

# Whole-genome resequencing reveals the polygenic architecture of dynamic laryngeal collapse in horses

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# Abstract

Complex performance traits often arise from the combined effects of numerous loci influencing multiple biological pathways, yet disentangling their genetic architecture remains challenging. Domestic animal populations offer powerful systems for studying such traits because artificial selection has generated marked phenotypic diversity while maintaining comparatively homogeneous genetic backgrounds. Dynamic laryngeal collapse (DLC), a performance-limiting upper airway disorder in horses associated with poll flexion, provides a natural model for investigating how distributed genomic variation contributes to multifactorial phenotypes involving neuromotor control, musculoskeletal function, and craniofacial morphology. Although previous SNP array analyses identified a suggestive locus on chromosome 7, the extent of genome-wide differentiation underlying susceptibility has remained unclear.

To investigate the genetic architecture of DLC, we performed whole-genome resequencing of 43 Norwegian-Swedish Coldblooded Trotters (25 affected and 18 unaffected) and analysed allele frequency divergence using fixation index ( $F_{ST}$ ) scans with 50 kb sliding windows and 25 kb steps. We identified 58 genome-wide significant regions ( $F_{ST} > 0.3$ ) distributed across the genome, including replication of the previously reported chromosome 7 locus.

Differentiated regions harbour genes involved in diverse but functionally connected processes, including neuromotor regulation (*ITPR1* and *ROBO1*), motor coordination and muscle tone (*RORA*), muscle contraction and force transmission (*TPM1* and *TLN2*), and craniofacial and airway development (*NR2F2*). Rather than supporting a single major-effect locus, these findings indicate coordinated differentiation across multiple biological systems contributing to phenotypic variation.

Our results provide genome-wide evidence that susceptibility to DLC reflects coordinated differentiation across multiple loci spanning interacting neuromotor, musculoskeletal and developmental pathways rather than a single major-effect variant. By resolving the systems-level organisation of this complex phenotype, our findings demonstrate how population genomic analyses can reveal biologically coherent polygenic architectures that may remain obscured in conventional single-locus association studies.

## Introduction

Understanding the genetic architecture of complex traits remains a central challenge in genetics and evolutionary biology. Many phenotypes arise through the combined effects of numerous variants distributed across interconnected biological pathways rather than a few major-effect loci, making it difficult to identify the mechanisms underlying phenotypic variation (Boyle et al. 2017; Timpson et al. 2018). Resolving these distributed architectures requires approaches capable of capturing genome-wide patterns of differentiation beyond single-locus association signals. Domestic animal populations provide particularly valuable systems for such investigations because strong artificial selection has generated

distinct phenotypes while maintaining relatively homogeneous genetic backgrounds (Andersson and Georges 2004).

Airway disorders that limit athletic performance represent an important class of complex phenotypes in which developmental, anatomical, and neuromuscular factors interact to influence disease susceptibility. In both humans and horses, laryngeal obstruction can arise not only from inflammation or injury but also from inherited anatomical predispositions that become apparent during exercise. The domestic horse is especially informative for studying these conditions, as selective breeding for speed, stamina, and conformational traits such as high head carriage has shaped upper airway anatomy in ways that can compromise respiratory function. Dynamic laryngeal collapse (DLC), a disorder triggered by head elevation and poll flexion during exercise, exemplifies this trade-off between performance-associated morphology and airway stability. Exercise-induced laryngeal obstruction (EILO) is increasingly recognised in human endurance athletes, including elite cross-country skiers, where transient upper airway obstruction can limit exercise capacity (Irewall et al. 2021). Although the anatomical triggers differ from those in horses, both EILO and DLC highlight the importance of interactions among airway anatomy, neuromuscular control, and the physiological demands of intense exercise. Comparative studies further suggest that neuromuscular coordination and craniofacial morphology may converge on common biological processes across mammals (Fretheim-Kelly et al. 2019), positioning DLC as a valuable natural model for investigating the genetic basis of complex airway phenotypes.

DLC has been reported in several breeds, including Icelandic Horses, Colombian Criollo Paso Horses, and Norwegian-Swedish Coldblooded Trotters (Hanche-Olsen et al. 2010; Velie et al. 2020; Joó et al. 2021). Clinical signs manifest during exercise when horses are driven in poll flexion with a high head and neck position but are absent at rest or during exercise with a free head carriage (Fig. 1a,c). Affected horses may experience severe upper respiratory tract obstruction that can lead to physical collapse because of airflow restriction (Fig. 1b,d; Supplementary Video). DLC is triggered by poll flexion-induced rein tension in horses with predisposing anatomical characteristics, particularly a more rostral positioning of the larynx relative to the mandible (Fjordbakk et al. 2013). Furthermore, DLC has been reported as a complication following laryngeal tie-forward surgery, with clinical signs indistinguishable from those observed in the naturally occurring condition (Vermedal and Strand 2020). Together, these observations indicate that individual anatomical predispositions play a central role in disease pathogenesis.

The marked differences in DLC prevalence among breeds and its strong association with individual anatomical characteristics suggest an underlying genetic component (Fjordbakk et al. 2013). In a previous genome-wide association study of Norwegian-Swedish Coldblooded Trotters, we identified a significantly associated region on chromosome 7; however, the resolution of the 670K SNP array was insufficient to refine the approximately 5 Mb interval containing multiple candidate genes (Velie et al. 2020). More broadly, SNP array-based analyses are constrained by the density and composition of predefined marker panels, which may incompletely capture the full spectrum of genomic variation underlying complex traits. Whole-genome sequencing (WGS), by contrast, provides near-comprehensive

characterisation of both coding and non-coding variation at nucleotide resolution, enabling a more comprehensive characterisation of the genomic architecture of multifactorial phenotypes. This capability has been substantially enhanced in horses through improvements in genome assembly and variant discovery associated with the EquCab3 reference genome (Kalbfleisch et al. 2018).

