UTU Publishing Group

Bulletin of Biomedical Science

Journal Homepage: www.utu.com.sg

REVIEW

NS3TP1 (ASNSD1/NBLA00058) and ASDURF: Unraveling Multifaceted Roles in Human Disease Pathogenesis and Therapeutics

Jun Cheng[1, 2], Hongping Lu[2], Liu Shi[3], Shumei Lin[3], Song Yang[1], Li Zhou[4]

[1] Liver Disease Center, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China
[2] Research and Development, Hebei Utu Pharmaceutical Com Ltd, Shijiazhuang, Hebei Province 052165, China
[3] Department of Infectious Diseases, First Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi Province 710061, China
[4] Department of Infectious Diseases, China-Japan Friendship Hospital, Ministry of Health, Beijing 100029, China

ARTICLE INFO

Received 10 July 2023
Accepted 12 August 2023
Online 6 September 2023

KEY WORDS:
NS3TP1;
ASDURF;
Multifaceted;
Pathogenesis;
Therapeutics;
ASNSD1;
Aspartate;
Fibrosis;
Steatosis;
Cancer;
Obesity;
Myosteatosis;
Huntinton's disease;
Osteoporosis;
Diabetes;
Medulloblastoma

Abstract

The gene NS3TP1, also known as ASNSD1 or NBLA00058, was initially identified within this cohort using the suppressive subtraction hybridization (SSH) technique as a target gene transactivated by the hepatitis C virus non-structural protein 3 in 2003. Subsequently, a small peptide encoded by a diminutive open reading frame (ORF) within the 5'-UTR of NS3TP1 mRNA was identified and designated as ASDURF. The regulatory mechanisms governing NS3TP1 expression encompass various levels, including the promoter region, miRNA-395/845 interactions, and other intricate pathways. Functional investigations have revealed that NS3TP1 plays a pivotal role in diverse pathological conditions, including liver fibrosis/cirrhosis, obesity, diabetes, cardiac-specific fibrosis, myosteatosis, Huntington's disease, osteoporosis, and cancer. Furthermore, ASDURF, as a small/short ORF-encoded peptide (SEP), not only contributes to the regulation of NS3TP1 gene expression but also holds significant potential in the context of medulloblastoma development in pediatric patients. As we embark on further exploration of the structural and functional aspects of the NS3TP1 gene and protein, the biological and medical implications of our findings stand to offer promising avenues for the treatment of human diseases.

Introduction

Following the completion of the Human Genome Project (HGP), the significance of functional genomics has escalated (Maxson et al., 2018). This burgeoning field has witnessed heightened attention on both coding and non-coding regions within the biomedical domain. The integration of large-scale sequencing and advanced bioinformatics techniques has

Corresponding author: Li Zhou, Department of Infectious Diseases, China-Japan Friendship Hospital, Ministry of Health, Beijing 100029, China. zhuimengzhouli@126.com

considerably advanced research on the structural and functional intricacies of the human genome (Way et al., 2021).

Three decades ago, chronic hepatitis B (CHB) and C (CHC) posed substantial health challenges in China. To unravel the molecular underpinnings of CHB and CHC, particularly their relevance to liver fibrosis/cirrhosis and hepatocellular carcinoma (HCC), molecular methodologies were employed to investigate the interactions between hepatitis B virus (HBV) and hepatitis C virus (HCV) with hepatocytes (Cheng et al., 2023). This endeavor resulted in the identification of 127 novel genes through diverse techniques. Among them, NS3TP1 (also known as ASNSD1 or NBLA00058) was unearthed using the suppressive subtraction hybridization (SSH) technique (Ji et al., 2004). Subsequent screening endeavors to elucidate the biological and medical significance of NS3TP1 unveiled an unexpected correlation with liver fibrosis/cirrhosis. Notably, aspartate (Asp) emerged as a regulator of NS3TP1 gene expression, in concordance with the presence of an asparagine synthetase domain within the NS3TP1 protein structure. Aspartate and its derivatives displayed promising therapeutic potential in a classic mouse model of CCl_4-induced liver fibrosis/cirrhosis (Shi et al., 2023; Zhou et al., 2023).

Recent investigations have unveiled a small open reading frame (ORF) within the 5'-UTR of NS3TP1 mRNA, leading to the identification of an encoded protein named ASDURF (Cloutier et al., 2020). ASDURF, serving as the twelfth subunit of PAQosome, assumes a pivotal role in regulating the expression of the NS3TP1 gene. Intriguingly, this protein exhibits a close association with the development of medulloblastoma in children.

In pursuit of a comprehensive comprehension of both NS3TP1 and ASDURF genes and their respective proteins, this review compiles the latest literature on their functions and mechanisms in liver fibrosis/cirrhosis, obesity, diabetes, cardiac-specific fibrosis, myosteatosis, Huntington's disease, osteoporosis, and cancer.

NS3TP1 gene and regulation

The NS3TP1 gene, also known as asparagine synthetase domain-containing 1 (ASNSD1) and indexed with GenBank accession No. AY116969, was first identified by our research group in 2004 (Ji et al., 2004). According to the National Center for Biotechnology Information (NCBI) database, the NS3TP1 gene spans 1392 base pairs and encodes a 642-amino-acid protein. Previous investigations have revealed that NS3TP1 is a target gene trans-activated by the hepatitis C virus nonstructural protein 3 (HCV NS3), as determined using the SSH technique. NS3TP1 exhibits widespread distribution throughout the human body (Vogel et al., 2020) and has been demonstrated to interact with the type 3 collagen α 1 chain (COL3A1) (Meienberg et al., 2010). NS3TP1 belongs to the family of Class II glutamine amido-transferases (GAT) d.153.1.1 (Linhorst et al., 2022) due to its classification as an asparagine synthetase domain-containing protein.

A dual Luciferase/Renilla expression technique was employed to identify the promoter for the NS3TP1 gene, situated within the 158/+30 base pair region. The selection of these regions and their boundaries was guided by the NRF1 and NFYB ChIP-seq datasets, which contain recognizable nuclear respiratory factor 1 (NRF1) peaks and NRF1 motifs (for GDPD1, ASNSD1, ZBTB17) (Benner et al., 2013). Understanding the regulation of NS3TP1 gene expression is complex and not fully elucidated. Gene expression primarily relies on two pivotal processes: transcription and translation. Traditionally, these processes have been considered distinct and largely uncoupled due to their varying timings, sites of action, and regulatory mechanisms, despite their shared purpose. However, there is potential for a connection between transcription and translation.

An unbiased screen of various human promoters has identified a TATA box on translation and revealed a general coupling between mRNA expression and translational efficiency. Employing a CRISPR-Cas9-mediated approach, genome-wide analyses, and in vitro experiments, it has been established that the transcription rate plays a pivotal role in governing translation efficiency. Additionally, m^6 A modification of mRNAs has been confirmed to occur co-transcriptionally and is contingent upon the dynamics of transcribing RNAPII. Suboptimal transcription rates can result in elevated m^6 A content, potentially leading to reduced translation. These findings have unveiled a widespread link between transcription and translation, regulated by the epigenetic modification of mRNAs.

Transcription and translation represent the fundamental layers of gene expression, with transcription being highly regulated at initiation, elongation, and termination stages. Recent studies in eukaryotes have revealed connections between transcription and other facets of mRNA regulation, such as alternative splicing, polyadenylation, localization, translation, and degradation. While splicing and polyadenylation are believed to be co-transcriptional processes, their influence on translation and degradation, which have distinct spatial and temporal dynamics, is more intricate to decipher. A proposed model suggests that transcription plays an imprinting role by recruiting coordinator proteins co-transcriptionally, which subsequently regulate the fate of the synthesized transcript.

Translation of mRNAs is predominantly controlled during initiation and elongation. Although recent studies have suggested certain dependencies between transcription and

translation, it remains unclear whether these interactions are limited to specific mRNA subgroups or represent a broader connection. N^6-methyladenosine (m^6 A) is one of the most prevalent RNA modifications, found in thousands of human transcripts. Several recent investigations have linked m^6 A to the regulation of splicing, translation, and degradation, suggesting its pivotal role in multiple mRNA regulatory levels. Using the ASNSD1 promoter as a model, the addition of a TATA box was observed to have a positive impact on translation efficiency under various conditions, indicating a robust phenomenon. The promoter region of the ASNSD1 gene produces better-translated Rluc mRNAs when supplemented with an artificial TATA box at its 3'-end. Across different stress conditions, the presence of the TATA box consistently enhanced translation efficiency.

Site-directed mutagenesis of the artificial TATA box reduced mRNA levels and prevented the super-induction of protein production. Additionally, when the TATA box sequence was mutated, Rluc mRNAs from ASNSD1 and SZT2 promoters exhibited differences in translation efficiency. RNA 5'-end analysis revealed that the TATA box facilitated precise focusing of the transcription start site (TSS) on the first ATG of the Rluc ORF.

These findings strongly indicate that m^6 A modification mediates transcription-responsive translation of NS3TP1/ASNSD1 gene expression (Slobodin et al., 2017). At the protein level, NS3TP1 regulates the SUMOylation of DNA damage response and repair proteins. Moreover, NS3TP1 mRNA is subject to regulation by small RNAs (Newton et al., 2019).

In a survey of expression quantitative trait loci (eQTLs) in lymphoblastoid cell lines (LCLs), differential eQTLs (deQTLs) were identified for 15 genes, including TBC1D4, MDGA1, CHI3L2, OAS1, GATM, ASNSD1, GLUL, TDRD12, PPIP5K2, OAS3, SERPINB1, ANKDD1A, DTD1, CYFIP2, and GSDME. NS3TP1 gene expression was found to be linked to polymorphisms in chromosome DNA sequences proximal to the NS3TP1 DNA region. While all 15 variants served as eQTLs for the deQTL genes in at least one cell or tissue type, the deQTLs for OAS1, OAS3, and GATM were not significant eQTLs in the CAP LCLs used in this study. It is noteworthy that statin treatment intensified the eQTL relationship for most deQTLs, but dampened it for others, such as ASNSD1. This is reflected in the weaker eQTL association with statin treatment compared to the eQTL association and the distinct directionality of the deQTL and eQTL associations. For instance, the association of the rs5742938 genotype with ASNSD1 statin-treated expression levels was less pronounced than its association with endogenous expression levels. These findings highlight the complex regulatory network of NS3TP1/ASNSD1 gene expression and its interaction with genetic and environmental factors (Theusch et al., 2020).

The length of the poly(A) tail in an mRNA plays a critical role in determining its translational efficiency, stability, and susceptibility to degradation. The regulation of polyadenylation and deadenylation processes in specific mRNAs is crucial for various biological processes, including oogenesis, embryonic development, spermatogenesis, and cell cycle progression (Akinci et al., 2009). Additionally, these processes are vital for synaptic plasticity.

To address these regulatory mechanisms, a novel technique for analyzing the length of poly(A) tails and fractionating mixed mRNA populations based on poly(A) tail length has been developed. This method can be applied to crude lysates or total RNA, offering speed, high reproducibility, and the ability to detect minor changes in the distribution of poly(A) tail lengths. To validate the technique, it was employed to analyze mRNAs known to undergo cytoplasmic polyadenylation during Xenopus laevis oocyte maturation. Subsequently, RNA from NIH3T3 cells was divided into two fractions based on the length of their poly(A) tails and subjected to microarray analysis. Combined with validation experiments, the results revealed that approximately 25% of expressed genes have poly(A) tails with fewer than 30 residues in a significant proportion of their transcripts. Interestingly, the NS3TP1/ASNSD1 gene was identified among the 47 mRNAs exhibiting short poly(A) tails (Meijer et al., 2007). Further investigation is required to elucidate the significance of a short poly(A) tail for NS3TP1 mRNA.

In the context of overwintering Tibetan frogs (*Nanorana parkeri*), the expression of the NS3TP1 gene was found to significantly decrease (Niu et al., 2023), suggesting tight regulation of NS3TP1 gene expression by body temperature, possibly linked to the determination of basal metabolic rate (BMR).

Integrin subunit α3 (ITGA3) has been associated with immune cell infiltration and serves as a favorable prognostic biomarker for breast cancer. DNA methylation, an epigenetic mechanism, plays a pivotal role in regulating gene expression. Notably, significant differences in methylation were observed for the top three genes between the ITGA3 gene expression-altered group and the unaltered group. These genes were identified as NPM1 (qValue = 1.73E−10), NS3TP1 (qValue = 20.1E−09), and MAK (qValue = 2.72E−08). These findings suggest that methylation modifications in the NS3TP1 promoter are dependent on the expression status of the ITGA3 gene (Li et al., 2021).

Regarding the transcription of a major human α satellite DNA in response to heat stress, the dynamics of "silent" H3K9me3 and "active" H3K4me2/3 histone marks were monitored at dispersed euchromatic and tandemly arranged heterochromatic α repeats. The study revealed an increase in H3K9me3 levels at dispersed α repeats immediately after

heat stress, along with downregulation of α repeat-associated genes in various human cell lines. NS3TP1 is among the genes associated with α repeats. Among genes linked to 20 dispersed α satellite repeats tested for insertion polymorphism, those exhibiting reliable expression levels in at least one cell line were selected. From the genes with α satellite elements located in introns, three showed very low expression levels, while the remaining seven genes were suitable for expression analysis. Additionally, 14 genes closest to intergenic α repeats were examined, with eight of them suitable for expression studies. It's important to note that SLC30A6, PRIM2, STAM, and MYO1E were associated with α repeats in introns, while SLC40A1, ASNSD1, ST6GAL1, HTRA3, ACOX3, INTS1, PHF20L1, and DIP2C were linked to intergenic α repeats. The study found that the presence of α satellite elements influenced the expression of associated genes. Expression was analyzed in MJ90 hTERT cell lines under standard conditions (no heat stress) and immediately following heat stress. These findings indicate a potential relationship between histone marks, α satellite DNA, and gene expression (Feliciello et al., 2020).

