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PRNP Gene: Non-Coding Variability in Norwegian Tundra Reindeer (R. t. tarandus) - A Bioinformatic Approach

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Introduction

1.1 Eurasian Tundra Reindeer in Norway:

The reindeer *(Rangifer tarandus* L.) is the only species belonging to the genus *Rangifer* (Gunn, 2016). It is a migratory species adapted to the seasonal shifts in the arctic and subarctic environment. The natural habitat stretches over Eurasia's and North America's arctic, alpine and boreal zones (Gunn, 2016; Harding, 2022). During the 20th century, reindeer were introduced into several areas outside their native range, including several islands in the Arctic and South Atlantic.

Rangifer tarandus, commonly known as reindeer or caribou, has multiple subspecies with distinct genetic and ecological traits. According to Harding (2022), the classification of these subspecies has historically been complex, with some being officially recognized and others remaining ambiguous. This often leads to broad generalizations across reindeer populations analyses, despite significant genetic differentiation revealed through modern molecular and phylogenetic analyses (Del Hoyo et al., 2011; Harding, 2022). In Eurasia *Rangifer tarandus tarandus* is the dominant sub-species and in Norway it is often referred as the Norwegian mountain reindeer. *R. tarandus tarandus* is adapted to the high-elevation alpine tundra, which exhibits characteristics like the Arctic tundra, where these reindeer exhibit specific adaptations.

After the last ice-age, reindeer was widely distributed in Europe (Banks et al., 2008). Today, only a small number of animals, all localized in Southern Norway, represent the remains of the former pan-European reindeer population. The wild reindeer populations in Norway are therefore considered unique, and they are specifically protected under the Bern Convention (Dorber et al., 2023). Norwegian authorities are managing its wild reindeer populations as a high priority "flagship" species. Reindeer in Norway, both wild and semi-domesticated, are a keystone species with significant ecological and cultural roles. Their grazing behavior shapes forest ecosystems by influencing soil chemistry, tree growth, and vegetation patterns, particularly benefiting epixylic lichen diversity (Mustonen, 2022; Olofsson et al., 2004). They affect soil carbon and nitrogen cycles, especially in subarctic regions, with long-lasting impacts on soil nutrient cycles and productivity (Mehli et al., 2000; Landauer et al., 2021)

Reindeer habitats are influenced by anthropogenic activities, elevation, and genetic interactions. They avoid areas near surface mining and prefer high-altitude regions to evade insect stress, steering clear of tourist areas and major roads (Eftestøl et al., 2019; Falldorf, 2013). Genetic changes due to introgression from domestic reindeer impact their adaptation to environmental conditions (Vistnes et al., 2008).

In addition to the wild reindeer populations, semi-domesticated reindeer, mostly held by the Sami people, are numerous in Norway (250 000), Sweden (250 000) and Finland (190 000). Interestingly, genetic evidence suggests that the semi-domesticated reindeer in Fennoscandia are of a different origin than the wild reindeer in Norway (Ministry of Agriculture and Food, 2023)

The wild tundra reindeer population in Norway is estimated to include a herd of about 25,000 animals (+/- 3,000 animals), with herd sizes approximately 20% larger in the summer. The largest wild reindeer population in Europe is found in Hardangervidda, Norway, with a winter herd of approximately 6,000 ± 200 animals.

1.2 Prion Protein (PrPC) and Prion Diseases:

The normal cellular prion protein (PrPC) encoded by the prion protein gene (PRNP) gene (Basler et al., 1986; Oesch et al., 1985), is a cell surface protein widely expressed in most mammalian tissue and particularly high in the central and peripheral nervous systems (Brown et al., 1990). Despite extensive research, the physiological functions of prions are still not fully understood (Wulf et al., 2017; Salvesen et al., 2019; Linden, 2017). Prions are unique pathogens comprising protein aggregates that induce incurable transmissible neurodegenerative diseases in mammals, including humans (Prusiner, 1998). These aggregates consist of a misfolded conformer (PrPSc) of PrPC (Glynn et al., 2020; Wang et al., 2020). During prion propagation, PrPSc binds to PrPC and templates the misfolding of PrPC into the PrPSc state, adding to the PrPSc aggregate. This process is most efficient when the primary structures (amino acid sequences) of the interacting PrP molecules are identical (Prusiner et al., 1990).

