



Article Unveiling the Genetic Complexity of Teratozoospermia: Integrated Genomic Analysis Reveals Novel Insights into IncRNAs' Role in Male Infertility

Maria-Anna Kyrgiafini ¹, Themistoklis Giannoulis ², Alexia Chatziparasidou ³, Nikolaos Christoforidis ³ and Zissis Mamuris ^{1,*}

- ¹ Laboratory of Genetics, Comparative and Evolutionary Biology, Department of Biochemistry and Biotechnology, University of Thessaly, Viopolis, Mezourlo, 41500 Larissa, Greece
- ² Laboratory of Biology, Genetics and Bioinformatics, Department of Animal Sciences, University of Thessaly, Gaiopolis, 41336 Larissa, Greece
- ³ Embryolab IVF Unit, St. 173-175 Ethnikis Antistaseos, Kalamaria, 55134 Thessaloniki, Greece
- Correspondence: zmamur@uth.gr

Abstract: Male infertility is a global health issue, affecting over 20 million men worldwide. Genetic factors are crucial in various male infertility forms, including teratozoospermia. Nonetheless, the genetic causes of male infertility remain largely unexplored. In this study, we employed whole-genome sequencing and RNA expression analysis to detect differentially expressed (DE) long-noncoding RNAs (lncRNAs) in teratozoospermia, along with mutations that are exclusive to teratozoospermic individuals within these DE lncRNAs regions. Bioinformatic tools were used to assess variants' impact on lncRNA structure, function, and lncRNA-miRNA interactions. Our analysis identified 1166 unique mutations in teratozoospermic men within DE lncRNAs, distinguishing them from normozoospermic men. Among these, 64 variants in 23 lncRNAs showed potential regulatory roles, 7 variants affected 4 lncRNA structures, while 37 variants in 17 lncRNAs caused miRNA target loss or gain. Pathway Enrichment and Gene Ontology analyses of the genes targeted by the affected miRNAs revealed dysregulated pathways in teratozoospermia and a link between male infertility and cancer. This study lists novel variants and lncRNAs associated for the first time with teratozoospermia. These findings pave the way for future studies aiming to enhance diagnosis and therapy in the field of male infertility.

Keywords: male infertility; teratozoospermia; long-noncoding RNA (lncRNA); variant

1. Introduction

Infertility is a significant global health concern that affects approximately 50 million couples worldwide [1]. It is estimated that around 12.5% of women and 10% of men experience infertility [2]. The World Health Organization (WHO) defines infertility as the inability to achieve pregnancy after engaging in regular unprotected sexual intercourse for a duration of 12 months or longer. The male factor contributes to 50% of infertility cases [3], and various sperm defects, such as quantity and quality issues, can be identified through semen analysis as causes of male infertility [4], though the etiology of sperm defects remains idiopathic in 30–50% of cases [5]. Teratozoospermia is defined as a percentage of morphologically normal spermatozoa below the lower reference limit. However, the definition of normality has significantly changed over the past few decades, ranging from 50% in the initial WHO classification in 1980 [6] to 4% in the most recent version published in 2010 [7]. In general, a morphologically normal spermatozoon is characterized by specific features, including a normal acrosome, an oval-shaped head with a width ranging from 2.5 to 3.5 μ m and a length between 5 and 6 μ m, a midpiece measuring approximately 4.0 to 5.0 μ m, and a tail that is approximately 50 μ m in length [8]. Teratozoospermia encompasses



Citation: Kyrgiafini, M.-A.; Giannoulis, T.; Chatziparasidou, A.; Christoforidis, N.; Mamuris, Z. Unveiling the Genetic Complexity of Teratozoospermia: Integrated Genomic Analysis Reveals Novel Insights into IncRNAs' Role in Male Infertility. *Int. J. Mol. Sci.* 2023, 24, 15002. https://doi.org/10.3390/ ijms241915002

Academic Editor: Elisabetta Baldi

Received: 26 August 2023 Revised: 3 October 2023 Accepted: 4 October 2023 Published: 9 October 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). a heterogeneous group of abnormal sperm phenotypes that can affect different parts of sperm, such as the head, neck, midpiece, and tail. These abnormalities may occur individually or concurrently [9].