Furthermore, glutamine metabolism has been implicated in the regulation of NS3TP1 gene expression. Analysis of RNA-sequence data from glutamine inhibitor-treated mouse cell lines, obtained from the GEO database, revealed significant downregulation of genes related to glutamine metabolism, including Asns, Gfpt1, Ctps1, Cad, Pfas, Gmps, Fasn, Ppat, NS3TP1/ASNSD1, Glyatl1, Nr1h4, Acly, Glud1, Ctps2, Bloc1s6, Glul, Cps1, Gls, Gls2, Gfpt2, Lgsn. Notably, the regulatory effects of glutamine on NS3TP1 gene expression were also confirmed in the context of Alzheimer's disease, where glutamine metabolism genes were associated with immunogenicity and immunotherapy efficacy (Wu et al., 2023).

Lastly, Y27632, a small molecule inhibitor of cytoskeletal dynamics, was found to down-regulate the NS3TP1 gene by more than 1.8-fold in melanoma tumors. This observation suggests that NS3TP1 plays a role in tumor initiation and progression (Spencer et al., 2011).

Expression of NS3TP1 changed in different diseases

The study of ancestry and genetics remains a contentious field, yet it holds profound implications for understanding health disparities. Americans of African ancestry are known to face a significantly elevated risk of type 2 diabetes and cardiovascular diseases, with mortality rates up to eight times higher than Americans of European ancestry. These disparities persist even after accounting for social factors and access to healthcare. Exploring the genetic basis of these differences, it is conducted a rigorous analysis of single nucleotide polymorphisms (SNPs) using genetic samples from subjects undergoing cardiac-related evaluations. In comprehensive approach identified 151 differentially expressed genes between Americans of African and European ancestry, including NS3TP1. However, the link between NS3TP1 and these health disparities remains unconfirmed, and the underlying mechanism remains enigmatic. Furthermore, in a meta-analysis aimed at identifying candidate genes and molecular networks associated with type 2 diabetes mellitus (T2DM), NS3TP1, along with 17 other genes, was identified as potentially relevant. Similarly, NS3TP1 has been associated with a selective sweep linked to an increased risk of schizophrenia (SCZ) within certain populations. However, the precise relationship between NS3TP1 and these health conditions, as well as the mechanisms at play, remains to be elucidated, highlighting the need for further research in this complex intersection of genetics and health disparities (Williams et al., 1999; Lencz et al., 2007; Rasche et al., 2008; Schisler et al., 2009; Ramey et al., 2013; Boitard et al., 2021).

NS3TP1 and aspartate involved in liver inflammation and fibrosis

NS3TP1 has emerged as a pivotal regulator of liver inflammation and fibrosis. Initially, its involvement in liver fibrosis remained uncertain, but our investigation probed 127 genes associated with liver fibrosis/cirrhosis (Cheng et al., 2023). Findings revealed a significant reduction in NS3TP1 expression within mouse fibrosis/cirrhosis tissue induced by CCl_4 when compared to normal mouse liver tissue, employing the qPCR technique (Zhou et al., 2023).

Subsequently, in vitro studies using the human stellate cell line LX-2 involved both overexpression and silencing strategies for NS3TP1. NS3TP1 expression exhibited a substantial decrease in TGFβ1-stimulated LX-2 cells and CCl_4-induced fibrotic disease models, indicating a potential association between NS3TP1 and liver fibrosis. NS3TP1 was also found to be downregulated in LPS-induced inflammatory models.

We explored whether NS3TP1 played a role in regulating liver fibrosis and inflammation through both overexpression and silencing approaches. Successful overexpression or silencing of NS3TP1 in LX-2 cells resulted in significant changes in the levels of α-SMA, FN, COL3A1, NLRP3, and IL-1β, providing evidence of NS3TP1's ability to suppress HSC activation and extracellular matrix (ECM) production in vitro.

To consider NS3TP1 as a potential therapeutic target for liver fibrosis/cirrhosis treatment, the need for a small molecule compound becomes apparent. Analyzing its protein structure reveals the presence of an asparagine synthetase domain, leading to its alternative name, asparagine synthetase domain-containing 1 (ASNSD1) (Ji et al., 2004).

Exploring the literature, we uncovered that the concentration of aspartate significantly increased in the supernatant of cultured murine macrophages isolated from the abdominal cavity of mice treated with lipopolysaccharide (LPS) and/or galactosamine (Gal). Aspartate exhibited the capacity to reduce the release of NLRP3, NF-κB, pro-IL1, and IL18 from cultured macrophages when administered at a concentration of 15 mM. Our in vivo research further established that aspartate could alleviate inflammation and mitigate liver damage in mice subjected to LPS and/or Gal treatment.

Moreover, our investigation involved the use of aspartate to treat the cultured human stellate cell line LX-2, employing a concentration of 15 mM. Remarkably, aspartate exhibited a substantial reduction in COL1A1, COL3A1, α-SMA, and Smad3 expression, underscoring the potential of aspartate (ASP), acting as an NS3TP1 regulator, to exert potent effects against liver fibrosis/cirrhosis (Zhou et al., 2023).

To investigate the role of aspartic acid (Asp) in regulating liver fibrosis, we established an animal model of liver fibrosis. In this model, 6-week-old C57BL/6J male mice were subjected to a standard CCl_4-induced liver fibrosis protocol. Over a 4-week period, these mice were exposed to 2% CCl_4 via intraperitoneal (IP) injection, administered three times a week at a dose of 0.5 mL/kg. Concurrently, different groups of mice received varying amounts of Asp or a phosphate-balanced solution (PBS) daily via stomach tube. While Asp did not significantly alter the serological levels of hyaluronic acid (HA), laminin (LN), COL3A1, and COL4A1 in CCl_4-induced fibrosis, it notably reduced the expression of histological fibrosis indicators such as α-SMA, COL1A1, COL1A2, and COL3A1, ameliorating liver injury as evidenced by reduced serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. Histopathological changes in the liver were confirmed through hematoxylin and eosin (H & E) staining, Masson's trichrome staining, and Sirius red staining. Asp co-treatment effectively mitigated the fibrogenic response induced by CCl_4 IP injection.

Activation of hepatic stellate cells (HSCs) plays a pivotal role in initiating and advancing liver fibrosis. To further elucidate this, we conducted in vitro studies using LX-2 cells, a well-established human HSC cell line. Initially, LX-2 cells were exposed to various concentrations of Asp for 24 hours, resulting in a dose-dependent decrease in the expression of genes associated with HSC activation. Subsequently, when treated with 15 mM Asp, LX-2 cells displayed a time-dependent reduction in α-SMA, COL3A1, and fibronectin expression. We also explored the effect of Asp on TGF-β1-induced HSC activation, revealing that Asp hindered TGF-β1-mediated remodeling and extracellular matrix (ECM) deposition, as reflected in the reduced expression of α-SMA, COL3A1, and FN at both mRNA and protein levels. These findings suggested that Asp promoted the return of TGF-β1-activated HSCs to a quiescent state.

Previous research had shown that Asp supplementation reduced NLRP3 and β-arrestin-2 (ARRB2) expression, mitigated hepatic inflammasome levels, and conferred protection against acute inflammatory liver injury (Farooq et al., 2014).

Considering that NS3TP1, also known as ASNSD1, is closely related to aspartic acid metabolism, and previous experiments have confirmed Asp's inhibitory effect on liver fibrosis, we explored whether NS3TP1 was also associated with liver fibrosis. Initial investigations revealed the expression of NS3TP1 in fibrotic liver tissue and activated HSCs. Remarkably, overexpression of NS3TP1 led to significant decreases in the levels of α-SMA, FN, COL3A1, NLRP3, and IL-1β, whereas NS3TP1 knockdown resulted in striking increases compared to the control group. These findings highlighted NS3TP1's role in suppressing HSC inflammation and ECM production in vitro. Intriguingly, NS3TP1 was downregulated in LPS-induced inflammatory models but upregulated following Asp administration.

Subsequently, we delved into investigating whether NS3TP1 played a role in regulating liver fibrosis and inflammation through both overexpression and silencing approaches. Successful overexpression or silencing of NS3TP1 in LX-2 cells resulted in significant changes in the levels of α-SMA, FN, COL3A1, NLRP3, and IL-1β, providing evidence of NS3TP1's ability to suppress HSC inflammation and extracellular matrix (ECM) production in vitro. To summarize, NS3TP1 appears to be a key regulator of liver fibrosis and inflammation.

In addition to its role in liver fibrosis, NS3TP1 has also been implicated in the process of heart fibrosis. Fibrosis, characterized by abnormal matrix remodeling, occurs in response to various stresses and poses therapeutic challenges. Previous studies involving aged plasminogen activator inhibitor 1 (PAI-1) knockout mice revealed spontaneous cardiac-selective fibrosis. Gene expression profiling in these hearts and kidneys identified several genes associated with processes such as immune responses, stress, cytokine signaling, cell proliferation, adhesion, migration, matrix organization, and transcriptional regulation. Importantly, genes related to profibrotic pathways, including ANKRD1, PI16, Egr1, SCX, TIMP1, TIMP2, KLF6, LOXL1, and KLOTHO, were deregulated in PAI-1 knockout hearts. Notably, NS3TP1 was found to be closely linked to the development of selective cardiac fibrosis (Ghosh et al., 2013).

NS3TP1 and calcitriol involved in liver inflammation and fibrosis

In our research, we made a significant discovery regarding the

NS3TP1 protein and its influence on liver fibrosis. Through gene chip technology, we observed that NS3TP1 upregulates the expression of transforming growth factor beta receptor I (TGFβRI), a pivotal molecule in the activation of the classical transforming growth factor beta 1 (TGFβ1)/Smad3 pathway implicated in liver fibrosis. Consequently, we postulate a strong association between NS3TP1 and the onset of liver fibrosis.

Moreover, our investigation revealed a correlation between calcitriol, NS3TP1, and liver fibrosis. Calcitriol, a conventional remedy for vitamin D deficiency-related rickets, governs biological responses by binding to the vitamin D receptor (VDR). Remarkably, VDR is abundantly expressed in hepatic stellate cells (HSCs) and Kupffer cells, although its expression in hepatocytes is relatively weak (Pop et al., 2022). Intriguingly, VDR's functionality in HSCs and Kupffer cells positions it as a crucial modulator of the fibrogenic activity of HSCs, signifying the significant role of active vitamin D in hepatic fibrosis.

We conducted a series of experiments to substantiate our findings regarding NS3TP1 and its role in hepatic fibrosis. The upregulation of NS3TP1 was evident in activated HSCs and the livers of mice treated with CCl_4. Both in vivo and in vitro assessments confirmed the involvement of NS3TP1 in hepatic fibrosis. In CCl_4-induced fibrotic mice, histological analyses using hematoxylin-eosin staining, Masson staining, Sirius red staining, and immunohistochemical staining for α-SMA confirmed the successful establishment of fibrosis models. Elevated levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in mouse plasma corroborated evident hepatocyte injury. Hepatic fibrosis scores further substantiated disease progression, with higher Ishak scores observed in the CCl_4 group.

We proceeded to explore the impact of NS3TP1 on hepatic fibrosis through overexpression and silencing techniques in LX-2 cells. Western blot analyses demonstrated that NS3TP1 overexpression led to increased levels of collagen I and α-SMA compared to the negative control group. Conversely, in LX-2 cells with NS3TP1 gene interference, we observed significant downregulation of collagen I, collagen III, collagen IV, and α-SMA at both the protein and mRNA levels. Additionally, we evaluated the effect of NS3TP1 on HSC growth using a Cell Counting Kit-8 (CCK-8) cell proliferation and activity assay. This analysis demonstrated that NS3TP1 promoted HSC proliferation, a phenomenon reversed by silencing NS3TP1. Furthermore, we assessed cell apoptosis through the analysis of Bcl-2 and Bax levels, revealing that NS3TP1 inhibited HSC apoptosis.

To delve deeper into the mechanisms, we explored the impact of NS3TP1 on the TGFβ1/Smad3 and NF-κB signaling pathways. We observed that NS3TP1 overexpression enhanced the activity of both pathways, while NS3TP1 gene silencing reduced their activity. Furthermore, we used Smad3-specific inhibitor (SIS3) and licochalcone D (LD) to inhibit Smad3 and NF-κB phosphorylation, respectively, in LX-2 cells. We confirmed that NS3TP1 was downstream of Smad3 and p65, as the downstream genes, including collagen I, α-SMA, p-smad3, and p-p65, displayed reduced expression levels following inhibition.

Coimmunoprecipitation (Co-IP) assays unveiled the binding of NS3TP1 to Smad3 and p65, further substantiating its role in modulating these pathways. Dual luciferase assays revealed that NS3TP1 augmented TGFβRI promoter activity, but TGFβ1 had no reciprocal impact on NS3TP1 promoter activity. Similarly, NS3TP1 did not influence p65 promoter activity, yet p65 boosted NS3TP1 promoter activity. Hence, NS3TP1 might suppress the phosphorylation of Smad3 or p65 at the protein level, thereby inhibiting the activity of both signaling pathways. Furthermore, NS3TP1 may regulate the TGFβ1/Smad3 pathway at the mRNA level by upregulating the TGFβRI promoter, while the regulation of NS3TP1 and NF-κB signaling pathways at the mRNA level may occur through the upregulation of the NS3TP1 promoter by p65.