PrPSc was first isolated from the sheep disease Scrapie. Aggregates of PrPSc accumulate in the lymphoid tissues, peripheral tissues, and the central nervous system (CNS), leading to severe neurological symptoms and death. Prion diseases, formerly known as transmissible spongiform encephalopathies (TSEs), include Bovine Spongiform Encephalopathy (BSE) in cattle, Creutzfeldt-Jakob Disease (CJD) in humans and Chronic Wasting Disease (CWD) in the *Cervidae*.

CWD is a fatal neurodegenerative disease affecting captive, free ranging and wild members of the *Cervidae* family, including deer, elk, moose, and reindeer (Baeten et al., 2007; Spraker et al., 1997; Williams and Young, 1980). CWD was identified at first in a captive mule deer (*Odocoileus hemionus hemionus*) at a research facility in Colorado, USA, in the late 1960s (Williams and Young, 1980), and it has since spread to both wild and captive *cervid* populations in North America. The disease is now found in 34 U.S. states (Bryan J Richards), five Canadian provinces (Williams and Miller, 2002; Bryan J Richards, 2024), Norway, Finland, Sweden, (Benestad et al. 2016) and South Korea (Sohn et al., 2002), raising significant concerns for wildlife health and management. Transmission occurs through direct contact between animals or indirectly through environmental contamination. Prions are shed in bodily fluids such as saliva, urine, feces, blood, and even antler velvet and can persist in soil and water for years, complicating the eradication efforts (Gough and Maddison, 2010; Georgsson et al., 2006)

In 2016, CWD was detected in Europe for the first time, with cases reported in wild reindeer in the Nordfjella mountain range in Norway (Benestad et al., 2016)). This represented the first natural occurrence of CWD in a Rangifer subspecies and the first case outside North America. An additional 19 reindeer from the Nordfjella population tested positive for CWD during a stamping-out procedure, with two more cases found in Hardangervidda and two moose (*Alces alces*) in Selbu in the same year. In 2022, three red deer (*Cervus elaphus*) were diagnosed with CWD. The Norwegian authorities responded with aggressive measures, including the culling of the entire infected reindeer population in Nordfjella zone 1 (Uehlinger et al., 2016), totaling approximately 2000 animals, to prevent further transmission (Mysterud et al., 2019). Different strains have since been detected in moose and red deer in Norway, although these were of different strains than those found in reindeer.

In reindeer, all CWD cases tested positive for PrPSc in lymphoid tissues, whereas in moose and red deer, PrPSc deposits appeared confined to the central nervous system (CNS), with lymphoid tissues testing negative (Vikøren et al., 2019; Pirisinu et al., 2018; Ågren et al., 2021). Further investigations confirmed that North American CWD strains differ from those observed in Europe,

with the European strains affecting reindeer, moose, and red deer being distinct (Nonno et al., 2020)

Molecularly, the PRNP consists of three exons and two introns in most mammals, except for the one in humans with two exons (Bagyinszky et al., 2018; Choi et al., 2006; Lee et al., 1998). Exon 3 contains the open reading frame (ORF) which encodes the 771 amino acid PrPC protein (Seabury et al., 2007). The PRNP is located on chromosome 7 in reindeer (Figure 1).

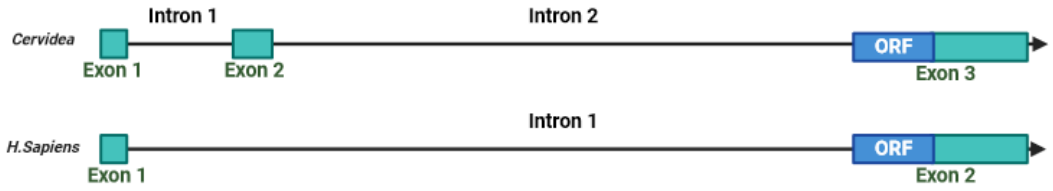

Figure 1 *The prion protein gene (PRNP) structure in Cervidae shown with three exons and two introns, compared to the PRNP structure for Homo Sapiens (H. Sapiens), which has only two exons and one intron. The open reading frame (ORF) that encodes the cellular prion protein (PrPc) is marked in blue in exon 3 for Cervidae and in exon 2 for H. Sapiens.*

1.3 Genetic Variation and its Role in Prion Disease

Genetic variation is crucial for the adaptability and survival of populations, offering resilience to environmental changes and diseases. Sequence variants (SVs), including single nucleotide polymorphisms (SNPs), insertions, and deletions, represent differences in DNA sequences among individuals within a population. These variations can significantly impact gene function and expression, influencing susceptibility to various diseases, including prion diseases such as CWD in cervids (Arifin et al., 2020).