Although significant progress has been made in investigating teratozoospermia, the molecular mechanisms underlying this condition in male infertility are not yet fully understood. In general, the identification of the molecular causes of male infertility is a major challenge. It is estimated that more than 4000 genes play a role in spermatogenesis, making the characterization and identification of their specific contributions a complex undertaking [10]. Furthermore, emerging evidence suggests that noncoding RNAs have crucial Molecular Functions [11], including a role in reproduction and male infertility [12]. Various techniques, such as real-time quantitative PCR, microarrays, and high-throughput next-generation sequencing, have been developed to detect and measure noncoding RNAs. These techniques provide valuable insights into the role of noncoding RNAs and the development of complex diseases, such as cancer, diabetes, cardiovascular diseases, etc. [13,14]. Regarding reproduction, attention has been drawn to a particular type of noncoding RNA, long-noncoding RNAs (lncRNAs). LncRNAs, a diverse group of RNA molecules larger than 200 nucleotides, despite lacking the ability to code for proteins, play important roles in cellular functions. They can be classified based on their chromosomal location in several categories, including antisense lncRNAs, intronic lncRNAs, divergent lncRNAs, intergenic lncRNAs, promoter-associated lncRNAs, transcription start site-associated lncRNAs, and enhancer RNAs [15]. LncRNAs also exhibit four distinct functions [15–18]: signaling, where they regulate gene transcription either independently or with proteins like transcription factors; decoying, in which they bind to proteins to impede interactions with DNA, mRNA, or miRNA; guiding, as they transport proteins to precise intracellular sites; and scaffolding, where they facilitate the assembly of macromolecular complexes for coordinated interactions.

When it comes to the involvement of lncRNAs in male infertility, particularly teratozoospermia, studies prove that these molecules play a role in the functions of spermatogonial stem cells, including the regulation of their differentiation, proliferation, and self-renewal processes [19]. Moreover, various studies have highlighted distinct expression patterns of lncRNAs between fertile and infertile men [12]. Nevertheless, our understanding of how lncRNAs precisely regulate reproduction and their association with teratozoospermia is still limited and subject to ongoing debate [20]. Except for studies reporting lncRNAs that are deregulated in teratozoospermia, there is also a significant knowledge gap in studying variants and mutations within lncRNA regions and investigating their role in teratozoospermia. Traditionally, research has primarily focused on genetic variations occurring within protein-coding regions to elucidate the molecular mechanisms underlying disease [21]. However, some genome-wide association studies (GWAS) have provided evidence suggesting that polymorphisms within lncRNA genes are linked to human diseases [21,22]. More specifically, single nucleotide polymorphisms (SNPs) in lncRNAs can have various consequences [21]. Firstly, variations in regulatory regions, such as transcription binding sites or lncRNA gene promoters, can affect lncRNA expression, resulting in the dysregulation of associated pathways [22,23]. SNPs can also disrupt the secondary structure of lncRNAs, thereby impacting their interactions with miRNAs, RNA-binding proteins, and other molecules [21,24]. Furthermore, the presence of a variant within the sequence of a lncRNA can potentially affect RNA turnover by altering the binding of proteins responsible for lncRNA's stability [21]. Lastly, similar to protein-coding genes, mutations in IncRNA regions can disrupt the splicing process and influence the structure and functionality of lncRNAs [21]. Numerous studies have demonstrated that variations in lncRNAs can contribute to the development of various diseases, such as different types of cancer [21,25] and a type of muscular dystrophy [26], through the mechanisms described above. However, despite indications of the significant role of lncRNAs in male infertility, no studies have been conducted to explore whether specific variants can impact lncRNA's expression or function, thereby leading to specific subtypes of male infertility, such as teratozoospermia. Thus, this study aims to explore the role of lncRNA variants in the pathogenesis of male infertility, specifically teratozoospermia. To achieve this, we utilized whole-genome sequencing (WGS) data and integrated these with RNA expression profiles from normozoospermic and teratozoospermic men. Our objectives are to (a) identify and characterize variants that present solely in teratozoospermic men and map to differentially expressed lncRNA regions and (b) investigate the impact of prioritized variants on the function, structure, and interactions of lncRNAs, particularly with microRNAs (miRNAs). By exploring these effects, we aim to shed light on the role of lncRNAs in teratozoospermia. The ultimate goal of this study is to provide a valuable reference for future research on teratozoospermia, as identified variants could hold significant importance in unraveling the genetic basis of teratozoospermia, potentially leading to improved diagnosis and treatment.

2. Results

2.1. WGS and RNA Expression Results—Identification of Exclusive Variants on DE lncRNAs

The primary objective of this study was to identify exclusive variants in teratozoospermic men that are mapped to differentially expressed (DE) lncRNA regions and explore their role in the pathogenesis of teratozoospermia as well as their impact on lncRNAs. To accomplish this, whole-genome sequencing (WGS) and RNA expression data were integrated.