Moreover, our research unveiled the potential of calcitriol in ameliorating liver fibrosis. Through a series of in vitro experiments, we determined the optimal concentration of calcitriol (16 μmol/L) and treatment duration (48 h) for mitigating HSC activation and promoting their return to quiescence. This effect was accompanied by enhanced apoptosis, lipid accumulation, and reduced migration of activated HSCs. In vivo, we established the efficacy of calcitriol in reducing collagen accumulation in mouse livers with CCl_4-induced fibrosis. This outcome was corroborated by various staining techniques and the Ishak scoring system, which collectively demonstrated the improvement of hepatic fibrosis. Additionally, the reduction in plasma ALT and AST levels highlighted the attenuation of liver inflammation following calcitriol treatment.

Lastly, we explored the mechanisms underlying the anti-fibrotic effects of calcitriol, specifically its influence on NS3TP1, the TGFβ1/Smad3, and NF-κB signaling pathways. Calcitriol not only inhibited NS3TP1 expression at the mRNA and protein levels in CCl_4-treated mouse livers but also exerted a dose- and time-dependent inhibitory effect on both signaling pathways in LX-2 cells. Furthermore, calcitriol significantly suppressed the LPS-activated NF-κB pathway in HSCs. Notably, when NS3TP1 was overexpressed in LX-2 cells, calcitriol effectively mitigated LX-2 cell activation, suggesting that calcitriol's anti-fibrotic action operates downstream of NS3TP1.

NS3TP1 and degenerative myopathy and myosteatosis

In the genetic and genomic analysis of musculoskeletal differences between the Berlin high (BEH) and Berlin low

(BEL) mouse strains, no Single Nucleotide Polymorphisms (SNPs) were identified within a 0.4 Mb region encompassing the Mstn gene (myostatin mutant [Mstn(Cmpt-dl1Abc)]), including the adjacent Hibch and Asnsd1 genes. This absence of SNPs in the vicinity of NS3TP1 suggests that there is no close association between the SNPs around NS3TP1 and musculoskeletal development (Lionikas et al., 2013; Doyle et al., 2020).

To identify genes linked to myopathy, an extensive evaluation of over 4000 gene knockout (KO) mice was conducted, assessing histopathological characteristics. Among these, 12 KO mouse lines exhibited lesions indicative of autosomal recessive myopathy. Remarkably, the inverted screen test only detected muscle weakness in four of these 12 lines, namely Scyl1, Plpp7, Chkb, and NS3TP1/ASNSD1, all of which had previously been identified and published. This led to an increased focus on exploring the relationship between NS3TP1 defects and myopathies (Vogel et al., 2021).

Recent comprehensive evaluations have investigated the connection between NS3TP1 and degenerative myopathy and myosteatosis. Mice harboring an inactivating mutation in the gene encoding asparagine synthetase domain-containing 1 (ASNSD1) develop a progressive degenerative myopathy that leads to severe sarcopenia and myosteatosis. Notably, ASNSD1 gene expression is most prominent in skeletal muscle across the entire body.

While ASNSD1-/- mice may appear normal upon casual inspection, they frequently exhibited handling-associated seizures between the ages of 3 and 12 months. However, histological examinations of the brains of ASNSD1-/- mice did not reveal any lesions. Subsequent primary phenotypic screens for body composition in these mice, performed on both chow and high-fat-diet-fed mice, revealed an increased percentage of body fat and decreased lean body mass, corroborating the initial findings. Grip strength measurements further confirmed the muscle weakness in ASNSD1-/- mice, while systolic blood pressure was notably decreased.

Furthermore, NS3TP1's involvement in metabolic regulation was corroborated in a study examining spatio-temporal transcriptome dynamics, shedding light on the rapid transition of core crop functions in 'lactating' pigeons. To delve deeper into nutrient metabolism within hypertrophied crops, the study scrutinized the expression of eight candidate gene sets associated with lipid and protein synthesis and transport—two major components of pigeon 'milk'. Unsurprisingly, genes related to lipid and protein synthesis were upregulated in the hypertrophied crops, including key genes involved in lipogenesis and amino acid synthesis. Importantly, ASNSD1, responsible for asparagine biosynthesis, was among the upregulated genes.

NS3TP1 and Huntington's disease

Huntington's disease (HD) is the most prevalent among the polyglutamine diseases, a group of dominantly inherited neurological disorders characterized by the expansion of a CAG trinucleotide repeat, resulting in an elongation of encoded glutamine residues within a specific protein (Lieberman et al., 2018). While these diseases share the common feature of an inverse correlation between age of onset and the length of the mutant CAG repeat, each presents unique neuropathological characteristics and clinical manifestations.

While correcting the estimated CAG repeat lengths had limited impact on the GWAS results, other than significantly reducing signals on chromosome 4, it is prudent to investigate the multiple loci identified by genome-wide significance in GWA12345 as potential modifiers of HD onset. In GWA123, modifier loci on chromosome 8 and chromosome 15 were identified, with the latter displaying two independent opposing effects. In subsequent investigations, a modifier locus on chromosome 3 reached genome-wide significance. These same loci resurfaced in GWA12345, alongside several novel loci that exhibited genome-wide significance in either continuous, dichotomous analyses, or both.

Similar to the chromosome 3 (MLH1) and chromosome 15 (FAN1) loci, new loci on chromosome 2 (PMS1), 5 (MSH3/DHFR), 7 (PMS2), and 19 (LIG1) all harbor genes associated with DNA repair processes. However, the chromosome 8 locus (RRM2B/UBR5) and additional modifier sites on chromosomes 5 (TCERG1) and 11 (CCDC82) may not be directly linked to such mechanisms. Two other loci, on chromosome 11 (SYT9) and chromosome 16 (GSG1L), exhibited significant signals only from single, very low-frequency SNP alleles, hinting at a possible statistical artifact due to extreme phenotypic outliers. To firmly establish these loci as genuine modifiers, a larger sample size and/or functional analysis will be necessary.

Although no significant difference in age at onset between genders was observed, male- and female-specific association analyses unveiled differences in relative effect size and significance for certain modifiers. The most notable example was the MSH3/DHFR locus, where common modifiers had a considerably greater effect in females. Additionally, the sex-specific analysis revealed three new loci on chromosomes 1, 12, and 18 that may contain male-specific modifiers or, since they are linked to very rare alleles, could be influenced by statistical outlier effects.

Furthermore, a transcriptome-wide association study (TWAS) was conducted to broadly investigate the association between gene expression and residual age at onset. This analysis identified four genes at three loci that remained significant after Bonferroni correction: FAN1 (p = 1.9E-22), MSH3 (1.9E-

8), PMS1 (6.1E-7), and NS3TP1/ASNSD1 (5.3E-6). In these cases, later onset was associated with increased expression of FAN1, PMS1, and ASNSD1 and decreased expression of MSH3. Comparing regional heritability estimates before and after conditioning on expression using HESS revealed that 40%, 57%, and 87% of the contribution of FAN1, PMS1, and MSH3, respectively, to the genetic age at onset liability could be explained by cis expression effects (Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium, 2019).

The effects of NS3TP1 and other genes may play a crucial role in influencing HD pathogenesis through alternative mechanisms. These findings have profound implications for understanding the pathogenesis of HD and other repeat diseases, challenging the fundamental premise that polyglutamine length solely determines the rate of pathogenesis in polyglutamine disorders.

It's important to note that NS3TP1 is not limited to its involvement in HD but also plays a role in other neurological diseases, such as epilepsy. The NS3TP1 gene is notably among the genes with high gene significance values (HGS). Another HGS gene, ASNSD1, is associated with asparagine biosynthesis and exhibits decreased expression in epilepsy patients. ASNSD1, involved in processes related to memory, displays reduced expression in individuals with severe memory impairment (Bando et al., 2021). Asparagine synthesis is vital for brain development, and asparagine deficiency has been linked to learning and memory defects. In a genome-wide association study (GWAS) examining calving performance using high-density genotypes in dairy and beef cattle, NS3TP1 was found to be closely related to direct calving difficulty, as confirmed by meta-analysis across three breeds (Purfield et al., 2015).

NS3TP1 gene and postmenopausal osteoporosis

To uncover potential risk pathways associated with postmenopausal osteoporosis (PMOP) and establish a microRNA (miRNA)-regulated pathway network (MRPN), a systematic approach was employed. Initially, it is identified differential pathways by computing gene- and pathway-level statistics using accumulated normal samples, leveraging the individual pathway aberrance score (iPAS). it is also extracted significant pathways by analyzing differentially expressed genes (DEGs) through the DAVID tool. The subsequent step involved pinpointing common pathways that were shared between the iPAS and DAVID methods. it is then proceeded with miRNA prediction, which was executed by calculating TargetScore values, utilizing precomputed inputs such as log fold change (FC), TargetScan context score (TSCS), and probabilities of conserved targeting (PCT). Subsequently, it is constructed an MRPN by considering the common genes within these shared pathways and the predicted miRNAs.

In total, 279 differential pathways were successfully identified. Among these pathways, it pinpointed 39 DEGs, which exhibited enrichment in 64 significant pathways as identified by the DAVID analysis. Remarkably, 27 pathways were found to be common to both iPAS and DAVID methodologies. Notably, the MAPK signaling pathway emerged as the most significantly enriched pathway, followed closely by the PI3K-Akt signaling pathway. These findings underscore the potential pivotal roles of the MAPK and PI3K/Akt signaling pathways in PMOP development.

Furthermore, it has been identified the top 5 significant DEGs, namely PTGES2, HAB1, SCT, ASNSD1, and TUT1. Of particular interest, ASNSD1 exhibited a notable decrease in expression levels, with a fold change of -3.32248. This finding strongly suggests that NS3TP1/ASNSD1 plays a crucial and influential role in the pathogenesis of PMOP (Shao, 2017).

NS3TP1 and cancer

The tumor microenvironment (TME), comprising a cellular niche surrounding tumor cells consisting of immune cells, fibroblasts, and endothelial cells, has garnered increasing attention due to its pivotal role in tumor progression, metastasis, therapy resistance, and immune evasion. Recent advancements in single-cell transcriptomics have unraveled the oncogenic functions of TME in the context of esophageal squamous cell carcinoma (ESCC) tumorigenesis. In this regard, single-cell transcriptomic datasets derived from primary tumor samples of ESCC patients were meticulously analyzed to comprehensively characterize the TME. Intriguingly, this in-depth analysis of TME revealed distinct networks governing the interplay between T cells, myeloid cells, and fibroblasts, ultimately delineating specific ESCC subtypes and immunosuppressive mechanisms. Notably, these findings shed light on the potential involvement of NS3TP1/ASNSD1 in TME, which could impact the sensitivity of immunotherapeutic interventions (Ko et al., 2023).

Sialic-acid-binding immunoglobulin-like lectins (siglecs), a novel family of immunoregulatory molecules, have gained increasing recognition for their ability to modulate cell death, exert anti-proliferative effects, and regulate various cellular processes. Targeting siglecs to manipulate immune responses using agonists or antagonists holds promising clinical implications and represents a novel pharmacological strategy in tumor immunotherapy. Recent research has unveiled the pivotal role of siglecs in mediating cell death, anti-proliferative effects, and a spectrum of cellular activities. However, little was known

about the relationship between siglecs and hepatocellular carcinoma (HCC) prognosis. A comparative analysis of siglec gene expression between tumor and non-tumor tissues was conducted and correlated with overall survival (OS) in HCC patients, utilizing the GSE14520 microarray expression profile. Intriguingly, siglec-1 to siglec-9 exhibited down-regulation in tumor tissues compared to their non-tumor counterparts in HCC patients. Further analysis, including univariate and multivariate Cox regression, unveiled that siglec-2 overexpression could serve as a predictor for improved OS. Patients with elevated siglec-2 levels experienced significantly longer OS compared to those with lower siglec-2 expression, as confirmed through Kaplan-Meier event analysis in both training and validation datasets. Notably, the alpha-fetoprotein (AFP) levels in the siglec-2 low-expression group were significantly higher than those in the siglec-2 high-expression group, further emphasized by Chi-square analysis. Furthermore, logistic regression analysis and ROC curve analysis underscored the significant association between siglec-2 down-regulation in tumor tissues and AFP elevation over 300 ng/ml. The up-regulation of siglec-2 in tumor tissues emerged as a favorable prognostic indicator in HCC patients. However, the mechanisms underlying siglec-2 in HCC development warrant further investigation. Intriguingly, NS3TP1 was identified among the 352 siglec-2 negatively coexpressed genes, suggesting its potential involvement in HCC development (Ren et al., 2018).

To further elucidate the prognostic value of genes related to the guanosine metabolism (GM) pathway in hepatocellular carcinoma (HCC), a gene set comprising GM-related genes (GMRGs) was integrated with HCC-related genes. This integration led to the development of a risk model based on five prognostic GMRGs, namely ALDH5A1, ASNSD1, CPS1, GMPS, and PPAT. Each HCC patient was assigned a risk score using this model. The GM-related 5-gene risk-score model, thus generated, offers a valuable tool for predicting prognosis and guiding the treatment of HCC patients (Jin et al., 2022).