Among Norwegian cervids, reindeer exhibit the highest number of non-synonymous variant positions in the PRNP coding locus, with seven identified alleles. In contrast, red deer and moose each have two alleles, and roe deer are monomorphic (Güere et al., 2022). The study by Güere et al.2020 compared PRNP allele variations (synonymous, nonsynonymous and indels) among Norwegian reindeer populations, including wild and semi-domestic sub-populations, have revealed significant differences. The PRNP alleles derived from nonsynonymous (six) and one indel variation were labeled A-G, showed varied frequencies, with allele G being extremely rare and excluded from detailed analysis. The most common genotypes were A/A, A/B, and B/B (Güere et al., 2022; Güere et al., 2020).

While variations within the coding region of PRNP are well-studied and it is known to influence CWD susceptibility, other regions of the gene can also play significant roles. Variations in the promoter region, pre-coding sections, and introns may affect gene expression and therefore the progression of prion diseases (Zink et al., 2020; Teferedegn et al., 2020; Seabury et al., 2007). For example, in bovines, certain deletions in the promoter region and intron 1 have been associated with an increased risk of Bovine Spongiform Encephalopathy (BSE), most likely due to variation in transcription rates caused by changes in the promoter's structure (Memon et al., 2018; Yang et al., 2018; Zink et al., 2020).

Despite the discovery of multiple non-synonymous variants in PRNP that are associated with susceptibility or resistance to prion disorders in various animals, such as goats and cats (Kdidi et al., 2021; Kim et al., 2020), the specific impact of intron changes on disease development has yet to be thoroughly understood (Kroll et al., 2022). Research into genetic variability in the PRNP in other species, such as quails, revealed novel SNPs in the 3'UTR but the impact of intronic changes on prion disease susceptibility has not thoroughly investigated (Kim et al., 2022)

Even not being translated into protein, the PRNP's non-coding sections, particularly introns and untranslated regions (UTRs), play a vital role in controlling mRNA synthesis and stability. In general, differences in gene UTRs greatly impact gene regulation by influencing transcription, translation, and post-transcriptional processes. UTR SNPs can influence miRNA binding, mRNA secondary structure stability, transcription factor binding locations, and gene expression levels, as well as the pathophysiology of disease (Shah et al., 2023; Wieder et al., 2023; Khan et al., 2022). Especially in disease-related genes. Longer 5'UTRs have related to genes that are intolerant to loss-of-function variants, showing the importance of post-transcriptional regulation to maintain proper mRNA and protein levels (Wieder et al., 2023). Changes in the 3' UTR length can lead to downregulation of gene expression, potentially causing diseases linked to the loss of specific miRNA binding sites (Navarro et al., 2021). These effects on mRNA could potentially modulate the susceptibility and progression of prion diseases such as CWD (Kroll et al., 2022).

In prion diseases, the role of intron variations in the PRNP is an emerging area of research. There is growing interest in understanding how variants in non-coding regions, including introns, contribute to disease susceptibility (Kroll et al., 2022).

1.4 Bioinformatics in PRNP

Initially, most of the molecular studies have focused on identifying cellular prion protein (PrPC) gene (PRNP) variations through techniques such as microsatellite analysis. These methods, while useful, offered limited resolution compared to more advanced tools available today. With the advent of high-throughput sequencing technologies, the focus shifted towards identifying genetic sequence variants (SVs), including single nucleotide polymorphisms (SNPs), gene insertions, and deletions. These variants represent differences in DNA sequences among individuals and can have significant impacts on gene function and disease susceptibility (Arifin et al., 2020).

State of the art bioinformatic tools like DeepVariant, GLnexus, and Beagel play a crucial role in uncovering non-coding areas of genes by using advanced computational analysis techniques. These tools help in identifying genetic variants that influence gene expression levels, such as expression quantitative trait loci (eQTLs), which can impact disease outcomes (Győrffy et al., 2018; Monti and Ohler, 2023; Zhernakova et al., 2013). DeepVariant, developed by Google, stands out as a cutting-edge, deep learning-based variant caller. It processes high-throughput sequencing data to locate SNPs and small indels with remarkable accuracy. When applied to whole-genome sequencing data, DeepVariant provides detailed variant calls, covering the entire gene in a population sample. This tool's ability to integrate data on somatic mutations and gene expression enables the detection of sequence alterations in non-coding regulatory regions, offering a comprehensive view of genetic variation (Poplin et al., 2018). To analyze the distribution of genetic differences within and between populations and species GLnexus is used, complementing DeepVariant by enabling the comparison of genetic variations across multiple individuals. This is crucial for understanding how evolution and selection pressures

changing genetic structures over short timescales, providing insights into disease outbreaks and gene function (Yun et al., 2021). In diseases like the prion diseases bioinformatics uncovers how genetic variants, including those in non-coding regions, influence gene expression and contributes to genetic diversity and disease susceptibility.