Here, we use WGS to investigate the genomic architecture of DLC in Norwegian-Swedish Coldblooded Trotters as a model of a complex multifactorial trait. Rather than focusing on the refinement of a single susceptibility locus, we apply fixation index ( $F_{ST}$ ) analyses and functional annotation to identify genome-wide patterns of differentiation between affected and unaffected horses. We show that DLC susceptibility is associated with multiple highly differentiated genomic regions containing genes involved in neuromotor coordination, muscle function, and craniofacial development, supporting a model in which disease risk arises through coordinated differentiation across interacting biological systems rather than a single major-effect variant. More broadly, our findings illustrate how population genomic approaches can resolve the distributed architecture of complex traits and provide biological insight into multifactorial phenotypes beyond conventional locus-centered association analyses.

## Materials and Methods

### Sample collection

This study included 43 horses: 25 cases (11 females, 6 geldings, and 8 males) and 18 controls (7 females, 4 geldings, and 7 males). These horses were selected from the larger cohort used in our previous GWAS study (Velie et al. 2020), based on the availability of blood or DNA samples suitable for WGS library preparation. The horses were selected from a larger cohort admitted to the Equine Teaching Hospital at the Norwegian School of Veterinary Science (Oslo, Norway) between 1999 and 2018 for evaluation of respiratory or performance-related concerns. All horses (affected and controls) underwent a standardized high-speed treadmill videoendoscopy examination, which included alternating 1-minute periods of free head carriage and poll flexion (driven on the bit) until fatigue (Fjordbakk et al. 2008; Strand et al. 2009; Velie et al. 2020). Diagnosis and categorization of DLC severity (mild, moderate or markedly affected) were conducted according to established criteria by two Board Certified Specialists in Equine Surgery with expertise in equine upper respiratory tract diagnosis (Fjordbakk et al. 2008; Velie et al. 2020). Peripheral blood samples were collected from cases diagnosed with only moderate or markedly affected DLC (Velie et al. 2020) as well as from control racehorses with no clinical evidence of any upper respiratory tract disorder based on this examination.

### DNA library preparation and whole genome sequencing

Genomic DNA was extracted from blood and quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher, Waltham, Massachusetts, USA). Fragmentation of 1 µg genomic DNA was performed using a Covaris E220 system targeting an insert size of 350-400bp. Individual DNA sequencing libraries were prepared using the Illumina TruSeq DNA PCR-Free kit (Illumina, San Diego, California, United States) and indexed using the Illumina TruSeq DNA CD Indexes kit (Illumina). The library preparation was

performed according to the manufacturer's instructions. The quality of the libraries was assessed using the TapeStation and the D1000 ScreenTape (Agilent Technologies, Santa Clara, California, USA). The adapter-ligated fragments were quantified by qPCR using the library quantification kit for Illumina (KAPA Biosystems, Basel, Switzerland) on a CFX384 Touch instrument (Bio-Rad, Hercules, California, USA) prior to cluster generation and sequencing.

Sequencing of paired-end 150bp reads was carried out on a single lane of an Illumina NovaSeq 6000 system, S4 flow cell, and v1.5 sequencing chemistry (Illumina). Demultiplexing and conversion to FASTQ format were performed using the bcl2fastq2 (v2.20.0.422) software, provided by Illumina. Additional statistics of sequencing quality were compiled with an in-house script from the FASTQ files, RTA and BCL2FASTQ2 output files.

## Bioinformatics analysis

The quality of raw fastq files was assessed with FastQC(Andrews 2015). The EquCab3 horse reference genome was downloaded from ensembl.org, indexed using BWA (v0.7.17) (Li and Durbin 2009), and used to align the fastq files using BWA mem (v0.7.17) (Li and Durbin 2009). SAMtools (Li et al. 2009) software was used to reorder and sort the BAM files that were generated. Duplicates were marked using Picard software (v1.92) ("Picard Toolkit." Broad Institute, GitHub Repository. <https://broadinstitute.github.io/picard/>; Broad Institute' 2019). The sequencing output of the 43 samples varied between 17.8 and 255.5 million reads with a 43% GC content. Indel realignment, variant calling, joint genotyping, and hard variant filtration were performed as previously described (Van der Auwera et al. 2013). Variant effects were annotated using SnpEff to predict the functional impact of identified variants (Cingolani et al. 2012). VCFtools (Danecek et al. 2011) was finally used to calculate the  $F_{ST}$  values following the Weir and Cockerham calculation method (Weir and Cockerham 1984). A statistical  $F_{ST}$  significance threshold for the autosomes was determined using the "qnorm" function, which comes as standard with the R software, to convert the  $F_{ST}$  values into Bonferroni-adjusted standardized Z scores, followed by determining the Z-score value that corresponds to the 95<sup>th</sup> percentile and its respective  $F_{ST}$  value. Similarly, an  $F_{ST}$  significance threshold was determined for the X chromosome separately.

## Results

To investigate the genetic predisposition to DLC, we performed whole-genome sequencing (WGS) on 43 horses, comprising 25 cases and 18 controls. Sequencing reads were aligned to the EquCab3 reference genome (Kalbfleisch et al. 2018), and genetic differentiation between cases and controls was assessed by calculating the fixation index ( $F_{ST}$ ) values using 50 kb sliding windows with a 25 kb step size.

This analysis identified 58 genomic regions that surpassed the standardized significance threshold, indicating a strong association with DLC. These regions collectively contained 171 significant windows, distributed across 53 autosomal regions and five regions on chromosome X (Fig. 2).

A previous study using the same set of case and control horses performed a pilot GWAS using a 670K SNP array and identified a region on chromosome 7 (Velie et al. 2020). Although the sample size was relatively small for GWAS, this pilot study suggested that a genetic component predisposes to DLC and that the identified region contributes to the disorder’s manifestation. Importantly, this region was among those identified in the current WGS analysis, providing an independent confirmation of this locus.