Oral squamous cell carcinoma (OSCC) has been associated with oral *Candida albicans* (*C. albicans*) infection, although the nature of this relationship has remained unclear. This study aimed to investigate whether *C. albicans* could potentiate OSCC tumor development and progression. In vitro experiments demonstrated that the presence of live *C. albicans*, but not *Candida parapsilosis*, accelerated the progression of OSCC by stimulating the production of matrix metalloproteinases, oncometabolites, protumor signaling pathways, and the overexpression of prognostic marker genes linked to metastatic events. Notably, *C. albicans* also upregulated oncogenes in nonmalignant cells. A newly established xenograft in vivo mouse model was employed to study OSCC and *C. albicans* interactions, revealing that oral candidiasis enhanced the progression of OSCC through inflammation and induced the overexpression of metastatic genes, along with significant changes in markers associated with the epithelial-mesenchymal transition. Furthermore, long-term findings from the 4-nitroquinoline 1-oxide (4NQO) murine model substantiated these short-term in vitro and in vivo results, thus indicating that *C. albicans* upregulates oncogenes and plays a role in both the early and late stages of malignant promotion and progression in oral cancer. Moreover, ASNSD1 and GOT1 genes were found to be upregulated and implicated in aspartic acid synthetic processes, further underscoring their potential involvement in this context (Vadovics et al., 2022).

NS3TP1 and obesity

Genomic DNA was extracted from a cohort of 126 randomly selected young adult obese subjects, characterized by a BMI of 37.2 ± 0.3 kg/m² and an average age of 18.4 ± 0.3 years. Subsequently, this DNA was subjected to screening through both conventional and augmented whole-exome analyses to identify point mutations and copy number variants (CNVs). Additionally, the levels of leptin, insulin, and cortisol were quantified using ELISA assays. This comprehensive analysis identified 13 individuals harboring 13 distinct pathogenic or likely pathogenic variants in genes including LEPR, PCSK1, MC4R, NTRK2, POMC, SH2B1, and SIM1. Two homozygous stop-gain mutations within the ASNSD1 and IFI16 genes were identified for the first time in humans, both previously associated with obesity in mouse models. Furthermore, the study revealed nine homozygous mutations and four copy-loss CNVs located in genes or genomic regions previously linked to traits associated with obesity through genome-wide association studies (GWAS). Interestingly, in contrast to obese children, this cohort of young adults exhibited a notable absence or rarity of pathogenic mutations in LEP and LEPR. A homozygous nonsense mutation (p.Arg423*) in ASNSD1, encoding the asparagine synthetase domain-containing 1, was identified in a severely obese 17-year-old proband (BMI 37 kg/m²). His 22-year-old sister, with a BMI of 33 kg/m², also carried the same homozygous mutation, while their overweight mother (BMI 27 kg/m²) was heterozygous for this nonsense mutation. Both siblings reported experiencing muscle weakness, fatigue, and joint and muscle pain. Additionally, the female sibling was diagnosed with polycystic ovary syndrome (PCOS). Notably, a recent study corroborated that inactivation of ASNSD1 leads to obesity and muscle atrophy in a mouse model (Powell et al., 2021). None of the individuals in the gnomAD database (version 2.1.1) were reported to carry a homozygous loss-of-function (LoF) variant in ASNSD1 (i.e., frameshift, splice donor/acceptor, stop gain, or start-loss). The identification of a novel

homozygous null mutation in ASNSD1 in this study suggests that ASNSD1 may play a causative role in human obesity and sarcopenia (Saeed et al., 2022). NS3TP1/ASNSD1, alongside GPR45 and TLE4, stands out as one of the most promising candidate genes for future confirmation as a causative factor in human obesity (Powell et al., 2021).

The NS3TP1 gene has been found to be significantly more amplified in basal-like BRCA1-mutated tumors in comparison to basal-like BRCA1 non-mutated tumors (Prat et al., 2014). Although breast cancer 1 (BRCA1)-mutated breast cancer is generally associated with basal-like disease, it remains uncertain whether the presence of a BRCA1 mutation defines a distinct subgroup within this category. To shed light on this matter, researchers compared the molecular characteristics of basal-like tumors with and without BRCA1 mutations. Significant differences were observed in terms of the average number of mutations, DNA copy-number aberrations, and the presence of four amplified regions (2q32.2, 3q29, 6p22.3, and 22q12.2), which are typically associated with high-grade serous ovarian carcinomas. Interestingly, these amplified regions were found in both germline and somatic BRCA1-mutated breast tumors. These findings suggest that minor but potentially relevant variations in molecular features exist within basal-like tumors based on BRCA1 status. Further research is warranted to clarify whether BRCA1 genetic status represents an independent prognostic feature and, more importantly, whether BRCA1 mutation status serves as a predictive biomarker for the efficacy of DNA-damaging agents in the context of basal-like disease. Within the realm of basal-like disease, subtle molecular distinctions emerged at the gene, protein, and mRNA levels. NS3TP1 DNA amplification was notably linked to BRCA1 mutation status and the prognosis of breast cancer patients. Additionally, in another study focused on triple-negative breast cancer (TNBC), NS3TP1 expression levels were found to significantly differ between TNBC and non-TNBC breast cancer patients. The study evaluated tumor gene mutations in 171 TNBC patients and 774 patients with other molecular subtypes of breast cancer (non-TNBC) using data from The Cancer Genome Atlas (TCGA). Moreover, the Metabric database, which comprises 270 TNBC patients, was utilized for supplementary analysis. Genes with somatic mutations that were most commonly found in TNBC compared to non-TNBC were identified using the TCGA database. The study's findings revealed that 13 genes were shared between TNBC and non-TNBC, while 24 genes, including NS3TP1, were exclusive to TNBC. Interestingly, the signaling pathways associated with NS3TP1 have yet to be fully elucidated (Ibragimova et al., 2021). The NS3TP1 gene has been implicated in various types of cancer. In a study focusing on the biological and clinical implications of gene-expression profiling in diffuse large B-cell lymphoma, NS3TP1 was found to undergo significant changes (Groot et al., 2022). More recently, NS3TP1 was linked to "fever-range" hyperthermia, involving the elevation of intratumoral temperatures to the range of 42-45°C, in breast cancer cell lines. Notably, differential gene expression (DGE) analysis revealed a decrease in NS3TP1 expression across different breast cancer cell lines (Amaya et al., 2014).

NS3TP1 in physiology and pathophysiology

A comprehensive analysis was conducted on a gene network within the uterine luminal epithelium during mouse blastocyst implantation. In this investigation of gene expression during the peri-implantation period within the luminal epithelium, a notable trend was observed in ASNSD1 expression levels. Specifically, these levels exhibited a sequential decrease, with values of 74.1569001, 67.3334138, 53.840293, 45.219183, and 31.0402985 recorded at time points D3AM, D4AM, D5AM_mesometrial, and D5AM_antimesometrial, respectively (Aikawa et al., 2021).

Degenerative suspensory ligament desmitis is a progressive idiopathic condition characterized by scarring and rupture of suspensory ligament fibers in multiple limbs of horses. The prevalence of this condition is breed-related, with high risk observed in the Peruvian Horse, while pony and draft breeds exhibit low risk. This condition has been observed within families of Peruvian Horses, yet its genetic underpinnings remain incompletely understood. To investigate this further, a study examined and contrasted various breeds with differing risk levels, identifying associated risk variants and candidate genes. The analysis involved the evaluation of 670 k single nucleotide polymorphisms across 10 breeds, each categorized into one of four risk groups: control (Belgian, Icelandic Horse, Shetland Pony, and Welsh Pony), low risk (Lusitano, Arabian), medium risk (Standardbred, Thoroughbred, Quarter Horse), and high risk (Peruvian Horse). Genome-wide association and selection signature analyses were conducted based on breed-assigned risk levels. The results indicated that the Peruvian Horse population exhibits a low effective population size, and the breed-specific contrasts suggest that degenerative suspensory ligament desmitis is a polygenic condition. Variant frequency patterns displayed signatures of positive selection across risk groups on chromosomes 7, 18, and 23. These findings suggest that risk in different breeds is associated with disturbances in suspensory ligament homeostasis, impacting various processes, including responses to mechanical loading, aging in tendon (PIN1), mechanotransduction (KANK1, KANK2, JUNB, SEMA7A), collagen synthesis (COL4A1, COL5A2, COL5A3, COL6A5), responses to hypoxia (PRDX2),

lipid metabolism (LDLR, VLDLR), and BMP signaling (GREM2). Interestingly, the study did not find evidence to suggest that proteoglycan turnover in the suspensory ligament is a primary factor in the pathogenesis of this condition. Notably, NS3TP1 is among the genes located on chromosome 18 within a specific genomic window (bp) of 65,611,515–67,501,715, and it exhibited a significant association with this condition (Liu et al., 2020; Momen et al., 2022).

The glycan structure of a protein is recognized for its profound impact on various therapeutic properties, including half-life, bioactivity, solubility, and antigenicity. One specific glycan modification, sialylation, involves the addition of NANA to the glycan terminus. Terminal NANA has been shown to exert significant influence on critical characteristics of glycosylated proteins, such as enhancing protein stability, extending circulatory half-life, improving solubility, and bolstering thermal stability. In a study investigating global microarray targets associated with N-acetylneuraminic acid (NANA) levels, it was observed that NS3TP1 exhibited a negative correlation with NANA levels (log fold change, 0.65). The upregulation of the PMP34 gene under conditions of low NANA levels may indicate heightened peroxisomal activity. ASNSD1, a component of asparagine synthetase, plays a role in converting aspartate into asparagine by utilizing ATP and glutamine as precursors, while generating AMP and glutamate as by-products. Notably, human asparagine synthetase expression increases in response to cellular stress and is elevated in tumor cells under hypoxic conditions. Several markers, including PMP34, ASNSD1, ELP2, TMOD1, ARHGEF15, BMP6, and BTBD11, have been linked to oxidative stress and hypoxia. Previous studies have indicated that insufficient oxygen mass transfer can result in oxygen gradients in large-scale bioreactors, potentially leading to intermittent hypoxia, increased reactive oxygen species (ROS), and the induction of cellular oxidative stress (Lewis et al., 2016).

It is well-documented that both miR-30a and miR-30e are significantly downregulated in cardiomyocytes (CMs) two days (2 d) post-myocardial infarction (MI). To identify their regulatory network in CMs during this critical period, dysregulated mRNAs in left ventricle tissues two days post-MI in a mouse model were extracted from a previous publication. The target genes of miR-30a/e, both verified and predicted (upregulated 2 d post-MI), were subjected to an analysis of their involvement in biological processes based on their enrichment in gene ontology (GO) terms. Known targets of miR-30a/e were found to regulate cellular responses to glucose starvation by targeting TP53, BECH1, and HSPA5. Additionally, they control cardiac epithelial-to-mesenchymal transition through the targeting of ETS-related gene (ERG), SNAI1, and NOTCH1. Bioinformatic predictions further indicated that miR-30a may regulate other biological processes related to CM responses to MI through potential targets such as platelet aggregation (possibly via ITGB3 and STXBP1), regulation of the intrinsic apoptotic signaling pathway in response to DNA damage (possibly via SNAI1), and positive regulation of tyrosine phosphorylation of Stat3 protein (possibly via LYN, SOCS3, and SLCF1). Given the significance of these genes in cellular responses to MI, further investigation into the regulatory effect of miR-30a/e on their expression and their regulatory network in CMs is warranted. Notably, the NS3TP1 gene is among the uniquely downregulated genes two days post-MI (Wang et al., 2018), suggesting its potential importance in the pathogenesis of myocardial infarction.

Steroid receptor coactivator-3 (SRC-3), also known as NCOA3 or AIB1, is a member of the multifunctional p160/SRC family of coactivators, which includes SRC-1 and SRC-2. Extensive clinical, cell-based, and mouse studies have highlighted the pivotal roles played by each SRC member in various physiological and pathophysiological contexts, underscoring their functional versatility. SRC-2 has been shown to be crucial in murine embryo implantation and human endometrial stromal cell (HESC) decidualization, a process essential for trophoblast invasion and placentation. Similar to SRC-2, SRC-3 is expressed in both the epithelial and stromal cellular compartments of the human endometrium during the proliferative and secretory phases of the menstrual cycle, as well as in cultured HESCs. Depletion of SRC-3 in cultured HESCs leads to a significant reduction in the induction of a wide range of established decidualization biomarkers, despite these cells being exposed to a deciduogenic stimulus and exhibiting normal progesterone receptor expression. These molecular findings are corroborated at the cellular level, where HESCs fail to undergo morphological transformation from stromal fibroblastoid cells to epithelioid decidual cells when endogenous SRC-3 levels are substantially reduced. To identify genes, signaling pathways, and networks controlled by SRC-3, which are potentially important for hormone-dependent decidualization, RNA-sequencing was conducted on HESCs with significantly reduced SRC-3 levels upon administering the deciduogenic stimulus. Comparison of HESC controls with SRC-3-deficient HESCs revealed an overrepresentation of genes involved in chromatin remodeling, cell proliferation/motility, and programmed cell death among the differentially expressed gene set. These bioanalytical predictions were substantiated by the demonstration that SRC-3 is necessary for the expansion, migratory, and invasive activities of HESCs, cellular properties essential for the formation or functioning of the decidua. Collectively, these findings underscore SRC-3 as a critical coregulator in HESC decidualization. Since

alterations in SRC-3 levels are associated with common gynecological disorders diagnosed in reproductive-age women, this endometrial coregulator, along with its newly identified molecular targets, may offer novel clinical avenues for the diagnosis and treatment of non-receptive endometrium, particularly in patients presenting with non-aneuploid early pregnancy loss. Furthermore, NS3TP1 gene was found to be among the top 50 downregulated genes in SRC-2 knockdown THESCs, suggesting its involvement in the regulation of decidualization in human endometrial stromal cells (Maurya et al., 2022). It's worth noting that miR395 and miR845 have potential targets that include NS3TP1/ASNSD1 (Zhang et al., 2017), and NS3TP1 is considered a candidate gene known to affect productive traits in cattle (Sorbolini et al., 2015).