1.5 Significance of the Study:

The PRNP gene encodes for the prion protein, with its regulation and coding sequences playing crucial roles in protein expression and function. Variations in the upstream regulatory regions and the 5' untranslated region (UTR) can alter transcriptional and translational efficiency, while changes in the coding sequence can affect the prion protein's structure and stability. Using the state of the art in throughput sequencing technologies and bioinformatics, this study looks at the variation in the pre-coding sequence and the intronic regions of the PRNP.

2. Material and Methods.

2.1 Short read sequencing

This study utilized two individuals of *Rangifer t. tarandus* (1071 and 557) from the Filefjell reindeer population, previously selected for a whole-genome sequencing project focused on investigating genetic variation related to chronic wasting disease (CWD) susceptibility. Genomic DNA was extracted from ear tissue samples using the QIAGEN DNeasy Blood & Tissue Kit (Qiagen, Germany), with DNA concentration and purity assessed using Nanodrop (Thermo Fisher Scientific, USA).

For SNP identification, the DNA samples were sent to Novogene UK. DNA libraries were prepared using the NEB Next Ultra II DNA Library Prep Kit (NEB, USA) and sequenced on the Illumina NovaSeq 6000 machine with S4 Flow-cells (Illumina, USA). This setup generated approximately 60Gb of pair-reads data per sample, corresponding to around 20X coverage of the reindeer genome.

2.2 Alignment

The sequencing reads were aligned to a partially assembled reindeer genome from Hardangervidda, Norway (GenBank accession GCA_019903745.1) (Kiel, 2021) and was used as a reference genome. BWA (version 0.7.17) tool was used for alignment. The reference genome was first indexed using the 'bwa index' command. The paired-end reads of each individual were then aligned to the reference genome using BWA mem 2 (Li, 2013), using the 'mem' parameter and generating SAM files for each sample. SAMtools (Danecek et al., 2021) was then used to convert and sort the SAM files directly into sorted BAM files using the command 'sammtool sort', organizing the reads to the right genomic position. The sorted BAM files were then indexed with 'samtools index' creating 'bai' files for downstream variant calling analysis.

2.3 Variant discovery

The variant calling analysis, was performed using the DeepVariant (version 1.6.0) tool (Poplin et al., 2018). The input data included the sorted BAM files and the masked and indexed reference genome. DeepVariant was configurated to use parameters tailored for whole genome

sequencing (WGS) data to generate the output files in VCF format with the identified variants and gVCF which including areas without detected variants.

2.4 Variation of PRNP-Gene

To examine variation in and around the PRNP in reindeer, the genomic position of this gene was identified by aligning a partly PRNP sequence (GenBank accession: DQ154293.1) (Happ, 2005) for the coding region of the PrP of a reindeer and a full genome PRNP sequence (accession: NW_018342841.1) from a white tail deer to the reindeer reference genome using Minimap2 (version 2.26.) (Li, 2021). Since a full sequence for the complete PRNP of *R. tarandus tarandus* was not available at the time of this study, the PRNP sequence from a white teil deer (*Odocoileus virginianus*) was used instead. The exact coordinate's location of the PRNP within the reference genome was then determined using SAMtools (Danecek et al., 2021) and an in-house written awk script. BED file was created based on the *R. tarandus tarandus* "JAHWTM010000007.1" and included the region spanning of the reindeer reference PRNP gene.

To explore variations within and surrounding the PRNP reading frame, the gVCF files produced by DeepVariant from each individual reindeer were combined into a single comprehensive file representing the reindeer population, for analyzing the collective genetic variation across all samples. The combination of VCF files was achieved using GLnexus (version 1.4.3) (github.com/dnanexus-rnd/GLnexus) producing a single binary BCF file.

A BCFtools (version 1.17) was used to convert the binary BCF file into a standard VCF format. This conversion was performed using 'pigz' to efficiently compress the output VCF file and then phased using the tool Beagle (version 5.2). Phasing with Beagle was specified with the parameter "`gt=`" allowing the identification of phased variants across the PRNP coordinates region previously detected, creating a phased variant calling file.

The variant positions within the PRNP genotypes were examined by manual inspection the aligned reads using the Integrative Genomics Viewer (IGV) (Lin et al., 2023).