Because DLC is a complex condition influenced by multiple genetic and anatomical factors rather than a single locus, focusing solely on outlier regions with the highest  $F_{ST}$  values could miss biologically relevant genes that contribute more subtly to the phenotype (Wood et al. 2014). To capture the full spectrum of genetic contributors, we therefore included all chromosomal regions that exceeded the Bonferroni-corrected genome-wide significance threshold in downstream analyses. This broader approach revealed 282 annotated genes, including 153 protein-coding genes, 114 lncRNAs, 5 miRNAs, 7 snRNAs, 2 snoRNAs, and 2 potential pseudogenes (Supplementary Table S1).

Protein-coding genes were prioritized for downstream mechanistic interpretation because they are associated with well-characterized biological processes relevant to DLC, including neuromuscular control, skeletal-muscle force generation, and craniofacial or laryngeal structural predispositions. Protein-coding genes encode the structural and enzymatic components that directly influence muscle contraction, motor coordination, and skeletal development, making them the most likely contributors to the observed phenotype. While non-coding RNAs may modulate gene expression, their effects are often indirect, so focusing the initial analysis on protein-coding genes enables a clearer framework for linking the genotype to the biological mechanisms underlying DLC.

## Top candidate genes associated with DLC

Among the 153 protein-coding genes located within the genome-wide significant regions, six genes stood out as the most functionally plausible candidates based on their known biological functions and potential relevance to DLC: *ITPR1*, *ROBO1*, *RORA*, *TPM1*, *TLN2*, and *NR2F2*. These genes, hereafter referred to as Tier 1 genes, were prioritized because they are directly implicated in neuromuscular control, skeletal-muscle force generation, and craniofacial or laryngeal development, all of which are processes critical for poll flexion-associated airway stability. Notably, within regions exceeding an  $F_{ST}$  of 0.332, only *TPM1* and *NR2F2* carried missense variants, suggesting potential functional alterations, whereas the other candidates contained non-coding or synonymous variants and likely exert regulatory or structural effects (Table 1). Additional variant information is provided in Supplementary File 1.

Table 1. Characterization of the top candidate genes (Tier 1).

| Gene symbol  | Gene full name                                      | Gene function                                                                                                                                                                                                                                                                                                               | $F_{ST}$ (Region-weighted) | Missense variants                                                                           |
|--------------|-----------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|---------------------------------------------------------------------------------------------|
| <i>ITPR1</i> | <i>inositol 1,4,5-trisphosphate receptor type 1</i> | A key calcium-release channel highly expressed in cerebellar Purkinje cells. Pathogenic variants in ITPR1 cause spinocerebellar ataxia, characterized by impaired motor coordination and cerebellar dysfunction (Zambonin et al. 2017).                                                                                     | 0.556                      | -                                                                                           |
| <i>ROBO1</i> | <i>roundabout guidance receptor 1</i>               | A receptor mediating Slit-Robo repulsive signals during axon pathfinding. Genetic disruption of Robo1/Robo2 in mouse models results in pronounced axon guidance errors in major motor tracts, indicating their central role in establishing neural circuitry underlying motor coordination (López-Bendito et al. 2007)      | 0.343                      | -                                                                                           |
| <i>RORA</i>  | <i>RAR related orphan receptor A</i>                | A transcription factor with established roles in both neuromotor control and craniofacial development. It has been associated with increased susceptibility to orofacial clefts in humans, likely through regulation of developmental genes such as SHH (Li et al. 2022)                                                    | 0.398                      | -                                                                                           |
| <i>TPM1</i>  | <i>tropomyosin 1</i>                                | A core sarcomeric protein that stabilizes actin filaments and regulates contraction in skeletal muscle. TPM1 is a strong candidate for influencing susceptibility to DLC, where robust and coordinated muscle function is required to stabilize the larynx during mechanical stress (England et al. 2017; Jin et al. 2016). | 0.398                      | c.115A>G<br>(p.Ser39Gly)<br><br>c.835G>C<br>(p.Glu279Gln)<br><br>c.1753A>G<br>(p.Ile585Val) |
| <i>TLN2</i>  | <i>talin 2</i>                                      | The major talin isoform in skeletal muscle, where it links integrins to the actin cytoskeleton at costameres and myotendinous junctions. Its loss leads to progressive myopathy and structural defects in muscle, particularly at sites of force transmission (Conti et al. 2008; Conti et al. 2009).                       | 0.398                      | -                                                                                           |
| <i>NR2F2</i> | <i>nuclear receptor subfamily 2</i>                 | A nuclear receptor critical for cranial neural crest-derived skeletal structures. Studies have                                                                                                                                                                                                                              | 0.375                      | c.1402G>C<br>(p.Glu468Gln)                                                                  |

|                     |                                                                                                                                                                                                                              |                            |
|---------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| group F<br>member 2 | demonstrated that NR2F2 regulates skeletal progenitor genes in post-migratory cranial neural crest cells, which form key craniofacial components such as the jaw and throat skeleton (Barske et al. 2018; Okeke et al. 2022) | c.1428C>A<br>(p.Leu476Leu) |
|                     |                                                                                                                                                                                                                              | c.1538C>T<br>(p.Thr513Ile) |
|                     |                                                                                                                                                                                                                              | c.1742A>G<br>(p.Asp581Gly) |
|                     |                                                                                                                                                                                                                              | c.1901A>G<br>(p.His634Arg) |

Collectively, the six genes listed in Table 1 underscore the multifactorial genetic architecture of DLC, where neuromuscular coordination, muscle contractility, and craniofacial positioning converge to jointly shape susceptibility. Together, they provide a strong mechanistic framework for functional and translational follow-up studies and may serve as candidates for developing genetic markers to support selective breeding strategies aimed at reducing DLC prevalence. In addition, we identified a secondary set of eight Tier 2 genes - *GABRB3*, *LMOD3*, *KBTD13*, *RSPO2*, *EOGT*, *SHH*, *ELOVL4*, and *RMND1* - that map to complementary pathways in musculoskeletal, neural, and metabolic development, broadening the potential biological context of DLC.