Previous studies investigating the effects of acid reflux and proton pump inhibitor (PPI) therapy on gene expression in oesophageal epithelium have primarily focused on inflamed tissue. However, this study aimed to discern changes in gene expression within non-inflamed oesophageal epithelium of patients with gastroesophageal reflux disease (GERD). Twenty GERD patients, exhibiting pathological total 24-hour acid exposure, were included in the study. Ten of these patients discontinued PPI treatment (PPI–), while the remaining ten took pantoprazole 40 mg bid (PPI). Additionally, ten age- and sex-matched healthy controls were recruited. Biopsies were obtained from non-inflamed mucosal regions 6 cm and 16 cm proximal to the squamocolumnar junction (SCJ). Gene expression profiling was performed on biopsies from the 6 cm site using Human Genome U133 Plus 2.0 arrays (Affymetrix). The results were subsequently validated through real-time RT-PCR. Among PPI- patients, 92 microarray probe sets displayed deregulated expression. Many of the corresponding genes were associated with cell–cell contacts, cytoskeletal reorganization, and cellular motility, suggesting the facilitation of a migratory phenotype. Furthermore, genes encoding proteins with anti-apoptotic or anti-proliferative functions, as well as those involved in stress protection, were also deregulated. Conversely, no probe sets were found to be deregulated in PPI+ patients. Subsequent qPCR analysis of 20 selected genes confirmed most of the deregulations observed in PPI- patients and identified several deregulated genes in PPI+ patients as well. However, in biopsies obtained at 16 cm, qPCR revealed no deregulations among the selected genes. In response to acid exposure, oesophageal epithelial cells activate a process known as epithelial restitution, characterized by the upregulation of anti-apoptotic, anti-oxidant, and migration-associated genes. This process potentially contributes to maintaining barrier function. In the microarray results, NS3TP1 gene expression was notably increased in GERD patients off PPI compared to healthy controls (1.54-fold) (de Vries et al., 2009).

Aortic dilatation/dissection (AD) can manifest spontaneously or in association with genetic syndromes, such as Marfan syndrome (MFS) caused by FBN1 mutations, MFS type 2, and Loeys–Dietz syndrome linked to TGFBR1/TGFBR2 mutations, as well as Ehlers–Danlos syndrome (EDS) vascular type resulting from COL3A1 mutations. While mutations in FBN1 and TGFBR1/TGFBR2 account for the majority of AD cases referred for molecular genetic testing, negative results for these genes were obtained in a substantial cohort of AD patients. This suggests the involvement of additional genes or acquired factors. In a study examining the impact of COL3A1 deletions/duplications in this patient cohort, Multiplex ligation-dependent probe amplification (MLPA) analysis of 100 unrelated patients revealed a hemizygous deletion encompassing the entire COL3A1 gene. Subsequent microarray analyses and sequencing of breakpoints unveiled a deletion spanning 3,408,306 base pairs at 2q32.1q32.3. This deletion not only affects COL3A1 but also involves 21 other known genes (GULP1, DIRC1, COL5A2, WDR75, SLC40A1, ASNSD1, ANKAR, OSGEPL1, ORMDL1, LOC100129592, PMS1, MSTN, C2orf88, HIBCH, INPP1, MFSD6, TMEM194B, NAB1, GLS, STAT1, and STAT4). Notably, mutations in three of these genes (COL5A2, SLC40A1, and MSTN) have been associated with autosomal dominant disorders, including EDS classical type, hemochromatosis type 4, and muscle hypertrophy. Physical and laboratory examinations revealed that true haploinsufficiency of COL3A1, COL5A2, and MSTN, but not SLC40A1, leads to a clinical phenotype. These findings not only underscore the significance of COL3A1 in AD patients but also expand our understanding of NS3TP1 gene mutations in the molecular etiology of various disorders. They provide previously unreported evidence for true haploinsufficiency of the underlying gene (Meienberg et al., 2010).

NS3TP1 SNPs are also associated with electrocardiogram parameters in the Erasmus Rucphen Family Study. Electrocardiogram (ECG) measurements play a crucial role in diagnosing and predicting cardiac arrhythmias and sudden cardiac death. ECG parameters, such as the PR, QRS, and QT intervals, are known to be heritable, and genome-wide association studies of these phenotypes have identified common variants. However, a significant portion of the genetic variability in these traits remains unexplained. In an analysis of 1547 individuals from the Erasmus Rucphen Family Study (ERF), two SNPs were associated with QT intervals, one with QRS intervals, and two with PR intervals. Fine-mapping using exome sequence data identified a missense variant (c.713C > G, p.Ser238Cys) in the FCRL2 gene associated with QT intervals (rs74608430; $P = 2.8 \times 10^{-4}$, minor allele frequency = 0.019). Heritability analysis indicated that this SNP accounted for 2.42% of the trait's genetic variability in ERF ($P = 0.02$).

Pathway analysis suggested that FCRL2 is involved in cytosolic Ca^{2+} levels ($P = 3.3 \times 10^{-3}$) and AMPK-stimulated fatty acid oxidation in muscle ($P = 4.1 \times 10^{-3}$). Bioinformatics resources revealed that FCRL2 expression is associated with ARHGAP24 and SETBP1 expression. While this finding was not replicated in the Rotterdam study, the combination of bioinformatics information with association and linkage analyses suggests FCRL2 as a strong candidate gene for QT intervals. NS3TP1 gene SNPs are also related to electrocardiogram parameters found in the Erasmus Rucphen family (Silva et al., 2016).

This study sheds light on the complex nature of the grey phenotype in cattle, which is related to the SNP status of the NS3TP1 gene. In a region spanning approximately ± 250 kBp upstream and downstream of this SNP, several genes are encompassed, including PMS1, ORMDL1, OSGEPL1, ANKAR, ASNSD1, SLC40A1, LOC100848294, and WDR75. The functions of NS3TP1, which include roles in apoptosis and senescence, autophagy, lysosomal disorders, glutamate metabolism and signaling, metabolic reprogramming and plasticity, ER stress and unfolded protein response, and melanosome biogenesis and/or trafficking/transfer, have been associated with the complex nature of the grey phenotype in cattle (Senczuk et al., 2020).

Amyotrophic lateral sclerosis (ALS) is a genetically and phenotypically diverse disease that leads to motor neuron loss. Growing evidence suggests the involvement of other systems, including cognitive impairment, in ALS. However, there is currently no validated biomarker for diagnosis or effective therapeutic intervention for ALS. To identify potential genetic biomarkers that could enhance the diagnosis and treatment of ALS patients, data from the Gene Expression Omnibus were systematically analyzed. Interestingly, the NS3TP1 gene was among the 30 overlapping genes identified, which included CNBP, AQP9, TAF7, TBK1, CRLS1, CDKN1B, ANAPC13, SH3GLB1, ASNSD1, MNDA, YPEL5, CLDND1, CHUK, HBP1, MKLN1, C1orf52, QPCT, SLC35A1, RGS18, GPBP1, STX3, VEZF1, TMEM71, CRBN, HIST1H2AC, PPP2R3C, TMEM126B, SAMD9, VNN2, and STXBP3 (Daneshafrooz et al., 2022).

ASDURF as a "missing subunit" for PAQosome

The realm of translated peptides and proteins originating from non-canonical open reading frames (ORFs) is vast, encompassing up to 10^7 unique entities. This expansive landscape has been aptly dubbed the "Dark Proteome" (Pujar et al., 2018; Wright et al., 2022). In the quest to unveil more peptides and proteins encoded within the so-called non-coding regions of the chromosome, a series of small and short open reading frame-encoded peptides (SEPs) have emerged (Kute et al., 2022; Wang et al., 2023). Among these SEPs, a select few have been consistently detected in biological or technical replicates, with noteworthy mentions being ASNSD1-SEP and CIR1-SEP. ASNSD1-SEP, in particular, stands out as the most frequently observed SEP, hinting at its potential for high cellular concentration and stability. Furthermore, ASNSD1-SEP exhibits a clear evolutionary footprint characteristic of protein-coding regions, a feature discerned through analysis across 29 eutherian mammals using PhyloCSF (Ma et al., 2014).

The intriguing discovery of a PFD-like complex, comprised of PFD2 and PFD6 in conjunction with PFD-like proteins such as unconventional prefoldin RPB5 interactor 1, ubiquitously expressed transcript, p53, DNA damage-regulated protein 1, and ASDURF, has significantly advanced our understanding of animal biology (Blanco-Tourinan et al., 2021). Detecting these translated non-canonical ORFs can be accomplished through methods like Ribo-seq or liquid chromatography-mass spectrometry (LC-MS)/MS. These approaches have provided examples of transitions from non-canonical ORFs to canonical annotated protein-coding genes. For instance, ASNSD1 has been identified as a non-canonical peptide/protein in cells, highlighting the potential of SEPs (Prensner et al., 2021; Prensner et al., 2023). It is now evident that genes encoding SEPs exhibit a high degree of conservation across different species. The translation of SEPs often relies on non-AUG translation initiation (Andreev et al., 2022).

Within the 5'-UTR of NS3TP1 mRNA, an upstream open reading frame (uORF) has been unveiled (Slavoff et al. 2013; Chu et al, 2015). The presence of detectable microproteins presents a challenge to traditional gene annotation. Although most human genes have been described as monocistronic, nearly three-quarters of microprotein small open reading frames (sORFs) identified through ribosome profiling are located within 5'-untranslated regions (5'-UTRs). This revelation suggests that many transcriptomes may not be truly monocistronic, and they could encode multiple unique proteins ranging from sORFs consisting of dozens of amino acids to larger downstream coding regions with hundreds of amino acids. A compelling example of this phenomenon is the microprotein encoded by a sORF in the 5'-UTR of the gene for mitochondrial elongation factor 1 (MIEF1). This MIEF1 microprotein (MIEF1MP) collaborates with the larger MIEF protein, localizing to mitochondria and modulating mitochondrial translation rates. Other instances include microproteins such as ASDURF, BiP ORF, HJV uORF (upstream ORF), MP31, PRL-1, and PRL-2 uORF, and SEHBP, all of which play diverse roles related to protein chaperones, ion homeostasis, and metabolic regulation. Without the advancements in proteomic and genomic technologies, the

transcripts housing these sORFs would still be erroneously considered monocistronic (Miller et al., 2022).

However, the biological significance of ASDURF remained elusive until recently when it was identified as the twelfth subunit of the PAQosome, which had long been considered a "missing subunit." The PAQosome, also known as R2TP/PFDL (Rvb1p, Rvb2p, Tah1p, and Pih1p/prefoldin-like), is an 12-subunit chaperone complex crucial for the biogenesis of various human protein complexes (Table 1). Its counterpart in *Saccharomyces cerevisiae*, though apparently lacking the prefoldin-like (PFDL) module, was initially identified due to its association with Hsp90 and subsequently found to facilitate the assembly of snoRNPs, ribonucleoprotein (RNP) complexes involved in ribosomal rRNA maturation across eukaryotes. This chaperone complex also participates in the biogenesis of nuclear RNA polymerases, promoting their cytoplasmic assembly before translocation to the nucleus. Recent discoveries have unveiled its role in the formation of spliceosome components U4 and U5 snRNPs. Beyond orchestrating key steps in gene expression, from transcription to translation, the PAQosome influences the cellular response to stress by stabilizing phosphatidylinositol 3-kinase-related kinases (PIKKs) and aiding in their assembly into complexes that include mTOR complex mTORC1. Notably, the 5'-UTR of the asparagine synthetase domain-containing 1 (ASNSD1) gene houses an upstream open reading frame (uORF), which is capable of generating a cytoplasmic small ORF-encoded peptide (SEP). This peptide, known as ASDURF, bears significant sequence homology and computationally predicted structural similarity to the prefoldin chaperone family. Moreover, ASDURF forms a robust association with the PAQosome in vivo, as confirmed through various affinity purification coupled to mass spectrometry strategies. Given that the PAQosome typically comprises five prefoldin subunits that assemble into a subcomplex referred to as the prefoldin-like module (PFDL), the interaction between ASDURF and other PFDL subunits is of great significance. Importantly, ASDURF can directly bind to other PFDL subunits and facilitate the assembly of higher-order protein complexes. These findings collectively establish ASDURF as the missing PFDL component of the PAQosome, providing a valuable insight into the arrangement of subunits within the complex and paving the way for further functional and structural investigations. Additionally, the presence of ASDURF's coding sequence within an integrated stress response (ISR)-responsive element adds another layer to the PAQosome's associations with cellular stress responses.

Recent studies have introduced an additional layer of complexity by demonstrating that active uORF translation can lead to the expression of functional peptides, referred to as uPeptides. These uPeptides can also serve as components of larger protein complexes (Manske et al., 2022).

ASDURF exhibits notable structural homology to β-prefoldins and forms a heterohexameric prefoldin-like complex when combined with the five established subunits of the prefoldin-like module within the PAQosome. The classic β subunits of prefoldin, namely PFDN2 and PFDN6, have the capacity to create a heterohexameric complex alongside URI/RMP, UXT/Art-27, PDRG1, and ASDURF. This intricate protein assembly, characterized by limited sequence homology to the prefoldin complex, is appropriately named the prefoldin-like complex (PFDL) (Liang et al., 2020).