3.Results

3.1 Manual inspection of sequencing variants of the PRNP coding region

The PRNP gene located on chromosome 7 was used for a variant calling for the two reindeer individuals 1071 and 557. After rRad mapping to the reference region, a total of 41 variants were detected in individual1071 and 37 variants in 577. Punctual analysis of the PRPN region detected 57 variations across individual 1071 and 557. Table 1 includes all of the variants manually extracted from the VCF file. Figure 2 the CVN panel shows all identified variants in the intron and exon region are illustrated based on the IGV output (Appendix 1). To determine the ORF's exact position within exon 3, a 771 bp sequence from a partially sequenced PRNP from reindeer was used. The rows marked in green in Table 1 were identified to be variants within the ORF and were excluded from this study to focus on non-coding regions within the gene's exon and intron regions.

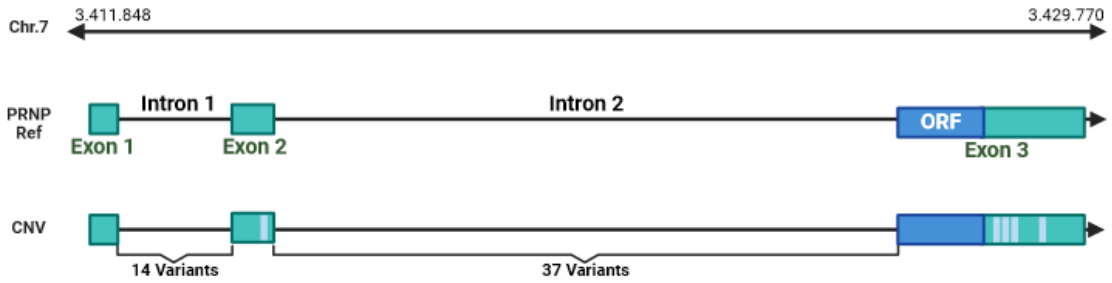

***Figure 2 PRNP:**: The prion protein gene (PRNP) shown in its structure with its three exons and two introns. Exon 3 includes the open reading frame (ORF) that encodes the cellular prion protein (PrPc). The "PRNP Ref" panel illustrates the structural composition of the PRNP gene in Cervidae. The "CNV" panel shows the number of variants identified in this study on chromosome JAHWTM010000007.1. (Chr.7). There are 14 variants found in Intron 1 and 38 variants in Intron 2. The exact positions of the variants within exons are marked by blue lines. These variants include single nucleotide polymorphisms (SNPs), insertions and deletions (indels), observed in the PRNP gene between the sampled individuals.*

3.1.1 Variation in the Exon Region

After the alignment of the full PRNP sequence from a white tail deer, the positions of the exon regions were identified on the chromosome 7 in the reindeer population and marked blue as illustrated in Figure 2. Since a complete PRNP sequence of a reindeer was not available at the time of the study, the PRNP sequence from a white tail deer was used. To identify the exact position of the ORF, the partial PRNP sequence from a reindeer was also aligned to the reindeer population.

Exon 1 was found to be located at the position 3411848 and spans 93bp. No variations were observed in exon 1, indicating high stability and conservation in this gene region. Exon 2 was located at position 3414375 with a length of 72bp. A single heterozygous SNP was detected at position 3414423 in the individual 1071. This SNP could reflect functional diversity or evolutionary adaptation in that position.

Exon 3, which includes the ORF begins at position 3428286 and extends over 1,485bp. Using the PRNP from the reindeer the start position of the 3'UTR was identified at position 3428999. The full length of the 3'UTR is 782bp. The 3'UTR plays an important role in the stability of the mRNA and potentially the production of PrP (Kroll et al., 2022). At positions 3429146 and 3429541, both individuals showed homozygous SNPs, indicating a fixed or very common variation in these regions. The other two variations in exon 3'UTR region of exon 3 were heterozygous and specific to each individual. At position 3429169, individual 1071 and at position 3429182, individual 557 displayed a heterozygous SNP.

3.1.2 Variation in the Intron Region

The highest number of CNVs were observed in the intronic region in the PRNP. Intron 1, located between position 3411848 and 3411940, contained a 55 bp insertion at position 3412764 along with a three base pair deletion at position 3412868, both of which were heterozygous for Individual 1071. At position 3412377 Individual 1071 shows a homozygous SNP.

Intron 2 starting at position 3414448 and extending 13,039 bp showed a total of five insertions and one deletion. Four of the insertion were structurally identical between the two individuals. An insertion at position 3419152 with 4 base pairs following the allele T was homozygous for individual 1017 and heterozygous in individual 557. Additionally, at position 3414494, 3419629, 3425991, 3426454 showed heterozygous 557 and homozygous 1071 SNPs, and 3415646, 3422295, 3427737 displayed insertions homozygous in both individuals.

