

# A *CDH23* missense variant in Beauceron dogs with non-syndromic deafness

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## Abstract

Congenital coat-colour-related deafness is common among certain canine breeds especially those exhibiting extreme white spotting or merle patterning. We identified a non-syndromic deafness in Beauceron dogs characterised by a bilateral hearing loss in puppies that is not linked to coat colour. Pedigree analysis suggested an autosomal recessive transmission. By combining homozygosity mapping with whole genome sequencing and variant filtering in affected dogs we identified a *CDH23:c.700C>T* variant. The variant, located in the *CHD23* (*cadherin related 23*) gene, was predicted to induce a *CDH23:p.(Pro234Ser)* change in the protein. Proline-234 of *CDH23* protein is highly conserved across different vertebrate species. *In silico* tools predicted the *CDH23:p.(Pro234Ser)* change to be deleterious. *CDH23* encodes a calcium-dependent transmembrane glycoprotein localised near the tips of hair-cell stereocilia in the mammalian inner ear. Intact function of these cilia is mandatory for the transformation of the acoustical wave into a neurological signal, leading to sensorineural deafness when impaired. By genotyping a cohort of 90 control Beauceron dogs sampled in France, we found a 3.3% carrier frequency. The *CDH23:c.[700C>T]* allele is easily detectable with a genetic test to avoid at-risk matings.

## KEYWORDS

cadherin, canine, *Canis lupus familiaris*, DFNB12, hearing loss, Usher syndrome

Congenital deafness is a relatively common condition among dogs with coat colours involving extreme white spotting or merle patterning. Variants in *MITF* (*melanocyte inducing transcription factor*) for white spotting and *PMEL* (*premelanosome protein* or *SILV: Silver*) for merle patterning are associated with increased risk of congenital deafness (Strain, 2015). In the Beauceron, a French dog breed also known as Berger de Beauce, two coat colours are recognised by the breed standard: black and tan and

merle patterning on a black and tan background ([www.fci.be](http://www.fci.be)). In 2014, the first cases of deaf Beauceron puppies were reported by French breeders. They were born to two unaffected parents and all of them had a black and tan coat colour, strongly arguing against a pigment-associated deafness. In the following years, a total of 12 deaf black and tan Beauceron puppies from several litters were identified. Affected puppies showed bilateral deafness assessed by abnormal behaviour or by BAER

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(brainstem auditory evoked response) examinations (Figure S1). No other sign was noticed by the owners and the attending veterinarians. Puppies grew normally except for hearing loss. Pedigree data supported an autosomal recessive inheritance pattern for this non-syndromic congenital deafness (Figure 1a). Samples from seven deaf dogs were available for genetic analyses. As control dogs had not been BAER examined, we could not rule out the hypothesis that control dogs included unilateral deaf dogs (Appendix S1). We therefore chose a combined homozygosity mapping and whole genome sequencing strategy to search for the locus and variants, using first bilateral deaf dogs. Forty-two Beauceron dogs (including seven deaf dogs) were genotyped using either the Illumina CanineHD 170k SNP array (six deaf dogs and one control dog) or the Affymetrix Axiom CanineHD 710k SNP array (one deaf dog and 34 control dogs). A total of 144 534 single nucleotide variants (SNVs) were shared on both arrays and yielded usable results (minor allele frequency >5%, genotyping rate >95%). All 42 dogs had genotyping rates >98% and remained in the analysis. Regions of homozygosity shared by the seven deaf dogs were identified using PLINK and genotypes for each chromosome were manually inspected (Appendix S1). Two regions located on chromosomes 4 and 25 were identified (Table S1). Only the region from chromosome 4 showed a noticeable difference in genotype frequencies between the seven cases and the 35 controls (Figure 1b; Table S1). This region encompassing 1.24 Mb lay between the markers BICF2P1043034 and BICF2S2418273 (Figure 1b).

