): protocol number: 17-021-O.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**VIDEO 1** Neurological examination of the proband, a 12-week-old Doberman Pinscher affected with deafness and vestibular syndrome
**TABLE 1** List of private variants in the affected dog