3.1.3 Amino acid changes

No structural variants were observed in the region between exon 2 and exon 3 within intron 2 that could lead to an early start codon. This could imply that the regulatory and splicing region flanking these exons are conserved, preserving the gene's functional integrity.

It is observed that individual 1071 exhibited a higher level of genetic variability with 41 sequence variations compared to 37 in individual 557. The outcome of the analysis showed that individual 1071 more homozygous variation, while individual 557 only has heterozygous variation, expect where homozygote variants were shared.

Table 1 lists all the genomic variations found in the PRNP gene across the specified genomic positions, detailing the reference allele and the alternative allele for two individuals, 1071 and 557. Alternative alleles are highlighted in orange to indicate the variations present in these individuals. Variations where individual 1071 is homozygous are marked in light orange. Blue highlights denote structural variations that are identical between individuals 1071 and 557.

Table 1 Genomic Variation in the PRNP Gene: *genomic variation found in the PRNP gene in the chromosome JAHWTM010000007.1. The genomic position detailing the reference allele and the alternative allele for two individuals 1071 and 557. The alternative alleles are marked in orange indicating the variations between the individuals. Variations where individual 1071 is homozygous are marked light orange. The blue markings indicate structural variation that are identical between 1071 and 557. Green highlights the variations in the PRNP coding region.*

	Position	Reference allele	Alternative allele	1071	557	Poly
Intron 1	3412318	G	A	0|0	0|1	SNP
Intron 1	3412377	G	A	1|1	0|0	SNP
Intron 1	3412465	G	T	0|1	0|0	SNP
Intron 1	3412605	G	A	0|0	0|1	SNP
Intron 1	3412703	T	C	0|0	0|1	SNP
Intron 1	3412760	C	A	0|0	0|1	SNP
Intron 1	3412764	A	AGGAGGGATCTCGGTCATAAGGAGACTGAGGGGAGGGGATCAGGCG	1|0	0|0	Insertion
Intron 1	3412840	A	T	1|0	0|0	SNP
Intron 1	3412868	CGAG	C	0|1	0|0	Deletion
Intron 1	3412872	G	A	0|0	0|1	SNP
Intron 1	3413289	G	C	0|0	0|1	SNP
Intron 1	3413814	C	T	0|1	0|0	SNP
Intron 1	3414003	A	G	0|0	1|0	SNP
Intron 1	3414085	C	T	0|1	0|1	SNP
exon2	3414423	C	T	1|0	0|0	SNP
Intron 2	3414494	T	C	1|1	1|0	SNP
Intron 2	3414761	A	G	0|1	0|0	SNP
Intron 2	3414923	C	CT	0|1	0|1	Insertion
Intron 2	3414967	TA	T	0|1	0|1	Deletion

Intron 2	3415447	T	C	1|0	1|0	SNP
Intron 2	3415625	G	C	0|1	0|1	SNP
Intron 2	3415646	A	ATATGGATATTTTTGTTGTTGT	1|1	1|1	Insertion
Intron 2	3415782	G	A	0|1	0|1	SNP
Intron 2	3416666	A	C	0|0	1|0	SNP
Intron 2	3416871	C	G	0|0	1|0	SNP
Intron 2	3418140	C	T	0|1	0|0	SNP
Intron 2	3418835	G	A	1|0	0|0	SNP
Intron 2	3419047	A	G	0|1	0|0	SNP
Intron 2	3419152	T	TAGAG	1|1	1|0	Insertion
Intron 2	3419301	A	G	0|0	0|1	SNP
Intron 2	3419629	A	G	1|1	1|0	SNP
Intron 2	3419662	G	A	0|1	0|0	SNP
Intron 2	3420027	T	C	0|1	0|0	SNP
Intron 2	3420615	A	G	1|0	0|0	SNP
Intron 2	3421203	G	C	0|0	1|0	SNP
Intron 2	3422295	G	GCTCCCCAAGCTGAAGCTGAA GCTGAAGCT	1|1	1|1	Insertion
Intron 2	3422349	A	C	1|0	0|0	SNP
Intron 2	3422535	T	C	0|0	1|0	SNP
Intron 2	3423067	C	G	1|0	0|0	SNP
Intron 2	3423220	G	A	0|1	0|1	SNP
Intron 2	3423251	A	G	0|1	0|0	SNP
Intron 2	3423381	C	A	0|0	0|1	SNP
Intron 2	3423936	T	G	0|1	0|0	SNP
Intron 2	3424725	A	T	1|1	1|1	SNP
Intron 2	3425991	G	A	1|1	1|0	SNP
Intron 2	3426393	G	C	0|1	0|0	SNP
Intron 2	3426454	C	A	1|1	0|1	SNP
Intron 2	3427435	G	A	0|1	0|0	SNP
Intron 2	3427445	C	G	0|0	1|0	SNP
Intron 2	3427737	G	GCTATTCAGAAAATTATTTAATGA T	1|1	1|1	Insertion
Intron 2	3428143	C	T	1|0	0|0	SNP
Intron 2	3428144	G	A	0|0	0|1	SNP
Intron 2	3428161	C	T	1|1	1|1	SNP
ORF	3428299	G	A	0|1	0|0	SNP
ORF	3428680	G	A	0|1	0|0	SNP
ORF	3428800	G	A	0|1	0|0	SNP
ORF	3428821	A	G	0|0	1|0	SNP
ORF	3428969	C	A	0|0	1|0	SNP
Exon 3 UTR	3429146	A	G	1|1	1|1	SNP
Exon 3 UTR	3429169	C	T	1|0	0|0	SNP
Exon 3 UTR	3429182	G	A	0|0	1|0	SNP
Exon 3 UTR	3429541	G	A	0|1	0|1	SNP