Specifically, 617,722 variants were found exclusively in teratozoospermic men, while 2,342,243 variants were present only in normozoospermic men. The identified variants were mapped to 34,603 and 22,022 genes and characterized as non-coding regions, such as miRNAs and lncRNA genes, in normozoospermic and teratozoospermic men, respectively. However, for this study, only the variants identified exclusively in teratozoospermic men were selected for further analysis, as the aim was to detect and investigate variants in lncRNA regions contributing to teratozoospermia.

Furthermore, to identify lncRNAs that are deregulated in teratozoospermia and, thus, that may play a role in male infertility, the analysis of Zhou and Wang (2020) [27] was utilized. Zhou and Wang (2020) [27], by applying a significance threshold of *p*-value < 0.05 and a fold change cutoff of [logFC] > 2, detected a total of 101 differentially expressed lncRNAs. Specifically, in comparison with the normozoospermic group, 68 lncRNAs were upregulated, and 33 lncRNAs were downregulated in the teratozoospermic group. Supplementary Table S1 provides a comprehensive list of these differentially expressed lncRNAs, as reported by Zhou and Wang [27].

Finally, the two datasets mentioned above, namely the differentially expressed (DE) lncRNAs and the exclusive variants in teratozoospermic individuals, were integrated. The aim was to identify exclusive variants found in teratozoospermic men that were mapped to DE lncRNAs. These variants have the potential to impact the structure, function, and interactions of lncRNAs, leading to altered gene expression and potentially contributing to the pathogenicity of teratozoospermia, as described in the Introduction [21]. Moreover, they have the potential to be used as biomarkers in teratozoospermia. Consequently, a total of 1166 variants found only in teratozoospermic men were identified to be mapped onto DE lncRNAs. The complete list of these unique variants mapped onto DE lncRNAs can be found in Supplementary Table S2. Subsequently, these variants were prioritized by applying a series of filters to investigate their consequences on lncRNAs and their role in teratozoospermia.

2.2. Exclusive Variants with a Functional Role

First, to comprehensively evaluate the impact of identified variants on lncRNA functionality and prioritize them, our analysis incorporated data from both RegulomeDB [28] and 3DSNP [29] databases. RegulomeDB [28] provides valuable insights into the regulatory significance of these variants by assessing their association with known regulatory elements, such as transcription factor binding sites and DNase I hypersensitive sites. On the other hand, the 3DSNP [29] database assesses variant significance by considering multiple factors, including thermodynamic stability, evolutionary conservation, protein binding sites, and structural dynamics. Therefore, in our study, we set stringent criteria, considering variants with a 3DSNP score greater than 10 and a RegulomeDB Rank ranging from 1a to 2c. Applying these criteria, we identified 64 variants on 23 lncRNAs that displayed a strong likelihood of influencing lncRNA functionality and contributing to teratozoospermia. These findings are presented in Table 1.

Table 1. Prioritized variants found exclusively in teratozoospermic men, which are mapped to differentially expressed lncRNAs, and have a strong likelihood of affecting lncRNAs' functionality, according to RegulomeDB [28] and 3DSNP [29] databases.

Variant	lncRNA	3DSNP Score	RegulomeDB Rank
rs11170045	LINC00592	42.35	1b
rs17126450	LINC00592	34.82	1b
rs7972661	LINC00592	28.40	1f
rs936329	LINC00592	26.09	1f
rs1870213	LINC00592	27.76	1f
rs143637901	LINC00592	27.63	1f
rs7301136	LINC00592	23.79	1f
rs7315889	LINC00592	44.57	1b
rs10783499	LINC00592	45.49	1f
rs7316130	LINC00592	45.37	1f
rs10876245	LINC00592	46.98	1f
rs10876246	LINC00592	14.97	1f
rs10876247	LINC00592	16.98	1f
rs10876248	LINC00592	12.32	1f
rs10783500	LINC00592	12.40	1f
rs7303604	LINC00592	10.60	1f
rs10783503	LINC00592	25.87	1f
rs10783504	LINC00592	125.41	1f
rs11170048	LINC00592	125.70	1f
rs11170049	LINC00592	125.39	1f
rs11170050	LINC00592	123.71	1f
rs56035420	HOXC-AS3	48.25	1b
rs1956568	FRMD6-AS1	25.01	1b
rs1956567	FRMD6-AS1	22.74	1f
rs566495825	FRMD6-AS1	16.98	2b
rs71266965	AQP4-AS1	23.77	2b
rs953369619	AQP4-AS1	38.38	2b
rs1265960	A1BG-AS1	23.04	1f
rs56822355	LINC01350	136.62	1b
rs41453048	LINC01350	18.39	1b
rs12029108	LINC01350	16.82	1f
rs72727706	LINC01350	12.63	1f
rs80202485	LINC00466	126.85	1f
rs72781367	LINC00276	11.09	1b