Subsequent to the homozygosity mapping we analysed the whole genome sequence of one deaf dog for variants across the candidate region. Paired-end reads (2 × 150 bp) were collected from a PCR-free DNA library from a deaf dog (project ID, PRJEB16012; sample ID, SAMEA6862928), achieving genome-wide coverage of 23×. SNVs and indels were called against the CanFam 3.1 reference genome. We searched for private homozygous variants in the deaf dog genome using 795 canine

control genomes (Jagannathan et al., 2019; Appendix S1, Table S2). We identified 4855 homozygous private variants (Table S3) including a strong candidate variant located in the candidate region of chromosome 4. This CDH23:c.700C>T missense variant was located in exon 8 of *CHD23* (*Cadherin Related 23*). *CDH23* lies in the homozygous region shared by all seven affected dogs (Figure 1b) and variants in *CDH23* were reported to cause non-syndromic (DFNB12) and syndromic (Usher syndrome) congenital deafness in humans and mice (Shearer et al., 1999; Wilson et al., 2001; OMIM #605516). *CDH23* encodes a calcium-dependent transmembrane glycoprotein localised near the tips of hair-cell stereocilia in the mammalian inner ear. Along with protocadherin-15 (PCDH15), *CDH23* forms a protein filament called the tip link. This filament is essential for hair-cell function. It was shown to be mandatory for delivering mechanical hearing signal to the mechano-electric transducer channels. Impaired transformation of the acoustical wave into a neurological signal leads to sensorineural deafness (Jaiganesh, Narui, et al., 2018; Richardson & Petit, 2019).

We genotyped a total of 75 Beauceron dogs related to deaf puppies including 12 deaf animals for the CDH23:c.700C>T variant. All 12 affected dogs were homozygous for the mutant allele. All five obligate carriers were heterozygous (Figure 1a, Table 1). We assessed the percentage of dogs carrying the CDH23:c.[700C>T] allele in a cohort of 90 control Beauceron dogs excluding first-degree relatives and found it to be 3.3% (Table 1).

As a *CDH23* missense variant was previously reported to be associated with increased anxious behaviour and deafness in a German pointer dog pedigree (Henthorn et al., 2006; Strain, 2015), we genotyped a cohort of 90 German pointer dogs. We failed to identify any carrier (Table 1).

The CDH23:c.700C>T variant was predicted to induce a CDH23:p.(Pro234Ser) change in the protein, predicted to be deleterious by MutPred2 (score = 0.725) and PROVEAN (score = -5.422). MutPred2 predicted this change to produce an altered transmembrane

**FIGURE 1** Non-syndromic congenital deafness in Beauceron dogs is governed by a recessive CDH23:c.[700C>T] missense allele. (a) Autosomal recessive inheritance pattern of the deafness. Partial pedigree tree of a large Beauceron family segregating a congenital non-syndromic deafness. Circles represent females, squares represent males. Deaf dogs are depicted with fully filled symbols. Obligate carriers are depicted with two-toned symbols. When available, the result of the genotyping assay for the CDH23:c.700C>T variant is mentioned. (b) Homozygosity mapping identified a single candidate region. SNV genotypes for each dog were manually inspected to identify homozygous regions shared by the seven deaf dogs. Two regions from chromosomes 4 and 25 were identified (Table S1) but only the region from chromosome 4 showed a clear difference in genotype frequencies between cases and controls. This region encompassing 1.24 Mb lay between SNV BICF2P1043034 and SNV BICF2S2418273. Markers from this region are depicted in bold. The disease-associated homozygous genotype is highlighted in blue. Heterozygosity is shown in white. Deaf dogs are depicted in brown. Missing genotypes were noted by '0'. Chr, Chromosome. Positions are in base pairs (CanFam 3.1 reference genome). (c) The canine CDH23:p.(Pro234Ser) change is predicted to produce a loss-of-function allele. Partial alignment of CDH23 protein sequences translated from the wild-type alleles reported in human, dog (wt-dog), cat, pig, cow, horse, mouse, xenopus, zebrafish and chicken and from the mutant allele of a deaf Beauceron dog (deaf-dog). The first 260 amino acids are shown and include the first (EC1, amino acids 40–137) and second (EC2, amino acids 138–246) extracellular cadherin (EC) domains (Appendix S1). Evolutionarily conserved residues are represented by dots compared with the reference human sequence. Dashes represent deletions. Non-conserved residues are represented by letters in the animal sequences. The red rectangle points out the human proline 234 (P234) residue that best aligns with proline 234 (P234) of the canine orthologue (Appendix S1, Figure S2, Table S4). The proline residue at this position is highly conserved among species and is part of the conserved XPXF/L motif involved in EC repeat stability (Jaiganesh, De-la-Torre, et al., 2018).





