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Duplication of *FGF3*, *FGF4*, *FGF19* and *ORAOV1* causes hair ridge and predisposition to dermoid sinus in Ridgeback dogs

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The dorsal hair ridge in Rhodesian and Thai Ridgeback dogs is caused by a dominant mutation that also predisposes to the congenital developmental disorder dermoid sinus. Here we show that the causative mutation is a 133-kb duplication involving three fibroblast growth factor (FGF) genes. FGFs play a crucial role in development, suggesting that the ridge and dermoid sinus are caused by dysregulation of one or more of the three FGF genes during development.

Dogs with a characteristic dorsal hair ridge seem to have been present in both Africa and Asia long before European colonization (**Fig. 1**). The Rhodesian Ridgeback dog (**Fig. 1a**), first registered in South Africa in 1924, is most likely a blend of European dogs (brought to Africa by early colonizers) and an extinct indigenous breed of Africa, the ridged Hottentot Khoi dog[1]. The Thai Ridgeback (**Fig. 1b**) and the Vietnamese Phu Quoc dog are two Asian breeds with a dorsal hair ridge closely resembling the one found in Rhodesian Ridgeback dogs.

Histology of the skin from a ridged dog, taken strictly from the dorsal median plane, showed cross-sectioned appendages (that is, hair follicles and sebaceous glands) of normal appearance but lateral orientation (**Fig. 1d**). In contrast, skin from the median plane of a ridgeless dog showed caudally oriented hair follicles (**Fig. 1e**). Ridgeback dogs are affected by the congenital malformation dermoid sinus