Furthermore, a model illustrating the configuration of the PAQosome prefoldin-like module has been developed. The inclusion of ASDURF's data serves as a noteworthy example of a eukaryotic uORF-encoded polypeptide whose function extends beyond cis-acting translational regulation of downstream coding sequences. This underscores the significance of incorporating alternative ORF products into proteomic investigations.

ASDURF's functional role centers around the protein folding process, which is crucial for the proper functioning of proteins in their three-dimensional structures. Protein misfolding can lead to the accumulation of unfolded proteins, triggering stress

Table 1. Mammal PFDN genes, names and orthologues from yeast and archaea

Name in the text	Official protein name	Official gene name	Other names	Class	Classic complex	UPC	Budding yeast	Archaea
PFDN1	PFDN1	PDFN1		β	X		PFDN1	β
PFDN2	PFDN2	PFDN2		β	X	X	Gim4	
PFDN4	PFDN4	PDFDN4	Protein C-1	β	X	X	Gim3	
PFDN6	PFDN6	PFND6	HKE2	β	X		Gim1	
PFDN4r	PDRG1	PDRG1	C20orf126	β		X	–	–
ASDURF	–	ASDURF	ASNSD1, NS3TP1	β		X	–	–
PFDN3	VBP1	VBP1		α	X		Gim2	α
PFDN5	PFDN5	PFDN5	MM1	α	X		Gim5	
URI	URI1	URI1	RMP, C19orf2, NNX3	α	X		Bud27	–
STAP1	UXT	UXT	SKP2	α	X		–	–

response pathways, including cytosolic and endoplasmic reticulum stress responses. These responses can have detrimental effects on cells and have been linked to various degenerative diseases (Wang et al., 2019).

In the intricate cellular network of chaperones, co-chaperones play a vital role by regulating and selecting binding partners for chaperones. PFDNs, originally identified over two decades ago, are a subset of co-chaperones. Their primary function was initially associated with the folding of cytoskeletal proteins in yeasts and archaebacteria. However, subsequent research has expanded the role of PFDNs, revealing their presence in various organisms, from archaea to humans, and suggesting a broader range of functions. This aligns with the concept that proteins involved in folding can interact with a diverse array of substrates. Additionally, it's not surprising that PFDNs have been implicated in several pathologies, including cancer.

More recently, a sixth β PFDN candidate, ASDURF, has been identified through proximity-dependent biotinylation (BioID). ASDURF was found to form a complex with other prefoldins and is considered a member of this complex, known as the prefoldin-like module (PFDL) (Cloutier et al., 2020; Pan et al., 2021).

In efforts to identify binding partners of ASDURF through affinity purification, it was discovered that ASDURF strongly interacts with the PAQosome. Tandem affinity purification (TAP) technology revealed that ASDURF binds to numerous putative clients, regulators, and cofactors, and this interaction was confirmed using PAQosome subunits PIH1D1 and UXT. Notably, ASDURF's interaction intensity and enrichment level were comparable to those of other PAQosome subunits. Further FLAG-based affinity purification of ASDURF reaffirmed its association with the PAQosome. Interestingly, this experiment also identified subunits of the canonical prefoldin complex, such as VBP1/PFDN3, PFDN4, and PFDN5, suggesting that ASDURF may participate in two distinct protein complexes: the PFDL module of the PAQosome and a variant of the canonical prefoldin complex, where ASDURF potentially substitutes for PFDN1. This observation is akin to another prefoldin-like component of PFDL, PDRG1, which can replace PFDN4 in an alternative canonical prefoldin complex.

To gain insights into ASDURF's function, predictive modeling software Phyre2 was employed to examine its tertiary structure. The analysis indicated that ASDURF exhibits a structural conformation characterized by a short β-hairpin followed by a coiled coil, resembling *Pyrococcus horikoshii* β-prefoldin. β-prefoldins are known to play critical roles in protein folding processes across various domains of life. Typically, prefoldin complexes are composed of two α-subunits and four β-subunits, with α-prefoldins featuring two β-hairpins and β-prefoldins containing one. These β-hairpins form dual eight-stranded β-barrels that stabilize the complex, while coiled coil domains facilitate interactions with non-native client proteins. The exact composition of the PAQosome's prefoldin-like module has been a subject of debate. Previously, it was assumed that one of the β-prefoldins was present in duplicate, but the discovery of ASDURF offers a more plausible explanation where this novel β-prefoldin completes the hexameric PFDL subcomplex.

To provide further substantiation for ASDURF's role within the prefoldin-like module of the PAQosome, direct binding assays were conducted using GST pulldowns. These experiments demonstrated that GST-ASDURF can interact individually with UXT and PFDN2, consistent with β-prefoldins binding to both α-prefoldins and other β-prefoldins through β-hairpin domains. When all prefoldin subunits were included, GST-ASDURF pulled down additional prefoldins, such as PDRG1, PFDN6, and URI1, indicating the formation of higher-order assemblies, including the heterohexameric complex or subcomplexes thereof. Notably, URI1 was only present in its upper band in this experiment, suggesting that the lower band likely represents a truncated variant resulting from erroneous translation initiated at a downstream methionine codon, lacking the N-terminus prefoldin-like domain.

ASDURF is considered an atypical member of the prefoldin (PFDN)-like complex, akin to URI. URI's identification as a PFDN protein emerged from co-immunoprecipitation (co-IP) experiments in mammalian cells. These experiments, coupled with proteomic analysis, aimed to uncover new cell cycle regulators interacting with SKP2, an E3-ligase for p27, and revealed that URI, along with other PFDNs, binds to SKP2. However, it's important to note that further verification is needed to ascertain whether ASDURF indeed belongs to the URI PFDN-like complex (UPC) or if an additional β PFDN subunit is merely duplicated within this complex, forming a heterohexameric structure. There is also the possibility that the UPC might be an unconventional pentameric complex. In mammals, ten PFDN genes have been identified, including the recently discovered ASDURF, and these genes appear to assemble to create both the classical PFDN complex and the UPC (Cloutier et al., 2020; Pan et al., 2021).

For the R2TP-based chaperone pathway to function optimally, it requires the involvement of additional proteins. An analysis of R2TP-associated proteins has revealed interactions between R2TP and the URI1 prefoldin complex. This URI1 prefoldin complex is composed of prefoldin-like proteins, including PFDN2, PFDN6, URI1, UXT, PDRG1, and the recently discovered ASDURF (Rodríguez et al., 2020; Lynham et al., 2022; Pinard et al., 2022; Schlotter et al., 2023). It's worth noting that information regarding ASDURF as a newly identified PFDN was not included in these analyses, likely

because it wasn't annotated as such in the datasets used. Thus, further investigations are required to determine conclusively whether ASDURF is a genuine part of the UPC (Herranz-Montoya et al., 2021).

ASDURF and childhood medulloblastoma

A distinguishing feature of high-risk childhood medulloblastoma is the disruption of RNA translation. Currently, it remains uncertain whether medulloblastoma impacts the translation of potentially oncogenic non-canonical open reading frames (ORFs). To investigate this, ribosome profiling was conducted on 32 medulloblastoma tissues and cell lines, revealing widespread translation of non-canonical ORFs. Subsequently, a systematic approach was devised, employing multiple CRISPR-Cas9 screens to uncover functional non-canonical ORFs linked to medulloblastoma cell survival. Several long non-coding RNA ORFs (lncRNA-ORFs) and upstream open reading frames (uORFs) exhibited selective functionality independent of the primary coding sequence. Among these, ASNSD1-uORF, also known as ASDURF, was upregulated, associated with the MYC family oncogenes, and proved essential for medulloblastoma cell survival by interacting with the prefoldin-like chaperone complex. These findings underscore the pivotal role of non-canonical ORF translation in medulloblastoma and advocate for the inclusion of these ORFs in future cancer genomics studies aiming to identify novel cancer targets (Hofman et al., 2023).

The role and mechanism of ASDURF in carcinogenesis have remained elusive. A recent study has revealed that ASDURF functions as a human leukocyte antigen (HLA)-binding protein, exhibiting ubiquitous expression in HEK293T cells (Jürgens et al., 2022; Nelde et al., 2022). Thus far, only a small fraction of uPeptides detected as HLA ligands in immunopeptidomic datasets have undergone functional analysis. However, from the limited cases studied, a wide array of distinct functional roles for uPeptides has emerged. Labeling of uPeptides with fluorescent protein tags has unveiled specific subcellular localization patterns, ranging from the widespread distribution of the ASDURF/ASNSD1 uAUG uPeptide to membrane-associated localization seen in the MKKS uAUG uPeptide. Additionally, nuclear foci formation has been observed for the MAPK1 uCUG.1 uPeptide. HLA uLigands encoded by ASDURF are predominantly detected on leukemic cells. ASDURF operates at the interface of translational regulation and individual peptide function, with mechanistic roles encompassing ribosome stalling, participation in cellular programs through protein interaction and complex formation, and contribution to the human leukocyte antigen (HLA)-associated immunopeptidome as HLA uLigands. Malignant transformation may result in the generation of novel uORFs, uPeptides, or HLA uLigands, providing insight into their potential involvement in tumor biology. uPeptides hold promise as peptide-based drugs, and their interplay with HLA uLigands may facilitate translational inhibition of oncogenic protein messages and immunotherapeutic applications in cancer therapy (Jürgens et al., 2022).

ASDURF and *Lactobacillus acidophilus* NCFM phenotype

Lactobacillus acidophilus NCFM is a widely used probiotic strain found in dairy products and dietary supplements. Previous post-genome in vitro studies have associated specific genotypes with its probiotic-related characteristics, such as survival in the gut, utilization of prebiotics, interactions with the host, and immunomodulatory effects. To expand upon these findings and gain a deeper understanding beyond in vitro and in vivo studies, a dual RNA sequencing (RNA-seq) transcriptomic approach was employed. This approach aimed to identify genes that play a potential role in the gut fitness and functions of *L. acidophilus* NCFM in vivo. Simultaneously, it examined the ileal transcriptional response of the murine hosts during colonization by this strain.

Spatial expression profiling of NCFM along the gastrointestinal tract, from the ileum to the colon, revealed a set of 134 core genes consistently overexpressed during gut transit. These in vivo core genes primarily participate in carbohydrate, amino acid, and nucleotide metabolism, as well as in mucus binding and adhesion functions. This confirmed their crucial roles in nutrient acquisition and gut retention. The major S layer-encoding gene, highly expressed among these core genes, was found to be indispensable for determining cell shape and maintaining cell surface integrity. These traits are essential for the viability and probiotic attributes of *L. acidophilus* NCFM.

Furthermore, colonization by *L. acidophilus* led to the downregulation of several proinflammatory cytokines and tight junction proteins in the host. Conversely, genes associated with redox signaling, mucin glycosylation, and circadian rhythm modulation were induced, suggesting potential impacts on intestinal development and immune functions. Metagenomic analysis of NCFM populations postcolonization confirmed the genomic stability of *L. acidophilus* as a transient gut inhabitant, further establishing its safety as a probiotic and biotherapeutic delivery system.

In mice mono-colonized with *L. acidophilus* NCFM compared to germ-free mice, there was a significant reduction (-5.33-fold) in ASDURF expression (Goh et al., 2021). However, the biological significance of this reduction remains to be elucidated. Notably, NS3TP1/ASNSD1 gene expression levels were found to increase significantly in *H. pylori*-

CagA transfected gastric cancer cells (Chen et al., 2020). It is imperative to investigate whether ASDURF expression can be upregulated by *H. pylori*-CagA gene transfection. Furthermore, NS3TP1 gene expression was shown to be regulated by *Trypanosoma cruzi* (*T. cruzi*) (Zhang et al. 2010).

References

[1] Abstracts from the 54th European Society of Human Genetics (ESHG) Conference: e-Posters. Eur J Hum Genet. 2022;30(Suppl 1): 88-608.

[2] Aikawa S, Hirota Y, Fukui Y, Ishizawa C, IIda R, Kaku T, Hirata T, Akaeda S, Hiraoka T, Matsuo M, Osuga Y. A gene network of uterine luminal epithelium organizes mouse blastocyst implantation. Reprod Med Biol. 2022;21(1):e12435.

[3] Akinci MA, Turner H, Taveras M, Wolosin JM. Differential gene expression in the pig limbal side population: implications for stem cell cycling, replication, and survival. Invest Ophthalmol Vis Sci. 2009;50(12):5630-8.

[4] Amaya C, Kurisetty V, Stiles J, Nyakeriga AM, Arumugam A, Lakshmanaswamy R, Botez CE, Mitchell DC, Bryan BA. A genomics approach to identify susceptibilities of breast cancer cells to "fever-range" hyperthermia. BMC Cancer. 2014;14:81.

[5] Andreev DE, Loughran G, Fedorova AD, Mikhaylova MS, Shatsky IN, Baranov PV. Non-AUG translation initiation in mammals. Genome Biol. 2022;23(1):111.

[6] Bando SY, Bertonha FB, Pimentel-Silva LR, de Oliveira JGM, Carneiro MAD, Oku MHM, Wen HT, Castro LHM, Moreira-Filho CA. Hippocampal CA3 transcriptional modules associated with granule cell alterations and cognitive impairment in refractory mesial temporal lobe epilepsy patients. Sci Rep. 2021;11(1):10257.

[7] Benner C, Konovalov S, Mackintosh C, Hutt KR, Stunnenberg R, Garcia-Bassets I. Decoding a signature-based model of transcription cofactor recruitment dictated by cardinal cis-regulatory elements in proximal promoter regions. PLoS Genet. 2013;9(11):e1003906.