### Justification for the deprioritization of additional genes

Several genes located within significant genomic intervals were deprioritized for functional interpretation despite showing statistical association with DLC. These include genes with **ubiquitous cellular roles** (e.g., *MRPL33*, *COMMD1*, *TFB2M*, *SMYD3*) where disruption typically causes systemic rather than tissue-specific effects; broadly **pleiotropic developmental regulators** (*BRAF*, *GRIP1*, *ZBED3*, *PDE8B*) whose known functions are multi-systemic and inconsistent with the localized, conformation-dependent nature and isolated presentation of DLC; and genes primarily associated with **smooth muscle biology, distal myopathy, or generalized excitability disorders** (*RBPM52*, *TAF44*, *KCNJ5*, *KLHL9*), which do not match the neuromuscular phenotype observed in affected horses. Finally, several genes linked to **ciliary dynamics and vesicle transport** (*WDPCP*, *EHBP1*, *VPS54*) have only indirect or speculative relevance to laryngeal or neuromuscular function. While these genes may act as secondary modifiers, their biological plausibility as primary contributors to DLC is weaker compared to the candidate genes with direct roles in neuromuscular coordination, contractile function, or craniofacial development.

## Discussion

Understanding how multiple genomic regions interact to shape complex phenotypes remains a major challenge in genetics. In this study, we used dynamic laryngeal collapse (DLC) as a model to investigate the genomic architecture of a multifactorial airway disorder involving coordinated neuromotor, musculoskeletal, and developmental processes. DLC affects several performance horse breeds, including Norwegian-Swedish Coldblooded Trotters, where it can cause moderate to severe upper airway obstruction during high-intensity exercise with important consequences for athletic performance and

welfare (Hanche-Olsen et al. 2010; Velie et al. 2020; Joó et al. 2021). The anatomical features predisposing to laryngeal compression during poll flexion have been well characterised (Fjordbakk et al. 2013), as has the associated physiological impairment of respiration during strenuous exercise (Strand et al. 2009).

Using whole-genome sequencing (WGS) and population genomic analyses, we identified 58 highly differentiated genomic regions associated with DLC susceptibility, providing evidence for a distributed polygenic architecture rather than a single major-effect locus. Among these, six genes—*ITPR1*, *ROBO1*, *RORA*, *TPM1*, *TLN2*, and *NR2F2*—emerged as the strongest candidates based on  $F_{ST}$  differentiation, expression in equine skeletal muscle, and functional relevance. A broader set of Tier 2 genes, including *GABRB3*, *LMOD3*, *KBTBD13*, *RSPO2*, *EOGT*, *SHH*, *ELOVL4*, and *RMND1*, implicates complementary pathways involved in neural development, musculoskeletal biology, and cellular metabolism. Collectively, these findings suggest that susceptibility to DLC reflects coordinated genomic differentiation across multiple interacting biological systems rather than disruption of a single molecular pathway.

Previous SNP array-based analyses identified a candidate locus for DLC on chromosome 7 but provided limited resolution to refine the underlying genetic architecture (Velie et al. 2020). By applying whole-genome sequencing together with population genomic analyses, we were able to characterise genomic differentiation at nucleotide resolution across the entire genome, revealing 58 significantly differentiated regions rather than a single dominant susceptibility locus. This pattern is consistent with a distributed polygenic architecture in which multiple loci collectively contribute to disease risk.

Although the regions with the highest  $F_{ST}$  values represent the strongest signals of allele frequency divergence between affected and unaffected horses, focusing exclusively on these intervals would risk overlooking biologically relevant contributors. We therefore considered all genome-wide significant regions in our functional interpretation while prioritising the six Tier 1 candidate genes because they showed the strongest combination of genomic differentiation, expression in equine skeletal muscle, and biological plausibility.

Interestingly, two regions exhibited exceptionally high differentiation ( $F_{ST} > 0.7$ ): one encompassing a T-cell receptor gene cluster on chromosome 1 and another on chromosome 10 spanning a non-coding interval upstream of a pseudogene and *ELOVL4*. Although *ELOVL4* has recognised functions in fatty acid metabolism, its connection to laryngeal biology is less direct than that of the principal neuromuscular candidates. Nevertheless, these highly differentiated regions raise the possibility that regulatory mechanisms or immune-related processes also contribute to the complex genetic architecture of DLC and warrant further investigation.

### **Neuromuscular coordination and neural development**

Two of the top candidates, *ITPR1* and *ROBO1*, are directly involved in motor coordination and the formation of neural circuits. *ITPR1* encodes a calcium-release channel in cerebellar Purkinje cells, essential for precise motor timing (Zamboni et al. 2017), while *ROBO1* mediates axon guidance critical

for establishing functional motor pathways (López-Bendito et al. 2007). These functional roles are consistent with a contribution to the fine neuromuscular coordination required to maintain upper airway stability during poll flexion.

*RORA* modulates muscle tone and contributes to motor function (Li et al. 2022). Variants in these genes could subtly impair laryngeal motor neuron output, reducing neuromuscular stability under the mechanical stress of exercise or poll flexion. Additional differentiated loci, including *SPG21*, *DOK5*, and *SEMA6A*, reinforce the role of neural development, axon guidance, and synaptic connectivity in maintaining laryngeal patency (Simpson et al. 2003; Wen et al. 2009; Kerjan et al. 2005). Together, these genes support a polygenic model in which variation affecting neural signalling and circuit formation contributes to susceptibility by altering the timing, strength, or reliability of laryngeal muscle activation.

### **Skeletal and craniofacial structure**

Structural predispositions of the upper airway are central to DLC. *NR2F2*, *RORA*, and *GABRB3* influence cranial neural crest-derived skeletal development and craniofacial morphogenesis. Dysregulation of these genes may alter laryngeal positioning, intermandibular spacing, and head/neck posture, increasing mechanical vulnerability (Li et al. 2022; Barske et al. 2018; Okeke et al. 2022; Culiati et al. 1995; Oh et al. 2010; Inoue et al. 2008). Additional candidate genes, including *EOGT* and *SHH* further support the involvement of developmental pathways influencing anatomical risk factors in DLC (Li et al. 2022; Alam et al. 2020; Pakvasa et al. 2021).