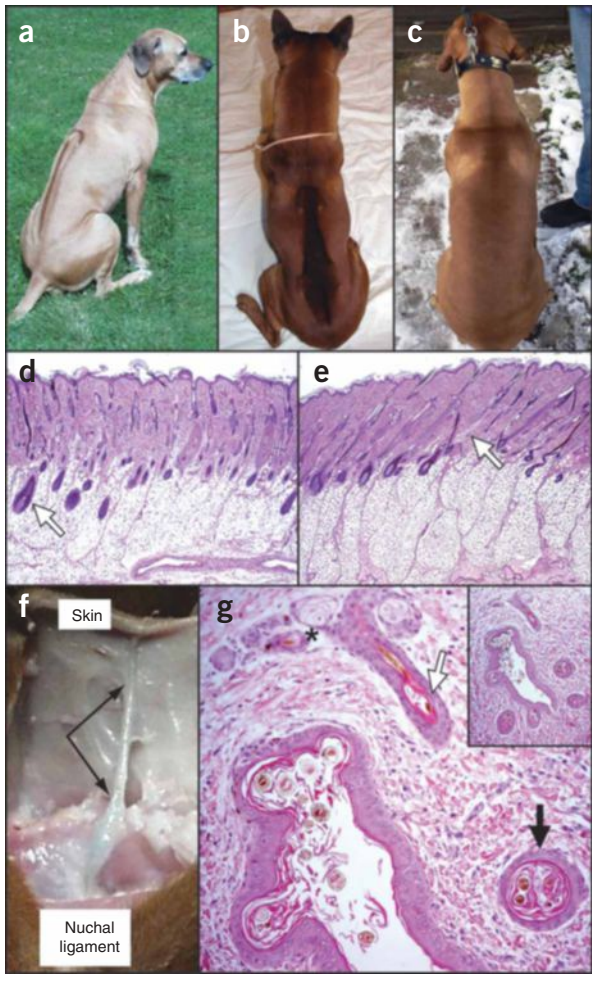

Figure 1 Phenotypes of Rhodesian and Thai Ridgeback dogs. (**a**,**b**) The dorsal hair ridge of Rhodesian Ridgeback (**a**) and Thai Ridgeback dogs (**b**). (**c**) A ridgeless Rhodesian Ridgeback dog. (**d**) Light micrograph of ridge skin taken from the dorsal median plane with cross-sectioned, laterally oriented hair follicles (arrow) and sebaceous glands in the dermis (objective lens × 4). (**e**) Corresponding cross-section from a ridgeless dog. Note that the hair follicles are caudally oriented in the median plane (arrow) (objective lens × 4). (**f**) The full extension of a dermoid sinus, extending from the upper dermis to the nuchal ligament, which overlays the cervical spinous process. The dermoid sinus–affected Rhodesian Ridgeback was 6 weeks old at the time of euthanasia. (**g**) Light micrograph of a cross-sectioned dermoid sinus with hair and keratin debris in the lumen covered by a stratified squamous keratinized epithelium. In the upper portion, appendages such as a hair follicle (filled arrow) and sebaceous glands (*) are seen; in the right corner, a hair follicle with multiple hair shafts is indicated (open arrow); (objective lens ×20). At lower magnification, the whole dermoid sinus is seen with the surrounding loose connective tissue (inset, objective lens ×10).

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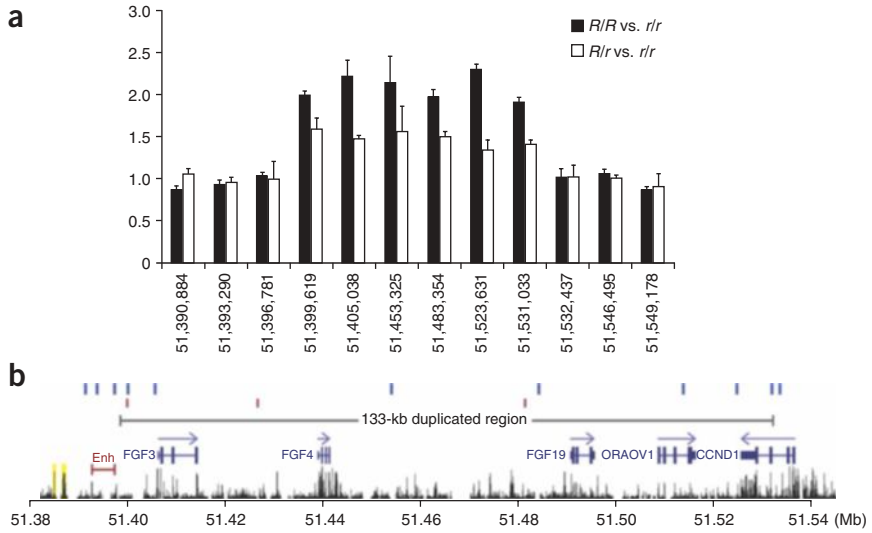

Figure 2 Duplication in Ridgeback dogs. (**a**) A 133-kb duplication on chromosome 18 in Ridgeback dogs detected using MLGA. Relative probe signals in two comparisons of a ridged dog and a ridgeless (*r/r*) dog; a value of ~1 indicates the same copy number in ridged and ridgeless dogs. One of the ridged dogs was interpreted as homozygous for the duplication (*R/R*, black staples) and the other was assumed to be heterozygous *(R/r,* unfilled staples). Error bars, s.d. (**b**) Genome annotation of the duplicated region on dog chromosome 18. Genome-wide mapping SNPs (pink bars) and MLGA probes (blue bars) identify a duplicated region that contains four complete genes (*FGF3*, *FGF4*, *FGF19* and *ORAOV1*) and the 3′ end of *CCND1*, but does not include conserved elements (yellow) and an enhancer region[7] (red) upstream of *FGF3*. Dog, human, mouse and rat sequence conservation from the University of California, Santa Cruz genome browser (http://www.genome.ucsc.edu) is shown in black.

(**Fig. 1f**), which closely resembles a neural-tube defect in humans that is usually termed dermal sinus[2]. In dogs, anatomical locations of dermoid sinus, anterior and posterior to the ridge, correspond with those found in humans. The dermoid sinus was characterized by a tubular indentation of the skin, with keratin and hair in the lumen; appendages such as sebaceous glands and aberrant hair follicles with multiple hair shafts were also observed (**Fig. 1g**). Dermoid sinus is closely associated with the ridge phenotype, and no ridgeless dogs with dermoid sinus have been reported[3]. Approximately 5–6% of Rhodesian Ridgebacks born in Sweden are ridgeless, and around 8–10% of ridged offspring have dermoid sinus[3,4]. Both ridgeless dogs and dogs with dermoid sinus are excluded from breeding.