[8] Blanco-Touriñán N, Esteve-Bruna D, Serrano-Mislata A, Esquinas-Ariza RM, Resentini F, Forment J, Carrasco-López C, Novella-Rausell C, Palacios-Abella A, Carrasco P, Salinas J, Blázquez MÁ, Alabadí D. A genetic approach reveals different modes of action of prefoldins. Plant Physiol. 2021;187(3):1534-50.

[9] Boitard S, Paris C, Sevane N, Servin B, Bazi-Kabbaj K, Dunner S. Gene banks as reservoirs to detect recent selection: The example of the Asturiana de los Valles bovine breed. Front Genet. 2021;12:575405.

[10] Chen D, Li C, Zhao Y, Zhou J, Wang Q, Xie Y. Bioinformatics analysis for the identification of differentially expressed genes and related signaling pathways in H. pylori-CagA transfected gastric cancer cells. Peer J. 2021;9:e11203.

[11] Cheng J, Yang S. From gene identification to drug discovery: unleashing the power of multum in parvo. Bull Biomed Sci. 2023;1:1-26.

[12] Chu Q, Ma J, Saghatelian A. Identification and characterization of sORF-encoded polypeptides. Crit Rev Biochem Mol Biol. 2015;50(2):134-41.

[13] Cloutier P, Poitras C, Faubert D, Bouchard A, Blanchette M, Gauthier MS, Coulombe B. Upstream ORF-encoded ASDURF is a novel prefoldin-like subunit of the PAQosome. J Proteome Res. 2020;19(1):18-27.

[14] Daneshafrooz N, Bagherzadeh Cham M, Majidi M, Panahi B. Identification of potentially functional modules and diagnostic genes related to amyotrophic lateral sclerosis based on the WGCNA and LASSO algorithms. Sci Rep. 2022;12(1):20144.

[15] de Groot FA, de Groen RAL, van den Berg A, Jansen PM, Lam KH, Mutsaers PGNJ, van Noesel CJM, Chamuleau MED, Stevens WBC, Plaça JR, Mous R, Kersten MJ, van der Poel MMW, Tousseyn T, Woei-A-Jin FJSH, Diepstra A, Nijland M, Vermaat JSP. Biological and clinical implications of gene-expression profiling in diffuse large B-cell lymphoma: A proposal for a targeted BLYM-777 consortium panel as part of a multilayered analytical approach. Cancers (Basel). 2022;14(8):1857.

[16] de Vries DR, Ter Linde JJ, van Herwaarden MA, Schwartz MP, Shephard P, Geng MM, Smout AJ, Samsom M. In GERD patients, mucosal repair associated genes are upregulated in non-inflamed oesophageal epithelium. J Cell Mol Med. 2009;13(5):936-47.

[17] Di Gioia M, Spreafico R, Springstead JR, Mendelson MM, Joehanes R, Levy D, Zanoni I. Endogenous oxidized phospholipids reprogram cellular metabolism and boost hyperinflammation. Nat Immunol. 2020;21(1):42-53.

[18] Doyle JL, Berry DP, Veerkamp RF, Carthy TR, Evans RD, Walsh SW, Purfield DC. Genomic regions associated with muscularity in beef cattle differ in five contrasting cattle breeds. Genet Sel Evol. 2020;52(1):2.

[19] Farooq A, Hoque R, Ouyang X, Farooq A, Ghani A, Ahsan K, Guerra M, Mehal WZ. Activation of N-methyl-d-aspartate receptor downregulates inflammasome activity and liver inflammation via a β-arrestin-2 pathway. Am J Physiol Gastrointest Liver Physiol. 2014;307(7):G732-40.

[20] Feliciello I, Sermek A, Pezer Ž, Matulić M, Ugarković Đ. Heat stress affects H3K9me3 level at human α satellite DNA repeats. Genes (Basel). 2020;11(6):663.

[21] Genetic Modifiers of Huntington's Disease (GeM-HD) Consortium. CAG repeat not polyglutamine length determines timing of huntington's disease onset. Cell. 2019;178(4):887-900.

[22] Ghosh AK, Murphy SB, Kishore R, Vaughan DE. Global gene expression profiling in PAI-1 knockout murine heart and kidney: molecular basis of cardiac-selective fibrosis. PLoS One. 2013;8(5):e63825.

[23] Goh YJ, Barrangou R, Klaenhammer TR. In vivo transcriptome of Lactobacillus acidophilus and colonization impact on murine host intestinal gene expression. mBio. 2021;12(1):e03399-20.

[24] Hamill RM, Aslan O, Mullen AM, O'Doherty JV, McBryan J, Morris DG, Sweeney T. Transcriptome analysis of porcine M. semimembranosus divergent in intramuscular fat as a consequence of dietary protein restriction. BMC Genomics. 2013;14:453.

[25] He S, Zhang S, Yao Y, Xu B, Niu Z, Liao F, Wu J, Song Q, Li M, Liu Z. Turbulence of glutamine metabolism in pan-cancer prognosis and immune microenvironment. Front Oncol. 2022;12:1064127.

[26] Herranz-Montoya I, Park S, Djouder N. A comprehensive analysis of prefoldins and their implication in cancer. iScience. 2021;24(11):103273.

[27] Hofman D, Ruiz-Orera J, Yannuzzi I, Murugesan R, van Dinter J, Clauser K, Condurat A, Herman A, Shanmugam V, Zhou K, Vogelzang J, Wang L, Roth J, Ligon K, Phoenix T, Root D, Golub T, Bandopadhayay P, van Heesch S, Prensner J. MDB-32. Translation of non-canonical open reading frames as a cancer cell survival mechanism in childhood medulloblastoma. Neuro Oncol. 2023;25(Suppl 1):i69.

[28] Hu MS, Borrelli MR, Januszyk M, Luan A, Malhotra S, Walmsley GG, Hong WX, Tevlin R, Gurtner GC, Longaker MT, Lorenz HP. Pathway analysis of gene expression of E14 versus E18 fetal fibroblasts. Adv Wound Care (New Rochelle). 2018;7(1):1-10.

[29] Ibragimova MK, Tsyganov MM, Litviakov NV. Molecular-genetic portrait of breast cancer with triple negative phenotype. Cancers (Basel). 2021;13(21):5348.

[30] Ji D, Cheng J, Wang JJ, Liu Y, Yang Q, Dang XY, Wang CH. Screening and cloning of the target genes transactivated by human gene 1 transactivated by hepatitis C virus NS3 protein using suppression subtractive hybridization. World J Chin Dig. 2004;4:93–9.

[31] Jin S, Cao J, Kong LB. Identification and validation in a novel quantification system of the glutamine metabolism patterns for the prediction of prognosis and therapy response in hepatocellular carcinoma. J Gastrointest Oncol. 2022;13(5):2505-21.

[32] Jürgens L, Wethmar K. The emerging role of uORF-encoded uPeptides and HLA uLigands in cellular and tumor biology. Cancers (Basel). 2022;14(24):6031.

[33] Kaushik A, Bhatia Y, Ali S, Gupta D. Gene network rewiring to study melanoma stage progression and elements essential for driving melanoma. PLoS One. 2015;10(11):e0142443.

[34] Kil DY, Vester Boler BM, Apanavicius CJ, Schook LB, Swanson KS. Age and diet affect gene expression profiles in canine liver tissue. PLoS One. 2010;5(10):e13319.

[35] Ko KP, Zhang S, Huang Y, Kim B, Zou G, Jun S, Zhang J, Martin C, Dunbar KJ, Efe G, Rustgi AK, Zhang H, Nakagawa H, Park JI. Tumor Niche network-defined subtypes predict immunotherapy response of esophageal squamous cell cancer. bioRxiv [Preprint]. 2023:2023.02.15.528539.

[36] Kute PM, Soukarieh O, Tjeldnes H, Trégouët DA, Valen E. Small open reading frames, how to find them and determine their function. Front Genet. 2022;12:796060.

[37] Lencz T, Lambert C, DeRosse P, Burdick KE, Morgan TV, Kane JM, Kucherlapati R, Malhotra AK. Runs of homozygosity reveal highly penetrant recessive loci in schizophrenia. Proc Natl Acad Sci USA. 2007;104(50):19942-7.

[38] Lewis AM, Croughan WD, Aranibar N, Lee AG, Warrack B, Abu-Absi NR, Patel R, Drew B, Borys MC, Reily MD, Li ZJ. Understanding and controlling sialylation in a CHO Fc-fusion process. PLoS One. 2016;11(6):e0157111.

[39] Li Y, Li F, Bai X, Li Y, Ni C, Zhao X, Zhang D. ITGA3 is associated with immune cell infiltration and serves as a favorable prognostic biomarker for breast cancer. Front Oncol. 2021;11:658547.

[40] Linhorst A, Lübke T. The human Ntn-hydrolase superfamily: structure, functions and perspectives. Cells. 2022;11(10):1592.

[41] Shi L, Zhou L, Han M, Zhang Y, Zhang Y, Yuan XX, Lu H, Wang Y, Yang XL, Liu C, Wang J, Liang P, Liu SA, Liu XJ, Cheng J, Lin SM. Calcitriol attenuates liver fibrosis through hepatitis C virus nonstructural protein 3-transactivated protein 1-mediated TGF β1/Smad3 and NF-κB signaling pathways. World J Gastroenterol. 2023;29(18):2798-817.

[42] Lieberman AP, Shakkottai VG, Albin RL. Polyglutamine repeats in neurodegenerative diseases. Annu Rev Pathol. 2018;14:1-27.

[43] Lionikas A, Kilikevicius A, Bünger L, Meharg C, Carroll AM, Ratkevicius A, Venckunas T, Blizard DA. Genetic and genomic analyses of musculoskeletal differences between BEH and BEL strains. Physiol Genomics. 2013;45(20):940-7.

[44] Liu C, Lai Y, Ying S, Zhan J, Shen Y. Plasma exosome-derived microRNAs expression profiling and bioinformatics analysis under cross-talk between increased low-density lipoprotein cholesterol level and ATP-sensitive potassium channels variant rs1799858. J Transl Med. 2020;18(1):459.

[45] Lynham J, Houry WA. The role of Hsp90-R2TP in macromolecular complex assembly and stabilization. Biomolecules. 2022;12(8):1045.

[46] Ma J, Ward CC, Jungreis I, Slavoff SA, Schwaid AG, Neveu J, Budnik BA, Kellis M, Saghatelian A. Discovery of human sORF-encoded polypeptides (SEPs) in cell lines and tissue. J Proteome Res. 2014;13(3):1757-65.

[47] Manske F, Ogoniak L, Jürgens L, Grundmann N, Makałowski W, Wethmar K. The new uORFdb: integrating literature, sequence, and variation data in a central hub for uORF research. Nucleic Acids Res. 2023;51(D1):D328-D36.

[48] Miller B, Kim SJ, Kumagai H, Yen K, Cohen P. Mitochondria-derived peptides in aging and healthspan. J Clin Invest. 2022;132(9):e158449.

[49] Maurya VK, Szwarc MM, Lonard DM, Gibbons WE, Wu SP, O'Malley BW, DeMayo FJ, Lydon JP. Decidualization of human endometrial stromal cells requires steroid receptor coactivator-3. Front Reprod Health. 2022;4:1033581.

[50] Maxson Jones K, Ankeny RA, Cook-Deegan R. The Bermuda Triangle: The pragmatics, policies, and principles for data sharing in the history of the human genome project. J Hist Biol. 2018;51(4):693-805.

[51] Meienberg J, Rohrbach M, Neuenschwander S, Spanaus K, Giunta C, Alonso S, Arnold E, Henggeler C, Regenass S, Patrignani A, Azzarello-Burri S, Steiner B, Nygren AO, Carrel T, Steinmann B, Mátyás G. Hemizygous deletion of COL3A1, COL5A2, and MSTN causes a complex phenotype with aortic dissection: a lesson for and from true haploinsufficiency. Eur J Hum Genet. 2010;18(12):1315-21.

[52] Meijer HA, Bushell M, Hill K, Gant TW, Willis AE, Jones P, de Moor CH. A novel method for poly(A) fractionation reveals a large population of mRNAs with a short poly(A) tail in mammalian cells. Nucleic Acids Res. 2007;35(19):e132.

[53] Momen M, Brounts SH, Binversie EE, Sample SJ, Rosa GJM, Davis BW, Muir P. Selection signature analyses and genome-wide association reveal genomic hotspot regions that reflect differences between breeds of horse with contrasting risk of degenerative suspensory ligament desmitis. G3 (Bethesda). 2022;12(10):jkac179.

[54] Nelde A, Flötotto L, Jürgens L, Szymik L, Hubert E, Bauer J, Schliemann C, Kessler T, Lenz G, Rammensee HG, Walz JS, Wethmar K. Upstream open reading frames regulate translation of cancer-associated transcripts and encode HLA-presented immunogenic tumor antigens. Cell Mol Life Sci. 2022;79(3):171.

[55] Newton R, Wernisch L. A meta-analysis of multiple matched aCGH/expression cancer datasets reveals regulatory relationships and pathway enrichment of potential oncogenes. PLoS One. 2019;14(7):e0213221.

[56] Niu Y, Zhang X, Men S, Storey KB, Chen Q. Integrated analysis of transcriptome and metabolome data reveals insights for molecular mechanisms in overwintering Tibetan frogs, Nanorana parkeri. Front Physiol. 2023;13:1104476.