### **Muscle development and contractile function**

Top candidates *TPM1* and *TLN2* encode sarcomeric and cytoskeletal proteins that maintain actin-myosin interactions and link the actin cytoskeleton to integrins, ensuring coordinated contraction of intrinsic laryngeal muscles. Disruption of these genes could compromise muscle performance during high mechanical demand (England et al. 2017; Jin et al. 2016; Conti et al. 2008; Conti et al. 2009). Other differentiated loci, including *LMOD3*, *KBTD13*, and *RSPO2* (Schultz et al. 2023; Yuen et al. 2014; Sambuughin et al. 2010; De Winter et al. 2024; Kazanskaya et al. 2004; Bentzinger et al. 2012), support sarcomere assembly, thin filament organization, and myogenesis, while *AGK*, *COX7A1*, *ELOVL4*, and *RMND1* highlight the importance of mitochondrial function, energy metabolism, and membrane integrity for muscular endurance (Mayr et al. 2011; Feng et al. 2022; Yeboah et al. 2021; Janer et al. 2015). Together, these genes illustrate how impairments in contractile machinery or cellular energy supply may reduce the ability of intrinsic laryngeal muscles to withstand the mechanical stresses imposed during poll flexion.

### **Cytoskeletal and regulatory modifiers**

Genes affecting cytoskeletal organization, membrane integrity, and intracellular signaling may modify DLC risk. Tier 2 candidates such as *ANXA2*, *CCT4*, and *UBA3* contribute to protein folding, structural maintenance, and cellular resilience (Defour et al. 2017; Vallin and Grantham 2019; Zhang et al. 2024),

thereby supporting the anatomical and contractile machinery essential for laryngeal stability. Disruptions in these processes may reduce the ability of laryngeal tissues to maintain structural integrity under the repetitive mechanical demands of poll flexion.

## Genomic architecture of a complex performance phenotype

Collectively, our findings support a model in which DLC susceptibility arises from coordinated genomic differentiation across multiple interacting biological systems rather than disruption of a single major-effect locus. The strongest candidate genes, including *ITPR1*, *ROBO1*, *RORA*, *TPM1*, *TLN2*, and *NR2F2*, implicate neuromotor regulation, muscle function, and craniofacial development as central components of disease susceptibility, while additional differentiated loci reinforce the contribution of complementary developmental and physiological pathways. Together, these results indicate that upper airway stability during exercise depends on variation distributed across interconnected neural, structural, and muscular processes. This genome-wide pattern of differentiation supports a polygenic architecture underlying a complex performance-associated phenotype.

## Declarations

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### Authors contribution statement

G.L. and E.S. conceived and designed the study. R.N. performed the bioinformatics and statistical analyses. G.L., R.N., and J.C. drafted the manuscript. C.F. and E.S. contributed to sample collection, clinical evaluation, and interpretation of the veterinary aspects of the study. B.V. read and revised the manuscript. All authors reviewed, edited, and approved the final manuscript.

### Competing interests

The authors declare that they have no competing interests.

# Research ethics statement

All experimental procedures and sample collection methods were approved by the Ethics Committee for Animal Experiments in Uppsala, Sweden [Number: C 121/14] and/or the Norwegian School of Veterinary Medicine, NMBU. Owners provided informed consent for their horses to be included in this study.

## Data availability

All raw FASTQ files are deposited in the European Nucleotide Archive (ENA) with accession number PRJEB105700.

# References

1. (2019). "Picard Toolkit." Broad Institute, GitHub Repository. <https://broadinstitute.github.io/picard/>; Broad Institute.
2. Alam SMD, Tsukamoto Y, Ogawa M, Senoo Y, Ikeda K, Tashima Y *et al* (2020). N-Glycans on EGF domain-specific O-GlcNAc transferase (EOGT) facilitate EOGT maturation and peripheral endoplasmic reticulum localization. *Journal of Biological Chemistry* **295**(25): 8560-8574.
3. Andersson L, Georges M (2004). Domestic-animal genomics: deciphering the genetics of complex traits. *Nat Rev Genet* **5**(3):202-12.
4. Andrews S (2015). FastQC: A quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
5. Barske L, Rataud P, Behizad K, Del Rio L, Cox SG, Crump JG (2018). Essential role of Nr2f nuclear receptors in patterning the vertebrate upper jaw. *Developmental cell* **44**(3): 337-347. e335.
6. Bentzinger CF, Wang YX, Rudnicki MA (2012). Building muscle: molecular regulation of myogenesis. *Cold Spring Harbor perspectives in biology* **4**(2): a008342.
7. Boyle EA, Li YI, Pritchard JK (2017). An Expanded View of Complex Traits: From Polygenic to Omnigenic. *Cell* **169**(7):1177-1186.
8. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L *et al* (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly (Austin)* **6**(2): 80-92.
9. Conti FJ, Felder A, Monkley S, Schwander M, Wood MR, Lieber R *et al* (2008). Progressive myopathy and defects in the maintenance of myotendinous junctions in mice that lack talin 1 in skeletal muscle. *Development* **135**(11): 2043–2053.
10. Conti FJ, Monkley SJ, Wood MR, Critchley DR, Müller U (2009). Talin 1 and 2 are required for myoblast fusion, sarcomere assembly and the maintenance of myotendinous junctions. *Development* **136**(21): 3597-3606.
11. Culiati CT, Stubbs LJ, Woychik RP, Russell LB, Johnson DK, Rinchik EM (1995). Deficiency of the  $\beta 3$  subunit of the type A  $\gamma$ -aminobutyric acid receptor causes cleft palate in mice. *Nature genetics*