	<i>CIC</i>	<i>CIT</i>	<i>TIT</i>	Total
Deaf Beauceron dogs	0	0	12	12
Obligate-carrier Beauceron dogs	0	5	0	5
Control Beauceron dogs related to deaf dogs	36	22	0	58
Control Beauceron dogs unrelated to deaf dogs	87	3	0	90
Control German pointer dogs	90	0	0	90
Total	213	30	12	255

**TABLE 1** Genotypes for the *CDH23:c.700C>T* variant

global alignment showed that canine wild-type protein displayed 95% identity with human protein and 94% identity with mouse protein. We found that there is full conservation of the proline residue between cadherins (Figure S2). Canine P234 residue of *CDH23* best aligns with P234 of its human orthologue (Figure 1c; Figure S2). In humans a *CDH23:p.(Pro234Ser)* change was also identified (SNP ID: rs530434456, [www.ensembl.org](http://www.ensembl.org)) and was predicted to be deleterious by MutPred 2 (score = 0.750), PROVEAN (score = -5.139), SIFT, PolyPhen2 and other *in silico* tools (Appendix S1). In human and dog, *CDH23* contains 27 extracellular cadherin (EC) repeats that are structurally similar and form most of the tip link of the protein (Jaiganesh, De-la-Torre, et al., 2018). Human and canine P234 have been located in the second extracellular cadherin (EC2) repeat. They belong to the conserved XPXF/L motif of a  $\beta$ -strand that is present in each EC repeat (Jaiganesh, De-la-Torre, et al., 2018). In human patients, both Usher syndrome and non-syndromic deafness were associated with missense variants affecting proline residues of the XPXF/L motif. These variants were found in EC3 and EC5 (P346S and P559S changes respectively; Shahin et al., 2010), EC11 (P1206R change; Astuto et al., 2002), EC17 (P1849A change, Atik et al., 2015), EC18 (P1957S change; Ammar-Khodja et al., 2009) and EC22 (P2400S change; Schultz et al., 2011). These changes may alter EC repeat stability through precluding proper folding of the  $\beta$ -strand (Jaiganesh, De-la-Torre, et al., 2018).

Finally, in the German pointer dog pedigree, the reported missense variant associated with increased anxious behaviour and deafness has been predicted to lead to a proline to serine change in *CDH23*, at an unspecified position (Henthorn et al., 2006; Strain, 2015).

Altogether, these convergent results strongly suggest that the proline residue at position 234 is essential for *CDH23* function in humans and dogs. The observation that its alteration in Beauceron dogs leads to a phenotype fully compatible with a loss-of-function mutation in *CDH23* strengthens the pivotal role of this residue in both species. The available evidence is sufficient to classify the variant as likely pathogenic and implies it as a compelling candidate causative variant for the non-syndromic congenital deafness in Beauceron dogs. Further genotyping and haplotyping studies are needed

to explore the origins of the Beauceron and German pointer dog *CDH23* variants.

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## CONFLICT OF INTEREST

Caroline Dufaure de Citres is an employee of Antagene, a company selling DNA tests for animals.

## DATA AVAILABILITY STATEMENT

SNV genotyping data were deposited at OSF ([https://osf.io/hr2mf/?view\\_only=1357bf9df1fd4da580bf7007b2517a58](https://osf.io/hr2mf/?view_only=1357bf9df1fd4da580bf7007b2517a58)). Accessions of the whole-genome sequence data are listed in Table S2. Genomic sequences of *CDH23* exon 8 from wild type and deaf dogs (*Canis lupus familiaris*) were submitted to GenBank. Accession numbers are (GenBank ID: ON462052) for the wild type allele and

(GenBank ID: [ON462053](#)) for the CDH23:c.[700C>T] mutant allele.

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## SUPPORTING INFORMATION

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

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