Variant	lncRNA	3DSNP Score	RegulomeDB Rank
rs2969359	LINC01116	84.20	1b
rs2969358	LINC01116	84.25	1f
rs2969357	LINC01116	74.60	1f
rs6723379	DCTN1-AS1	44.15	1f
rs1727884	ARHGEF26-AS1	18.68	1f
rs1713828	ARHGEF26-AS1	55.43	1f
rs1713827	ARHGEF26-AS1	125.75	1f
rs71281391	PRICKLE2-AS3	23.94	2b
rs901028815	LINC00877	11.67	2b
rs59058260	LINC00877	15.06	2a
rs10016533	SEC24B-AS1	111.37	1f
rs553142360	LINC01091	56.16	2b
rs17491036	LINC01091	24.33	2b
rs1560738	LINC01091	21.52	1b
rs58833496	LINC01091	10.36	1f
rs78146337	NNT-AS1	12.66	1b
rs3734931	C7orf69	50.17	2b
rs4595031	C7orf69	10.44	1f
rs7823346	ZNF252P-AS1	205.05	2b
rs2978419	ZNF252P-AS1	106.11	1f
rs7832026	ZNF252P-AS1	54.79	1f
rs10107110	LZTS1-AS1	20.92	1b
rs12681283	STAU2-AS1	52.30	2b
rs199889712	OTUD6B-AS1	75.01	1b
rs3217986	CDKN2B-AS1	75.44	2b
rs1368574245	CDKN2B-AS1	92.75	2b
rs73652847	CDKN2B-AS1	12.19	2b
rs17694555	CDKN2B-AS1	14.83	1f
rs564311323	CDKN2B-AS1	17.41	2b
rs112111321	RAP2C-AS1	216.01	1a

Table 1. Cont.

2.3. Exclusive Variants Affecting lncRNAs' Structure

Among the 1166 variants exclusively observed in teratozoospermic men and identified within DE lncRNAs, the variants affecting the secondary structure of lncRNAs were prioritized, as they could contribute to the development of teratozoospermia. Even minor changes in the nucleotide sequence, such as single nucleotide polymorphisms (SNPs), have the potential to disrupt the thermodynamic stability of RNA secondary structures. Consequently, these alterations can impact the overall three-dimensional conformation and stability of lncRNAs, potentially influencing their interactions with other molecules, including proteins and DNA. Furthermore, since lncRNAs lack protein-coding capacities and rely on their structural integrity for proper functioning, SNPs can induce significant structural alterations that can affect the functionality of lncRNAs [30–32]; thus, they were prioritized. In this study, variants with a *p*-value < 0.2, a cutoff indicating an impact on lncRNAs' structure as determined by lncRNASNP v3 [33], were selected. Overall, the analysis revealed 7 SNPs that affected the structure of 4 specific lncRNAs, as summarized in Table 2.

Table 2. Prioritized variants found exclusively in teratozoospermic men, which are mapped to differentially expressed lncRNAs, and have an impact on the structure of lncRNAs, as determined by lncRNASNP v3 database [33].

Variant	lncRNA	Transcript	<i>p</i> -Value
rs139506376	LINC00944	NONHSAT031816.2	0.1688
rs200712585	PRMT5-AS1	NONHSAT035833.2	0.0739
	PRMT5-AS1	NONHSAT168171.1	0.0814
	PRMT5-AS1	NONHSAT168172.1	0.1409
	PRMT5-AS1	NONHSAT035834.2	0.0818
rs4792409	PRMT5-AS1	NONHSAT145734.2	0.1078
	COX10-AS1	NONHSAT145753.2	0.0946
	COX10-AS1	NONHSAT145752.2	0.0946
ma150264102	COX10-AS1	NONHSAT145754.2	0.0946
15150504102	COX10-AS1	NONHSAT145751.2	0.0921
	COX10-AS1	NONHSAT175957.1	0.0946
	COX10-AS1	NONHSAT175958.1	0.0946
mo7E122619	COX10-AS1	NONHSAT175957.1	0.1591
r\$75155618	COX10-AS1	NONHSAT175958.1	0.1591
rs142992890	MIR663AHG	NONHSAT189547.1	0.1669
rs1285843394	MIR663AHG	NONHSAT189547.1	0.1941