**11(3): 344-346.**

12. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA et al (2011). The variant call format and VCFtools. *Bioinformatics* **27(15): 2156-2158.**
13. De Winter JM, Molenaar JP, Yuen M, Van Der Pijl R, Shen S, Conijn S et al (2024). KBTBD13 is an actin-binding protein that modulates muscle kinetics. *The Journal of clinical investigation* **130(2): 754-767.**
14. Defour A, Medikayala S, Van der Meulen JH, Hogarth MW, Holdreith N, Malatras A et al (2017). Annexin A2 links poor myofiber repair with inflammation and adipogenic replacement of the injured muscle. *Human molecular genetics* **26(11): 1979-1991.**
15. England J, Granados-Riveron J, Polo-Parada L, Kuriakose D, Moore C, Brook JD et al (2017). Tropomyosin 1: Multiple roles in the developing heart and in the formation of congenital heart defects. *Journal of molecular and cellular cardiology* **106: 1-13.**
16. Feng Y, Xu J, Shi M, Liu R, Zhao L, Chen X et al (2022). COX7A1 enhances the sensitivity of human NSCLC cells to cystine deprivation-induced ferroptosis via regulating mitochondrial metabolism. *Cell death & disease* **13(11): 988.**
17. Fjordbakk C, Chalmers H, Holcombe S, Strand E (2013). Results of upper airway radiography and ultrasonography predict dynamic laryngeal collapse in affected horses. *Equine Veterinary Journal* **45(6): 705-710.**
18. Fjordbakk CT (2014). Dynamic laryngeal collapse associated with poll flexion in harness racehorses: clinical and pathophysiological aspects (Philosophia Doctor Thesis 2014:33; ISBN 978-82-575-1930-8; ISSN 1503-1667). Norwegian University of Life Sciences, Ås, Norway.
19. Fjordbakk CT, Strand E, HANCHE-OLSEN S (2008). Surgical and conservative management of bilateral dynamic laryngeal collapse associated with poll flexion in harness race horses. *Veterinary Surgery* **37(6): 501-507.**
20. Fretheim-Kelly ZL, Halvorsen T, Clemm H, Roksund O, Heimdal J-H, Vollsæter M et al (2019). Exercise induced laryngeal obstruction in humans and equines. A comparative review. *Frontiers in Physiology* **10: 1333.**
21. Hanche-Olsen S, Rannem L, Strand E (2010). Bilateral dynamic laryngeal collapse associated with collection in high poll flexion in a gaited Icelandic horse. *Pferdeheilkunde* **26: 810-813.**
22. Inoue H, Kayano S, Aoki Y, Kure S, Yamada A, Hata A et al (2008). Association of the GABRB3 gene with nonsyndromic oral clefts. *The Cleft palate-craniofacial journal* **45(3): 261-266.**
23. Irewall T, Bäcklund C, Nordang L, Ryding M, Stenfos N (2021). High Prevalence of Exercise-induced Laryngeal Obstruction in a Cohort of Elite Cross-country Skiers. *Med Sci Sports Exerc* **53(6):1134-1141.**
24. Janer A, Van Karnebeek CD, Sasarman F, Antonicka H, Al Ghamdi M, Shyr C et al (2015). RMND1 deficiency associated with neonatal lactic acidosis, infantile onset renal failure, deafness, and multiorgan involvement. *European Journal of Human Genetics* **23(10): 1301-1307.**

25. Jin Y, Peng Y, Lin Z, Chen Y-C, Wei L, Hacker TA et al (2016). Comprehensive analysis of tropomyosin isoforms in skeletal muscles by top-down proteomics. *Journal of muscle research and cell motility* **37**: 41-52.
26. Joó K, Betancourt DD, Marin TV, Moyano LAP (2021). Evaluation of Overground endoscopy findings in Colombian criollo Paso horses. *Journal of Equine Veterinary Science* **99**: 103374.
27. Kalbfleisch TS, Rice ES, DePriest Jr MS, Walenz BP, Hestand MS, Vermeesch JR et al (2018). Improved reference genome for the domestic horse increases assembly contiguity and composition. *Communications biology* **1**(1): 197.
28. Kazanskaya O, Glinka A, del Barco Barrantes I, Stannek P, Niehrs C, Wu W (2004). R-Spondin2 is a secreted activator of Wnt/ $\beta$ -catenin signaling and is required for *Xenopus* myogenesis. *Developmental cell* **7**(4): 525-534.
29. Kerjan G, Dolan J, Haumaitre C, Schneider-Maunoury S, Fujisawa H, Mitchell KJ et al (2005). The transmembrane semaphorin Sema6A controls cerebellar granule cell migration. *Nature neuroscience* **8**(11): 1516-1524.
30. Li H, Durbin R (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**(14): 1754-1760.
31. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N et al (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**(16): 2078-2079.
32. Li M, Olotu J, Buxo-Martinez CJ, Mossey PA, Anand D, Busch T et al (2022). Variant analyses of candidate genes in orofacial clefts in multi-ethnic populations. *Oral diseases* **28**(7): 1921-1935.
33. López-Bendito G, Flames N, Ma L, Fouquet C, Di Meglio T, Chedotal A et al (2007). Robo1 and Robo2 cooperate to control the guidance of major axonal tracts in the mammalian forebrain. *Journal of Neuroscience* **27**(13): 3395-3407.
34. Mayr JA, Zimmermann FA, Horváth R, Schneider H-C, Schoser B, Holinski-Feder E et al (2011). Deficiency of the mitochondrial phosphate carrier presenting as myopathy and cardiomyopathy in a family with three affected children. *Neuromuscular Disorders* **21**(11): 803-808.
35. Oh W-J, Westmoreland JJ, Summers R, Condie BG (2010). Cleft palate is caused by CNS dysfunction in *Gad1* and *Viaat* knockout mice. *PLoS One* **5**(3): e9758.
36. Okeke C, Paulding D, Riedel A, Paudel S, Phelan C, Teng CS et al (2022). Control of cranial ectomesenchyme fate by *Nr2f* nuclear receptors. *Development* **149**(23): dev201133.
37. Pakvasa M, Haravu P, Boachie-Mensah M, Jones A, Coalson E, Liao J et al (2021). Notch signaling: Its essential roles in bone and craniofacial development. *Genes & diseases* **8**(1): 8-24.
38. Sambuughin N, Yau KS, Olivé M, Duff RM, Bayarsaikhan M, Lu S et al (2010). Dominant mutations in *KBTBD13*, a member of the BTB/Kelch family, cause nemaline myopathy with cores. *The American Journal of Human Genetics* **87**(6): 842-847.
39. Schultz LE, Colpan M, Smith Jr GE, Mayfield RM, Larrinaga TM, Kostyukova AS et al (2023). A nemaline myopathy-linked mutation inhibits the actin-regulatory functions of tropomodulin and leiomodulin. *Proceedings of the National Academy of Sciences* **120**(47): e2315820120.