We assumed a genetic model in which (i) ridgeless dogs are homozygous (*r/r*) for the wild-type allele, (ii) ridged dogs without dermoid sinus are heterozygous or homozygous for the *Ridge* allele (*R/r* or *R/R*) and (iii) ridged dogs with dermoid sinus are homozygous *R/R*. We were able to assign the *Ridge* locus to a 750-kb region on chromosome 18 using only nine ridgeless and 12 ridged dogs, of which 11 had dermoid sinus, in a genome-wide association analysis[5]. The results indicated that 10 of 11 dogs with dermoid sinus were homozygous for a haplotype not present among ridgeless dogs. Notably, all Rhodesian ridgebacks with dermoid sinus were heterozygous for a SNP (SNP_51,399,353) within the 750-kb region associated with the phenotype. Further analysis showed that 43 of 45 ridged Rhodesian Ridgebacks were heterozygous for this SNP. This significant deviation ($P < 0.001$) from Hardy-Weinberg proportions suggested that the SNP is part of a duplicated region, and that the two copies tend to carry different SNP variants.

We used the recently developed multiple ligation–dependent genome amplification (MLGA) technique[6] to test whether the *Ridge* allele is associated with a duplication (**Supplementary Methods** online). We designed MLGA probes with 100-kb spacing over a 2-Mb region (**Supplementary Table 1** online). This analysis showed conclusively that ridged Rhodesian and Thai Ridgebacks are heterozygous or homozygous for a large duplication (>100 kb) (**Fig. 2a**). We defined the precise location of the duplication breakpoints using additional interspersed probes in the critical intervals. Using PCR, we then amplified and sequenced the duplication breakpoint between the two tandem copies. The 133.4-kb duplication spans from nucleotide position 51,398,518 to position 51,531,941 in the CanFam2.0 genome assembly, with a single-nucleotide insertion at the breakpoint (**Fig. 2b** and **Supplementary Fig. 1** online). Notably, sequencing of ~1.7 kb flanking the breakpoint from Rhodesian and Thai Ridgebacks revealed identical breakpoints, although mitochondrial DNA analysis did not reveal a close relationship between the breeds (**Supplementary Note**, **Supplementary Tables 2** and **3** and **Supplementary Fig. 2** online). This strongly suggests that the duplication is the causative mutation for the ridge. Furthermore, it implies that either the *Ridge* allele has introgressed from one population to the other, or the mutation is old enough to have been present in the early domestic dog population.

The nucleotide sequence within 3 kb of the breakpoint did not contain any known repetitive elements that could have acted as target sites for the recombination event creating the duplication. Duplicated loci often show copy-number instability, but the MLGA analysis gave no clear indication of *Ridge* haplotypes with more than two copies of the duplicated region. However, this needs to be further investigated in a larger sample of ridged dogs, as it may be relevant for the incidence of dermoid sinus.

Table 1 Genotype-phenotype relationships in regard to 133-kb duplication

	Duplication genotype			
Phenotype	–/–	+/–	+/+	*n*
Rhodesian Ridgebacks				
Ridgeless, DS–	10	0	0	10
Ridged, DS–	0	16	4	20
Ridged, DS+	0	2	10	12
Thai Ridgebacks				
Ridged, DS–	0	3	6	9
Ridged, DS+	0	0	3	3
Other breeds[a]				
Ridgeless, DS–	37	0	0	37

Shown are genotype-phenotype relationships relating to the presence of the 133-kb duplication associated with the dorsal hair ridge in Rhodesian and Thai Ridgeback dogs. DS, dermoid sinus.
[a]Including Basenji, beagle, border collie, boxer, bull terrier, Dalmatian, fox terrier, Dachshund and wolf.

We detected a complete association between the duplication and the ridge phenotype in more than 50 Rhodesian and Thai Ridgebacks (**Table 1**). This is consistent with a fully penetrant, dominant inheritance of the ridge. Furthermore, 13 of 15 Ridgebacks with dermoid sinus were homozygous for the duplication. Thus, the mutation predisposes to dermoid sinus with low penetrance in duplication heterozygotes and with high penetrance in homozygotes. Data indicate that about 80% of healthy ridged dogs in the Swedish Rhodesian Ridgeback population are heterozygous for the duplication, suggesting that about 16% of newborn dogs ought to be ridgeless ($0.8^2 \times 1/4$). This is threefold higher than the estimated rate of 5–6%[3,4], suggesting either that the frequency of the undesired ridgeless phenotype is under-reported, or that *R/R* dogs without dermoid sinus are over-represented in the breeding population.