2.4. Exclusive Variants Affecting miRNA–lncRNA Interactions and Investigation of Target Genes of Affected miRNAs

Exclusive variants on teratozoospermic men were also prioritized to identify those that affect miRNA–lncRNA interactions. These variants can lead to the gain or loss of miRNA target sites within lncRNAs, consequently altering the regulation of gene expression [34]. Through their intricate interactions, miRNAs and lncRNAs orchestrate important cellular processes and can contribute to the development and progression of various diseases [35], such as teratozoospermia. Therefore, according to the lncRNASNP v3 database [33], out of the 1166 exclusive variants identified on DE lncRNAs, 37 SNPs were found to affect interactions between 17 lncRNAs and 151 miRNAs. The complete list of variants and the corresponding affected interactions (lncRNAs and miRNAs) can be found in Supplementary Table S3.

Subsequently, an investigation was carried out to determine the overlap of target genes among affected miRNAs, aiming to identify common pathways that become deregulated in teratozoospermia due to lncRNA variants. According to miRTargetLink 2.0 [36], affected miRNAs targeted a total of 198 common genes. The complete list of these genes and their interactions with miRNAs is provided in Supplementary Table S4. It is important to note that only strong and validated interactions were selected for constructing miRNA–mRNA interaction networks.

Additionally, to gain further insights into the role of target genes, Gene Ontology (GO) Enrichment analysis [37,38] and KEGG pathways analysis [39] were performed. These analyses revealed dysregulation in processes such as cell proliferation and programmed cell death, with many miRNAs and miRNA target genes being involved in pathways associated with various types of cancer. In terms of Cellular Components, most of these genes were associated with the Bcl-2 family protein complex, while the most enriched category for



the Molecular Function consisted of genes encoding proteins with tyrosine kinase activity (Figure 1a–d).

Figure 1. Significant (**a**) GO Biological Process, (**b**) GO Molecular Function, (**c**) GO Cellular Component, and (**d**) KEGG pathway terms associated with the overlap gene targets of miRNAs that are affected by variants in lncRNA regions. The size and color of the dots represent the number of genes and the range of statistical significance, respectively. The red color indicates higher -log10(FDR) values, followed by pink, purple and blue colors. The *y*-axis represents the GO and KEGG terms, and the *x*-axis represents the fold enrichment. The *p*-values were corrected for multiple tests using the false discovery rate (FDR).

2.5. Variants Affecting Both Structure or Function of IncRNAs and miRNA-IncRNA Interactions

Then, we identified prioritized variants that had potential effects on both the function or structure of lncRNAs and on miRNA–lncRNA interactions. These variants, which are listed in Table 3, are particularly significant as they are exclusively found in teratozoospermic men and are mapped to lncRNAs, which are known to be deregulated in teratozoospermia.

Variants	lncRNAs	Impact on IncRNAs
rs150364102	COX10-AS1	Structure and miRNA-IncRNA interactions
rs56035420	HOXC-AS3	Function and miRNA-IncRNA interactions
rs2969359	LINC01116	Function and miRNA-IncRNA interactions
rs2969358	LINC01116	Function and miRNA-IncRNA interactions
rs2969357	LINC01116	Function and miRNA-IncRNA interactions
rs1713827	ARHGEF26-AS1	Function and miRNA-IncRNA interactions

Table 3. Exclusive variants mapped on DE lncRNAs that affect both the structure or function of lncRNAs and miRNA–lncRNA interactions.

2.6. IncRNAs with Multiple Variants Affecting Their Function, Structure, or Interactions with miRNAs

As a final step in this study, we identified lncRNAs with multiple prioritized variants that affect both the function or structure of lncRNAs and their interactions with miRNAs. The lncRNAs are presented in Table 4. These findings shed light on the potential mechanisms underlying teratozoospermia and provide valuable insights into the role of lncRNAs in male fertility issues.