40. Simpson MA, Cross H, Proukakis C, Pryde A, Hershberger R, Chatonnet A et al (2003). Maspardin is mutated in mast syndrome, a complicated form of hereditary spastic paraplegia associated with dementia. *The American Journal of Human Genetics* **73**(5): 1147-1156.
41. Strand E, Fjordbakk C, Holcombe S, Risberg A, Chalmers H (2009). Effect of poll flexion and dynamic laryngeal collapse on tracheal pressure in Norwegian Coldblooded Trotter racehorses. *Equine veterinary journal* **41**(1): 59-64.
42. Timpson NJ, Greenwood CMT, Soranzo N, Lawson DJ, Richards JB (2018). Genetic architecture: the shape of the genetic contribution to human traits and disease. *Nat Rev Genet* **19**(2):110-124.
43. Vallin J, Grantham J (2019). The role of the molecular chaperone CCT in protein folding and mediation of cytoskeleton-associated processes: implications for cancer cell biology. *Cell Stress and Chaperones* **24**(1): 17-27.
44. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A et al (2013). From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr Protoc Bioinformatics* **43**: 11 10 11-33.
45. Velie B, Smith P, Fjordbakk C, Solé M, Jäderkvist Fegraeus K, Rosengren M et al (2020). Exploring the genetics underpinning dynamic laryngeal collapse associated with poll flexion in Norwegian-Swedish Coldblooded Trotter racehorses. *Equine veterinary journal* **52**(2): 174-180.
46. Vermedal H, Strand E (2020). Dynamic laryngeal collapse associated with poll flexion as a complication of laryngeal tie-forward surgery in three harness racehorses. *Veterinary Surgery* **49**(3): 600-606.
47. Weir BS, Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. *evolution*: 1358-1370.
48. Wen J, Xia Q, Wang C, Liu W, Chen Y, Gao J et al (2009). Dok-5 is involved in cardiomyocyte differentiation through PKB/FOXO3a pathway. *Journal of molecular and cellular cardiology* **47**(6): 761-769.
49. Wood AR, Esko T, Yang J, Vedantam S, Pers TH, Gustafsson S et al (2014). Defining the role of common variation in the genomic and biological architecture of adult human height. *Nature genetics* **46**(11): 1173-1186.
50. Yeboah GK, Lobanova ES, Brush RS, Agbaga M-P (2021). Very long chain fatty acid-containing lipids: a decade of novel insights from the study of ELOVL4. *Journal of lipid research* **62**: 100030.
51. Yuen M, Sandaradura SA, Dowling JJ, Kostyukova AS, Moroz N, Quinlan KG et al (2014). Leiomodrin-3 dysfunction results in thin filament disorganization and nemaline myopathy. *The Journal of clinical investigation* **124**(11): 4693-4708.
52. Zamboni JL, Bellomo A, Ben-Pazi H, Everman DB, Frazer LM, Geraghty MT et al (2017). Spinocerebellar ataxia type 29 due to mutations in ITPR1: a case series and review of this emerging congenital ataxia. *Orphanet journal of rare diseases* **12**(1): 121.
53. Zhang H, Yang J, Song Q, Ding X, Sun F, Yang L (2024). UBA3 promotes the occurrence and metastasis of intrahepatic cholangiocarcinoma through MAPK signaling pathway: UBA3 promotes