4. Discussion

In this study, we conducted a variant calling analysis on the PRNP gene of two tundra reindeer individuals from a Norwegian population, focusing on polymorphisms in the non-coding regions surrounding exon 3, the gene's main coding area. Our goal was to identify any polymorphisms that could influence expression levels of PrPC and potentially be associated with increased susceptibility to Chronic Wasting Disease (CWD).

4.1 Variant Distribution

Reindeer is characterized by a high genetic variant among the species and population (Solovieva et al., 2022; Svishcheva et al., 2022b). Previously it was shown that in that even in conserver gene coding areas, reindeer exhibits the highest variation compared to other species (Svishcheva et al., 2022a). Same was seen in the variation distribution in individual 557 and 1071 in the intron and exon region of the PRNP gene. Table 1 includes 57 variants from which 14 marked in blue showed identical structural characteristics in both individuals, suggestion this variation may be fixed in the population. This pattern could reflect evolutionary pressures maintaining certain genetic traits within this reindeer population maintaining essential functions or population specific adaptation in the PRNP gene. Since this research used only two individuals further research will be needed for serenity.

4.2 Exon Variation

No variation was detected in exon 1, showing a high conservation in this region. Such stability could be either to a significant evolutionary pressure to maintain the integrity of the sequence or probably due to exon 1 critical functional role.

The single heterozygous SNP observed in exon 2 at position 3414423 in individual 1071 suggests a site of functional diversity or evolutionary adaptation. This variation could influence the regulatory process either downregulate or overregulate the production of PrP.

Exon 3 showed four distinct variations in the 3' UTR region. This region is crucial for the mRNA stability and translation efficiency impacting the PrP production. At positions 3429146 and 3429541, both individuals had homozygous SNPs, suggesting these are likely fixed variations within the population. The other two variations were heterozygous and unique to each of the individual. Individual 1071 had a SNP at position 3429169, and individual 557 had one at position 3429182. Theses variations could have an effect in mRNA stability or localization. Influencing the translation and therefore the regulation of the production level of PrP.

These findings overlap with the results of other studies. While exon 1 and exon 3 with the coding area can be found in all species, the exon 2 is not present in humans. Exon 3 and the coding area differ in size in different species, showing a higher liking for variation changes (Choi et al., 2006; Juan et al., 2013)

4.3 Intronic Variations

Intronic regions, historically viewed as non-functional, are now recognized for their importance in evolutionary processes within protein-coding genes. Recent studies have highlighted that intronic variations, such as copy number variants (CNVs), are prevalent and can significantly

influence gene expression by affecting regulatory mechanisms and splicing (Rigau et al., 2019; Juan et al., 2013). In-silico analysis here presented revealed significant variations in the intronic regions. In intron 1, individual 1071 showed at position 3412764 a long insertion with 55bp. The longer intron in individual 1071 could alter the regulation of the PRNP either over or under regulate the production of the PrPc. Intron 2 was more complex, with five insertions and one deletion. Four insertions were identical in both individuals, indicating these might be conserved population variations. At position 3419152, an insertion following the allele T was homozygous in individual 1071 and heterozygous in individual 557. This result showed the genetic diversity between the two individuals.