Table 4. DE lncRNAs with exclusive variants that affect both their function or structure and lncRNAmiRNA interactions.

lncRNAs	Number of Variants	Impact of Variants
COX10-AS1	7	Structure and miRNAs-IncRNAs
MIR663AHG	6	Structure and miRNAs-IncRNAs
HOXC-AS3	2	Function and miRNAs-IncRNAs
AQP4-AS1	5	Function and miRNAs-IncRNAs
A1BG-AS1	6	Function and miRNAs-IncRNAs
LINC01116	7	Function and miRNAs-IncRNAs
DCTN1-AS1	2	Function and miRNAs-IncRNAs
ARHGEF26-AS1	6	Function and miRNAs-IncRNAs
PRICKLE2-AS3	2	Function and miRNAs-IncRNAs
STAU2-AS1	2	Function and miRNAs-IncRNAs
RAP2C-AS1	2	Function and miRNAs-IncRNAs
CDKN2B-AS1	7	Function and miRNAs-IncRNAs

3. Discussion

Previous studies have demonstrated that variants in lncRNAs can impact their function, structure, and interactions with other molecules [21]. Consequently, these variants may lead to the dysregulation of gene expression and contribute to disease development [14,21,40]. While the functions of the majority of estimated ~20,000 lncRNAs remain unknown [21], studies suggest that they play an essential role in spermatogenesis regulation [19,20]. Furthermore, lncRNAs have been associated with male infertility and its specific subtypes [12], such as teratozoospermia, which is characterized by morphological abnormalities in spermatozoa. However, no studies have investigated the consequences of lncRNA variants and their role in teratozoospermia. Therefore, this study aimed to combine WGS and RNA expression profile data to identify exclusive variants found only in teratozoospermic men that are also mapped to lncRNAs with a deregulated expression in teratozoospermia. Subsequently, this study assessed the impact of these variants on the structure, functionality, and interactions of lncRNAs. In summary, out of the 1166 exclusive variants identified, 64 variants on 23 lncRNAs displayed a strong likelihood of influencing lncRNA functionality. Additionally, 7 SNPs affected the structure of 4 lncRNAs, while 37 SNPs were found to affect the interactions between 17 lncRNAs and 151 miRNAs. The affected miRNAs' overlapping target genes were primarily associated with cancer and processes such as cell proliferation, apoptosis, programmed cell death, etc.

3.1. Impact of Variants on the Functionality of lncRNAs

At first, we assessed the impact of variants on the functionality of lncRNAs. Among the variants identified with potential functional roles, only one had previously been associated with a specific disease. Specifically, the variant rs3217986, mapped on *CDKN2B-AS1 (AN-RIL)*, has been associated with cutaneous melanoma [41] and coronary artery disease [42]. Regarding the other lncRNAs on which these variants were mapped, *LINC00592* has been demonstrated in several studies to play a role in various cancer types through transcription regulation [43,44]. Other lncRNAs identified as potentially involved in cancer include *FRMD6-AS1* [45,46] and *LINC00466* [47,48], which have also been shown to promote tumor growth in vivo alongside [49] *LINC00877* [50], *LINC01091* [51], *NNT-AS1* [52–54], *ZNF252P-AS1* [55], *LZTS1-AS1* [56], and *OTUD6B-AS1* [57,58]. Of particular significance is also the fact that the host gene of *NNT-AS1*, *NNT*, encodes an inner mitochondrial membrane protein and produces large amounts of NADPH. *NNT* deficiency has been reported to cause complete germ line loss and azoospermia [59]. Additionally, a separate study has associated *NNT* mutations with impairments in gonadotropic function and genitalia [60].

3.2. Impact of Variants on the lncRNAs' Structure

Variants can also have significant effects on the structure of lncRNAs. These alterations can disrupt stable secondary structures within lncRNAs, potentially impacting base-pairing interactions and binding sites for other molecules, or can influence alternative splicing, resulting in distinct structural conformations of lncRNA isoforms [30–32,61]. It should be noted that any of the structural alterations described above can significantly impact functionality. These changes can alter binding affinity, subcellular localization, stability, and the accessibility of functional domains within lncRNAs. Consequently, they can influence lncRNAs' role in gene regulation and cellular processes. In the present study, none of the variants identified to affect lncRNAs' structure had been previously associated with male infertility or other diseases. Regarding the lncRNAs on which these were found, *PRMT5-AS1* is highly expressed in the testis, but its role and function have not been extensively studied. Baytak et al. (2017) [62] suggested its involvement in lymphoma and its potential impact on cell growth. Interestingly, however, its host gene, PRMT5, is essential for germ cell development and the preservation of genomic integrity [63]. Another study also demonstrated that the spermatogonia-specific deletion of Prmt5 leads to germ cell loss and male infertility [64]. LINC00944 is another lncRNA-harboring exclusive variant that affects its structure. While this lncRNA has been associated with various cancer subtypes, most studies have focused on its role in renal cell carcinoma [65,66], where it functions as an oncogene [67]. Variants affecting structure were also found on COX10-AS1 and MIR663AHG.