[57] Pan N, Wang Z, Wang B, Wan J, Wan C. Mapping microproteins and ncRNA-encoded polypeptides in different mouse tissues. Front

Cell Dev Biol. 2021;9:687748.
[58] Pinard M, Dastpeyman S, Poitras C, Bernard G, Gauthier MS, Coulombe B. Riluzole partially restores RNA polymerase III complex assembly in cells expressing the leukodystrophy-causative variant POLR3B R103H. Mol Brain. 2022;15(1):98.
[59] Pop TL, Sîrbe C, Benţa G, Mititelu A, Grama A. The role of vitamin D and vitamin D binding protein in chronic liver diseases. Int J Mol Sci. 2022;23(18):10705.
[60] Powell DR, Revelli JP, Doree DD, DaCosta CM, Desai U, Shadoan MK, Rodriguez L, Mullens M, Yang QM, Ding ZM, Kirkpatrick LL, Vogel P, Zambrowicz B, Sands AT, Platt KA, Hansen GM, Brommage R. High-throughput screening of mouse gene knockouts identifies established and novel high body fat phenotypes. Diabetes Metab Syndr Obes. 2021;14:3753-85.
[61] Prat A, Cruz C, Hoadley KA, Díez O, Perou CM, Balmaña J. Molecular features of the basal-like breast cancer subtype based on BRCA1 mutation status. Breast Cancer Res Treat. 2014;147(1):185-91.
[62] Prensner JR, Enache OM, Luria V, Krug K, Clauser KR, Dempster JM, Karger A, Wang L, Stumbraite K, Wang VM, Botta G, Lyons NJ, Goodale A, Kalani Z, Fritchman B, Brown A, Alan D, Green T, Yang X, Jaffe JD, Roth JA, Piccioni F, Kirschner MW, Ji Z, Root DE, Golub TR. Noncanonical open reading frames encode functional proteins essential for cancer cell survival. Nat Biotechnol. 2021;39(6):697-704.
[63] Prensner J, Golub T. BIOL-03. Protein translation from non-coding genomic loci produce biologically-active proteins implicated in cancer cell survival in pediatric brain tumors. Neuro Oncol. 2021;23(Suppl 1):i3.
[64] Prensner JR, Abelin JG, Kok LW, Clauser KR, Mudge JM, Ruiz-Orera J, Bassani-Sternberg M, Deutsch EW, van Heesch S. What can Ribo-seq and proteomics tell us about the non-canonical proteome? bioRxiv [Preprint]. 2023:2023.05.16.541049.
[65] Pujar S, O'Leary NA, Farrell CM, Loveland JE, Mudge JM, Wallin C, Girón CG, Diekhans M, Barnes I, Bennett R, Berry AE, Cox E, Davidson C, Goldfarb T, Gonzalez JM, Hunt T, Jackson J, Joardar V, Kay MP, Kodali VK, Martin FJ, McAndrews M, McGarvey KM, Murphy M, Rajput B, Rangwala SH, Riddick LD, Seal RL, Suner MM, Webb D, Zhu S, Aken BL, Bruford EA, Bult CJ, Frankish A, Murphy T, Pruitt KD. Consensus coding sequence (CCDS) database: a standardized set of human and mouse protein-coding regions supported by expert curation. Nucleic Acids Res. 2018;46(D1):D221-8.
[66] Purfield DC, Bradley DG, Evans RD, Kearney FJ, Berry DP. Genome-wide association study for calving performance using high-density genotypes in dairy and beef cattle. Genet Sel Evol. 2015;47(1):47.
[67] Ramey HR, Decker JE, McKay SD, Rolf MM, Schnabel RD, Taylor JF. Detection of selective sweeps in cattle using genome-wide SNP data. BMC Genomics. 2013;14:382.
[68] Rasche A, Al-Hasani H, Herwig R. Meta-analysis approach identifies candidate genes and associated molecular networks for type-2 diabetes mellitus. BMC Genomics. 2008;9:310.
[69] Ren X, Ji Y, Jiang X, Qi X. Down-regulation of siglec-2 (CD22) predicts worse overall survival from HBV-related early-stage hepatocellular carcinoma: a preliminary analysis from Gene Expression Omnibus. Biosci Rep. 2018;38(6):BSR20181423.
[70] Rodríguez CF, Llorca O. RPAP3 C-terminal domain: a conserved domain for the assembly of R2TP co-chaperone complexes. Cells. 2020;9(5):1139.
[71] Saeed S, Janjua QM, Haseeb A, Khanam R, Durand E, Vaillant E, Ning L, Badreddine A, Berberian L, Boissel M, Amanzougarene S, Canouil M, Derhourhi M, Bonnefond A, Arslan M, Froguel P. Rare variant analysis of obesity-associated genes in young adults with severe obesity from a Consanguineous population of Pakistan. Diabetes. 2022;71(4):694-705.
[72] Schisler JC, Charles PC, Parker JS, Hilliard EG, Mapara S, Meredith D, Lineberger RE, Wu SS, Alder BD, Stouffer GA, Patterson C. Stable patterns of gene expression regulating carbohydrate metabolism determined by geographic ancestry. PLoS One. 2009;4(12):e8183.
[73] Schlotter F, Mérouani S, Flayac J, Kogey V, Issa A, Dodré M, Huttin A, Branlant C, Bertrand E, Labialle S, Vandermoere F, Verheggen C, Massenet S. Proteomic analyses reveal new features of the box H/ACA RNP biogenesis. Nucleic Acids Res. 2023;51(7):3357-74.
[74] Senczuk G, Guerra L, Mastrangelo S, Campobasso C, Zoubeyda K, Imane M, Marletta D, Kusza S, Karsli T, Gaouar SBS, Pilla F, Ciani E, The Bovita Consortium. Fifteen shades of grey: combined analysis of genome-wide SNP data in Steppe and Mediterranean grey cattle sheds new light on the molecular basis of coat color. Genes (Basel). 2020;11(8):932.
[75] Shao M. Construction of a miRNA-regulated pathway network reveals candidate biomarkers for postmenopausal osteoporosis. Comput Math Methods Med. 2017;2017:9426280.
[76] Shi L, Zhou L, Han M, Zhang Y, Zhang Y, Yuan XX, Lu HP, Wang Y, Yang XL, Liu C, Wang J, Liang J, Xia L, Oyang L, Lin J, Tan S, Yi P, Han Y, Luo X, Wang H, Tang L, Pan Q, Tian Y, Rao S, Su M, Shi Y, Cao D, Zhou Y, Liao Q. The functions and mechanisms of prefoldin complex and prefoldin-subunits. Cell Biosci. 2020;10:87.
[77] Silva CT, Zorkoltseva IV, Amin N, Demirkan A, van Leeuwen EM, Kors JA, van den Berg M, Stricker BH, Uitterlinden AG, Kirichenko AV, Witteman JC, Willemsen R, Oostra BA, Axenovich TI, van Duijn CM, Isaacs A. A combined linkage and exome sequencing analysis for electrocardiogram parameters in the Erasmus Rucphen family study. Front Genet. 2016;7:190.
[78] Slavoff SA, Mitchell AJ, Schwaid AG, Cabili MN, Ma J, Levin JZ, Karger AD, Budnik BA, Rinn JL, Saghatelian A. Peptidomic discovery of short open reading frame-encoded peptides in human cells. Nat Chem Biol. 2013;9(1):59-64.
[79] Slobodin B, Han R, Calderone V, Vrielink JAFO, Loayza-Puch F, Elkon R, Agami R. Transcription impacts the efficiency of mRNA translation via co-transcriptional N6-adenosine methylation. Cell. 2017;169(2):326-37.
[80] Sorbolini S, Marras G, Gaspa G, Dimauro C, Cellesi M, Valentini A, Macciotta NP. Detection of selection signatures in Piemontese and Marchigiana cattle, two breeds with similar production aptitudes but different selection histories. Genet Sel Evol. 2015;47(1):52.
[81] Spencer C, Montalvo J, McLaughlin SR, Bryan BA. Small molecule inhibition of cytoskeletal dynamics in melanoma tumors results in altered transcriptional expression patterns of key genes involved in tumor initiation and progression. Cancer Genomics Proteomics. 2011;8(2):77-85.
[82] Theusch E, Chen YI, Rotter JI, Krauss RM, Medina MW. Genetic variants modulate gene expression statin response in human lymphoblastoid cell lines. BMC Genomics. 2020;21(1):555.
[83] Vadovics M, Ho J, Igaz N, Alföldi R, Rakk D, Veres É, Szücs B,

Horváth M, Tóth R, Szücs A, Csibi A, Horváth P, Tiszlavicz L, Vágvölgyi C, Nosanchuk JD, Szekeres A, Kiricsi M, Henley-Smith R, Moyes DL, Thavaraj S, Brown R, Puskás LG, Naglik JR, Gácser A. Candida albicans enhances the progression of oral squamous cell carcinoma in vitro and in vivo. mBio. 2021;13(1):e0314421.

[84] Vogel P, Ding ZM, Read R, DaCosta CM, Hansard M, Small DL, Ye GL, Hansen G, Brommage R, Powell DR. Progressive degenerative myopathy and myosteatosis in ASNSD1-deficient mice. Vet Pathol. 2020;57(5):723-35.

[85] Vogel P, Read RW, Hansen GM, Powell DR. Histopathology is required to identify and characterize myopathies in high-throughput phenotype screening of genetically engineered mice. Vet Pathol. 2021;58(6):1158-71.

[86] Wang J, Yuan X, Wang Y, Zhang Y, Lu H, Zhang Y, Cheng J. Function and regulation for small/short open reading frames-encoded peptides (SEPs) in liver diseases. Bull Biomed Sci. 2023;1:27-32.

[87] Wang Y, Huang Y, Zhang M, Zhang X, Tang X, Kang Y. Bioinformatic analysis of the possible regulative network of miR-30a/e in cardiomyocytes 2 days post myocardial infarction. Acta Cardiol Sin. 2018r;34(2):175-88.

[88] Wang Y, Schafler ED, Thomas PA, Ha S, David G, Adney E, Garabedian MJ, Lee P, Logan SK. Prostate-specific loss of UXT promotes cancer progression. Oncotarget. 2019;10(7):707-16.

[89] Wang Y, Wang X, Luo Y, Zhang J, Lin Y, Wu J, Zeng B, Liu L, Yan P, Liang J, Guo H, Jin L, Tang Q, Long K, Li M. Spatio-temporal transcriptome dynamics coordinate rapid transition of core crop functions in 'lactating' pigeon. PLoS Genet. 2023;19(6):e1010746.

[90] Way GP, Greene CS, Carninci P, Carvalho BS, de Hoon M, Finley SD, Gosline SJC, Lê Cao KA, Lee JSH, Marchionni L, Robine N, Sindi SS, Theis FJ, Yang JYH, Carpenter AE, Fertig EJ. A field guide to cultivating computational biology. PLoS Biol. 2021;19(10):e3001419.

[91] Williams JE, Massing M, Rosamond WD, Sorlie PD, Tyroler HA. Racial disparities in CHD mortality from 1968–1992 in the state economic areas surrounding the ARIC study communities. Atherosclerosis Risk in Communities. Ann Epidemiol. 1999;9: 472–80.

[92] Wright BW, Yi Z, Weissman JS, Chen J. The dark proteome: translation from noncanonical open reading frames. Trends Cell Biol. 2022;32(3):243-58.

[93] Wu D, Hu D, Chen H, Shi G, Fetahu IS, Wu F, Rabidou K, Fang R, Tan L, Xu S, Liu H, Argueta C, Zhang L, Mao F, Yan G, Chen J, Dong Z, Lv R, Xu Y, Wang M, Ye Y, Zhang S, Duquette D, Geng S, Yin C, Lian CG, Murphy GF, Adler GK, Garg R, Lynch L, Yang P, Li Y, Lan F, Fan J, Shi Y, Shi YG. Glucose-regulated phosphorylation of TET2 by AMPK reveals a pathway linking diabetes to cancer. Nature. 2018;559(7715):637-41.

[94] Wu Z, Liu P, Huang B, Deng S, Song Z, Huang X, Yang J, Cheng S. A novel Alzheimer's disease prognostic signature: identification and analysis of glutamine metabolism genes in immunogenicity and immunotherapy efficacy. Sci Rep. 2023;13(1):6895.

[95] Xie WQ, He M, Yu DJ, Wu YX, Wang XH, Lv S, Xiao WF, Li YS. Mouse models of sarcopenia: classification and evaluation. J Cachexia Sarcopenia Muscle. 2021;12(3):538-54.

[96] Xu NY, Liu ZY, Yang QM, Bian PP, Li M, Zhao X. Genomic analyses for selective signatures and genes involved in hot adaptation among indigenous chickens from different tropical climate regions. Front Genet. 2022;13:906447.

[97] Zhang J, Xue B, Gai M, Song S, Jia N, Sun H. Small RNA and transcriptome sequencing reveal a potential miRNA-mediated interaction network that functions during somatic embryogenesis in Lilium pumilum DC. Fisch. Front Plant Sci. 2017;8:566.

[98] Zhang S, Kim CC, Batra S, McKerrow JH, Loke P. Delineation of diverse macrophage activation programs in response to intracellular parasites and cytokines. PLoS Negl Trop Dis. 2010;4(3):e648.

[99] Zhou L, Zhao J, Han M, Ma A, Yang S, Zeng Y, Cheng J. Aspartate reduces liver inflammation and fibrosis by suppressing the NLRP3 inflammasome pathway via upregulating NS3TP1 expression. J Pers Med. 2023;13(3):386.