The identified intronic variations in the two individuals could influence splicing efficiency or introduce alternative splicing patterns, potentially producing prion protein isoforms which potentially could influence resilience or susceptibility towards CWD. To be certain more work needs to be done to understand how variation in the intronic region of the PRNP can affect the susceptibility in the prion diseases.

4.4 Regulatory Elements and Splicing Conservation

No structural variants in the region between exon 2 and exon 3 within intron 2 that could lead to an early start codon were found. This suggests that the regulatory and splicing regions flanking these exons are conserved, maintaining the gene's functional integrity. Such conservation is crucial for proper gene function and avoiding harmful effects on protein production and function.

Variations in exon 2 might contain specific regulatory elements, such as upstream open reading frames (uORFs), internal ribosome entry sites (IRES), or secondary structures, which are critical for controlling the translation rate and efficiency. Alterations in these regulatory elements could disrupt translation, leading to abnormal levels of prion protein, which is a key factor in the pathology of prion diseases (Movassat et al., 2019; Rigau et al., 2019). Nonetheless, further research I s necessary proper understand the impact of these variations

4.5 Genetic Variability in the Population and CWD control

The variations identified, especially those in the regulatory and intronic regions, could have significant implications for the expression and function of the prion protein. For example, improper splicing due to variations at splice donor or acceptor sites could lead to the retention of intronic sequences or exon skipping, producing non-functional or aberrant prion proteins. Such misfolding of prion proteins is central to prion diseases like CWD.

The 5’ UTR region primarily affects mRNA stability and translation. Variations in this region, resulting from improper splicing, could reduce the efficiency of ribosome loading and translation initiation, thereby decreasing protein production (Iacono et al., 2005). This could have downstream effects on the folding and function of the prion protein, critical factors in the pathology of prion diseases.

A downregulation of PrPc production may slow the progression of prion CWD. However, this could delay the first symptoms, potentially prevent the detection and removal of infected animals in time, which could exacerbate the spread of the disease, leading to delayed isolation and culling of infected individuals. A characteristic feature of CWD is a long incubation period, sometimes lasting several years, during which infected animals appear healthy, but can still spread the disease. As the disease progresses, affected animals develop exhibit a range of clinical signs, including weight loss, behavioral changes, excessive salivation, difficulty

swallowing, and lack of coordination (Sigurdson, 2008; Williams and Miller, 2002; Williams, 2005)

Management of CWD relies heavily on surveillance, testing, and containment strategies. Infected populations are monitored through regular testing of hunter-harvested animals, targeted culling, and environmental sampling (Mysterud et al., 2021).Currently, the market offers only a single test for detecting CWD (Burgener et al., 2022) and is at the current time not a common practice due to it novelty.

A significant challenge in controlling CWD is the persistent nature of the PrPsc in the environment. PrPsc can remain viable in soil and water for years and can be transmitted through all body fluids and tissues of an infected animal (Maddison et al., 2010; Sigurdson, 2008). This persistence poses a serious risk to herd animals, where one infected individual can potentially contaminate a large area and affect many others. Historical outbreaks of prion diseases in livestock provide a stark warning. For instance, during the Bovine Spongiform Encephalopathy (BSE) crisis in the 1990s and 2000s, millions of cattle were culled to prevent the spread of the disease (Nathanson et al., 1997; Beck et al., 2007). The rapid and widespread culling was a response to the highly infectious nature of the prion protein and the severe consequences of its spread (Nathanson et al., 1997; Marín-Moreno et al., 2016). Therefore, this shows, that more research in the areas around the main-coding area of the PRNP Gene is crucial, to understand and prevent the spread of CWD in reindeer and other *Cervidae*.

5. Conclusion

To the best of our knowledge, this work is the first uses high-throughout sequencing and variant calling to determine the polymorphic variation in the non-coding area of the PRNP in the *Rangifer t. tarandus*i. This research highlights the complexity genetic variations of the PRNP gene and the potential impact on the CWD resistance in reindeer. While the intronic variations we identified do not alter the protein sequence directly, they may have significant regulatory effects that could influence susceptibility to CWD. Nonetheless, further research is needed to understand the functional consequences of these variations, particularly their potential roles in splicing and translation regulation, and how they might contribute to disease resistance or susceptibility in reindeer populations.

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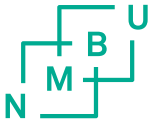
Appendix 1

Figure 2 IGV output: Exon 1 and exon 2

Figure 3 IGV output: Exon 3 in the upper panel and the Open reading frame in the lover panel.



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