3.3. Impact of Variants on miRNA–lncRNA Interactions and Processes and the Pathways Affected

Studying miRNA–lncRNA interactions is crucial due to their significant role in gene regulation and their implications in disease development [35]. In this study, the variant rs62560775, found on *CDKN2B-AS1 (ANRIL)*, is linked to lung cancer [68]. Numerous lncRNAs with variants identified in this study, including *LINC01359* [69], *LINC01305* [70], *SLC16A1-AS1* [71,72], and *FIRRE* [73,74], are associated with cancer. *LINC01359* is also deregulated in asthenozoospermia [75] and oligozoospermia [76], whereas the host gene of *SLC16A1-AS1*, *SLC16A1*, is connected to spermatogenic defects [77]. Additionally, *FIRRE*, located on the X chromosome, plays a role in the chromosomal organization and nuclear position of the inactive X chromosome (Xi) in female cells [78].

Furthermore, regarding miRNA–lncRNA interactions, we examined the gene targets of affected miRNAs using GO and KEGG Enrichment analyses. Our findings revealed a significant presence of gene targets within the Bcl-2 protein family complex. The BCL2 protein family plays a pivotal role in apoptosis regulation and maintaining cellular balance, with members like BCL2 supporting cell survival and others like BAX promoting cell death. These proteins also regulate mitochondrial membrane permeability [79]. In the context of male infertility, our study validates prior research, highlighting the importance of BCL2 family proteins in sperm development and function [80]. Apoptosis is a dynamic process in spermatogenesis affecting germ cell divisions, differentiation, and sperm formation [81]. Imbalances in BCL2 family protein levels can disrupt spermatogenesis and fertility. In teratozoospermia, the dysregulation of the apoptotic machinery due to altered miRNA–lncRNA interactions may result in spermatozoa with abnormal morphology "es-caping" apoptosis, contributing to the condition.

KEGG pathway analysis has revealed a significant association between gene targets affected by miRNAs and cancer, reinforcing the existing link between male infertility and cancer. Previous studies have suggested a higher cancer risk in infertile men, suggesting shared molecular pathways [82–87]. Our study, supported by GO Biological Process Enrichment, identified the gene targets affected by miRNAs, including genes associated with apoptosis, programmed cell death, cell proliferation, and differentiation—all critical in cancer development [86]. Similarly, in male infertility, dysregulated apoptosis and cell processes can lead to teratozoospermia. This condition can also result from disruptions in cell proliferation and differentiation during spermatogenesis: a complex process involving spermatogonial cell growth, meiotic divisions, and spermatid maturation [88]. While common genetic pathways might link cancer and male infertility, their precise molecular mechanisms require further investigation.

Finally, GO Biological Process Enrichment and KEGG analyses identified deregulated signaling pathways in teratozoospermia, including TNF, FoxO, MAPK, and PI3K-Akt pathways. More specifically, FoxO transcription factors regulate the cell cycle, apoptosis, and oxidative stress response, impacting germ cell balance and sperm quality [89,90]. The MAPK pathway is also essential for spermatogenesis, influencing proliferation, differentiation, apoptosis, and sperm quality [91–93]. PI3K-Akt regulates the hypothalamus–pituitary–gonad (HPG) axis, spermatogonia, somatic cells, and sperm autophagy, affecting germ cell survival and spermatogenesis [94], while TNF, as an inflammation-related cytokine, is linked to testicular dysfunction, impaired sperm function, and increased apoptosis, all impacting sperm production and quality [95,96]. Additionally, our study revealed that many affected miRNAs target kinases, which are essential for regulating these processes and various sperm development stages in the testis [97,98].

3.4. Common Variants and lncRNAs with Multiple Pioritized Variants

In the present study, we also identified variants that affected both the function or structure of lncRNAs and their interactions with miRNAs, as presented in Table 3. These findings suggest the dual impact of the above variants on lncRNA functionality and interactions, potentially contributing to teratozoospermia. Therefore, these variants hold potential for future investigation as biomarkers, and conducting functional experiments could help validate their impact on the functionality and structure of lncRNAs, unveiling the molecular mechanisms underlying teratozoospermia.