## Figures

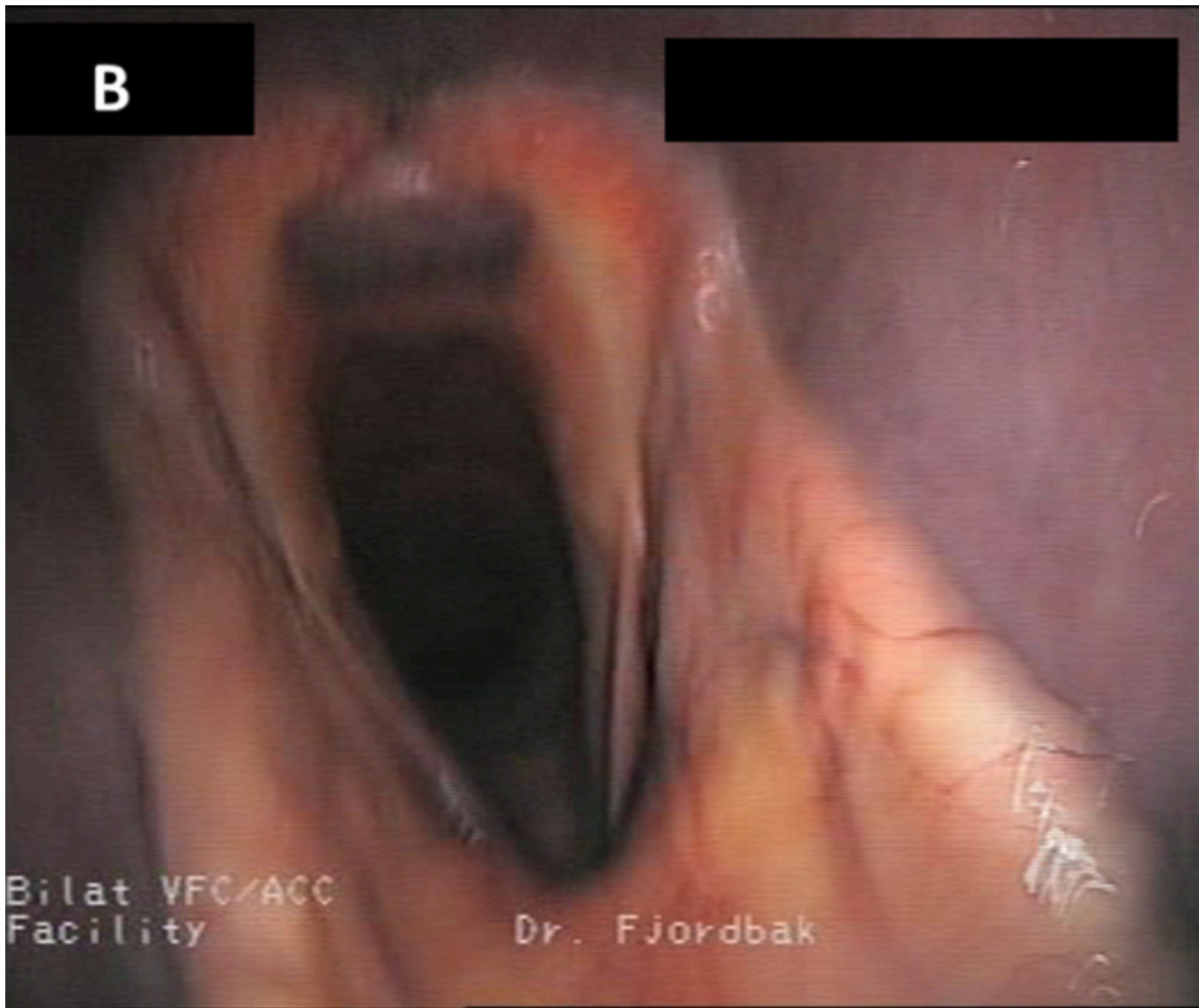


**Figure 1**

Photographs and corresponding endoscopic images during treadmill exercise of a Norwegian-Swedish coldblooded trotter horse diagnosed with DLC. In a neutral head position (A), the endoscopic appearance of the larynx is within normal limits (B). In poll flexion (C), bilateral collapse of the arytenoid cartilages and vocal folds is evident (D). Image adopted from Fjordbakk (2014).

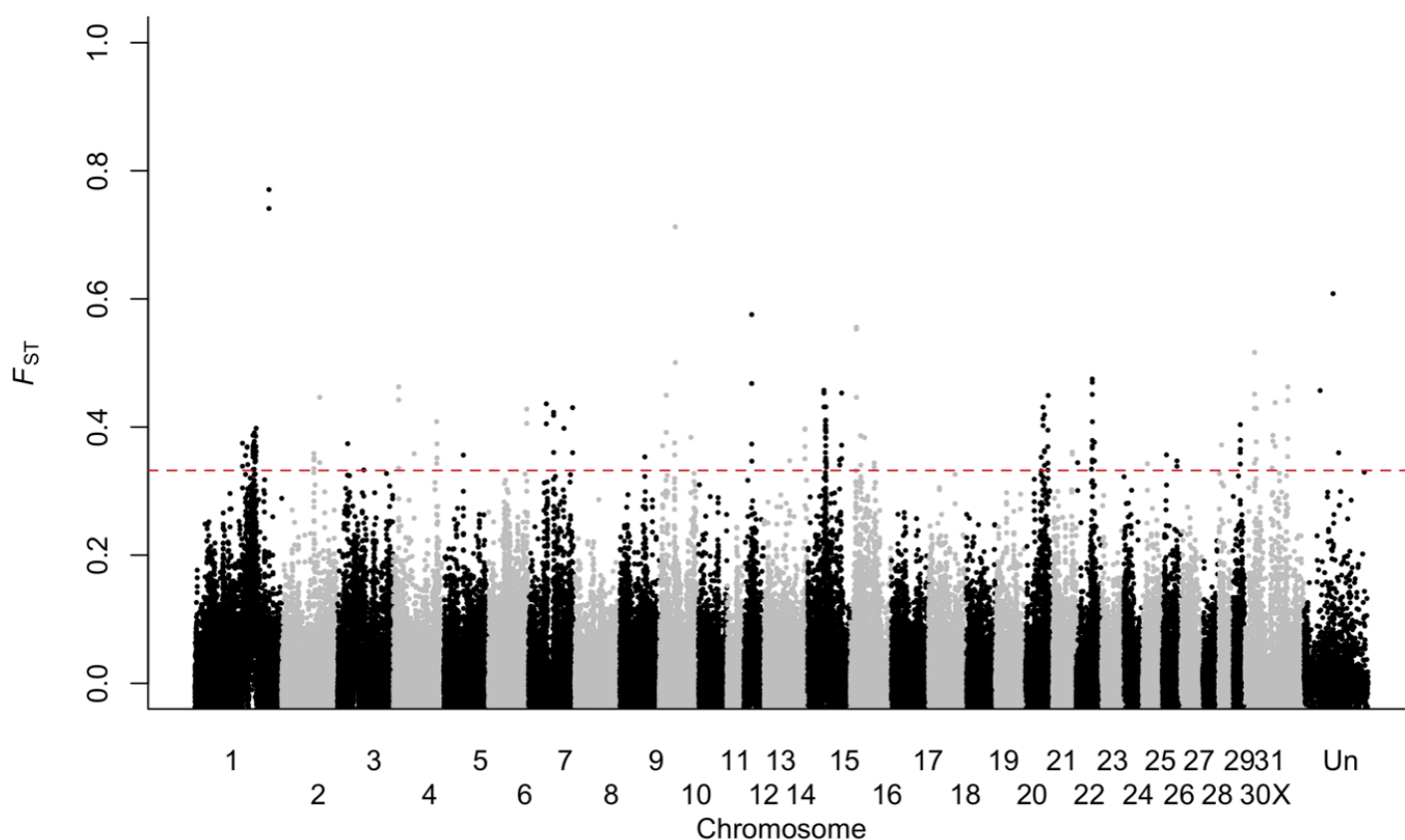


**Figure 2**

Manhattan plot displaying the  $F_{ST}$  values calculated between Norwegian-Swedish Coldblooded Trotters diagnosed with DLC and confirmed healthy controls. The red horizontal line represents the Bonferroni-adjusted significance threshold (0.332).

## Supplementary Files

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