The duplication includes *FGF3*, *FGF4*, *FGF19*, *ORAOV1* and the 3′ end of *CCND1* encoding cyclin D1 (**Fig. 2b**). The duplication may act as a regulatory mutation, either through a dosage effect or by disrupting the location of coding sequences in relation to important regulatory elements. The proximal duplication breakpoint is ~10 kb upstream of *FGF3* but downstream of an enhancer critical for embryonic expression of *FGF3* (**Fig. 2b**)[7]. The next gene resides ~200 kb upstream, suggesting that multiple regulatory elements essential for normal expression of these FGFs may be located in this region. Both the hair ridge and dermoid sinus are most likely caused by dysregulated gene expression during early embryogenesis. *CCND1* is unlikely to contribute to these phenotypes, because only the 3′ end of the gene is duplicated. *ORAOV1* (oral cancer overexpressed 1) is a poorly characterized proto-oncogene whose function during development is unknown[8]. In contrast, it is well established that tight regulation of FGF expression is crucial during embryonic development, including hair follicle morphogenesis[9]. Several of the 22 known mammalian FGF genes have been implicated in regulation of both hair growth and skin development[10]. Ridgeback dogs apparently have a mild defect of the planar cell polarity system, which is required both for a normal orientation of hair follicles[11] and for neural-tube closure[12]. The dorsal hair ridge in Ridgeback dogs resembles the global disorganization of hair orientation in frizzled 6 (*Fzd6*) knockout mice[11]. Notably, *Fzd3 Fzd6* double-knockout mice also show severe defects in neural tube closure[13]. Thus, we propose that dysregulated FGF expression along the dorsal midline during embryonic development leads to disorganized hair follicles and an increased risk of dermoid sinus in Ridgeback dogs. We did not have access to embryonic tissue to test this hypothesis, but expression of *ORAOV1* mRNA was about twofold higher in postnatal tissue from homozygous ridged dogs compared with ridgeless dogs, consistent with a chromosome duplication (**Supplementary Note** and **Supplementary Fig. 3** online).

Our results have implications for the breeding of Ridgeback dogs. Neither ridgeless dogs nor those with dermoid sinus are allowed for breeding by Rhodesian Ridgeback clubs. This leads to overdominance, because the heterozygote is the favored genotype: it expresses the ridge and has a low incidence of dermoid sinus. The problem with dermoid sinus could be virtually eliminated by allowing ridgeless dogs in breeding and by avoiding matings between ridged dogs.

In humans, dermal sinus is often associated with spina bifida occulta, but it may also occur independently[14]. Both disorders are collectively categorized as neural-tube defects, with a poorly understood genetic background[15]. An obvious topic for future research will be to test whether mutations in the region corresponding to the *Ridge* duplication contribute to human malformations.

URL. Information on the CanFam2.0 genome is available at http://www.genome.ucsc.edu.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

N.H.C.S.H. was responsible for collection of field material, histopathological confirmation of dermoid sinus and autopsies together with E.H., phenotypic characterization of the field material, break-point analyses together with M.I., and real-time PCR analyses; M.I. and M.N. were responsible for the MLGA analyses; E.K.K. carried out the SNP association and bioinformatics analyses; E.H. carried out the histological analyses; G.R.P. did pyrosequencing, P.S. carried out the sequencing of mitochondrial DNA and performed phylogenetic analyses; C.M.W. contributed to the SNP screen, H.v.E. took part in the collection of tissue samples; U.G. provided technical assistance; Å.H. provided advice for the clinical part of the study; K.L.-T., L.A. and G.A. co-directed the study; and N.H.C.S.H., L.A., G.A., and K.L.-T. were responsible for preparation of the manuscript with input from the other authors.

COMPETING INTERESTS STATEMENT

The authors declare competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturegenetics/.

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