Furthermore, we identified lncRNAs with multiple prioritized SNPs, as depicted in Table 4. Among the above lncRNAs, COX10 antisense RNA 1 (*COX10-AS1*) stands out as it harbors seven variants that are found only in teratozoospermic men and is the only one that has been linked in the past with male infertility as it dysregulates not only in teratozoospermia but also other types of male infertility, such as asthenozoospermia [75] and oligozoospermia [76]. While its role in human diseases was previously poorly understood, several studies have associated *COX10-AS1* with various types of human cancers, suggesting its involvement as an oncogene [99–101]. These studies have shed light on

the complex interactions between *COX10-AS1* and miRNAs, promoting cell proliferation and inhibiting apoptosis in cancer cells [102,103]. The emerging evidence of *COX10-AS1*'s significance in both male infertility and cancer highlights its potential as a crucial player in these Biological Processes, warranting further investigation.

CDKN2B-AS1 (ANRIL) is also a captivating lncRNA with implications in various cancer types, where it serves as a critical regulator of cell proliferation [61–63]. Additionally, it has been linked to several non-malignant diseases, such as idiopathic pulmonary fibrosis, endometriosis, inflammatory bowel disease, intracranial aneurysm, diabetes mellitus, coronary artery diseases, and atherosclerosis [64]. However, intriguingly, no studies to date have associated this lncRNA with male infertility or proposed its role in spermatogenesis. Despite its extensive involvement in different diseases, the absence of investigations regarding its connection to male fertility warrants further research to explore its potential relevance in this context.

Among the identified lncRNAs mentioned above, several have also been associated with cancer. *LINC01116*, for instance, has a well-established role in cancer development, with numerous studies supporting its involvement in promoting cell proliferation, invasion, migration, and apoptosis [104]. Similarly, *HOXC-AS3* is extensively implicated in tumorigenesis through the promotion of proliferation [105,106]. *A1BG-AS1* has been linked to cancer in some studies [107,108], while *ARHGEF26-AS1* is a ferroptosis-related lncRNA with a role in tumorigenesis [109,110]. Additionally, *MIR663AHG* has been found to be downregulated in psoriatic tissues [111] and acts as a tumor suppressor, inhibiting the development of colon cancer by cis-binding to miR663a/pre-miR663a [112].

On the other hand, the available studies are limited for some lncRNAs. *RAP2C-AS1*, which has been implicated in esophageal cancer [113], *AQP4-AS1*, while also being associated with retinal neurovascular dysfunction [114], possibly cancer [102], and *DCTN1-AS1*, potentially contributing to Alzheimer's disease development [103], has undergone scarce investigations. Furthermore, no studies explore the role and function of *STAU2-AS1* and *PRICKLE2-AS3*. Importantly, for all the mentioned lncRNAs, no studies have implicated their role in male infertility or spermatogenesis. While these lncRNAs have been linked to cancer and other diseases, their involvement in male reproductive health remains unexplored, presenting an avenue for further research to understand their potential significance in male fertility and spermatogenesis.

4. Materials and Methods

4.1. Whole Genome Sequencing (WGS)—Identification of Exclusive Variants on Teratozoospermic Men

Blood and semen samples were collected from Greek volunteers participating in the Spermogene (Fertilaid) research program (Grant number T1E Δ K-02787) in collaboration with the "Embryolab IVF Unit" in Thessaloniki, Greece. This study obtained ethical approval from the Ethics Committee of the University of Thessaly, and all participants willingly provided written informed consent for their participation in the program.

All recruited volunteers underwent an andrological examination, and their semen samples were subjected to comprehensive analysis. Sperm samples were collected via masturbation, following a minimum abstinence period of two to three days. The semen analysis followed the guidelines outlined in the fifth edition (2010) of the World Health Organization (WHO). This analysis included evaluating parameters such as semen volume, sperm count, motility, morphology, etc. Cell vision counting slides (Tek-Event) were utilized for cell counting, and Nikon Eclipse TS100, E200, and Ts2 microscopes (Minato, Japan) were employed for observation during semen analysis. WHO guidelines were employed for the processing and classification of human sperm. To elaborate, the samples underwent classification using the seminogram results and reference values stipulated in the WHO guidelines. The classification categorized the samples into two groups: those with morphology falling below the 5th percentile of fertile subjects. It is important to note that this classification

is in line with the latest version of the WHO guidelines, specifically the 2021 edition (accessible at https://www.who.int/publications/i/item/9789240030787, accessed on 25 August 2023). This approach replaces the use of the terms "teratozoospermic" and "normozoospermic", respectively.

Then, genomic DNA was extracted from the blood samples of five individuals with teratozoospermia and ten individuals with normozoospermia using the PureLink Genomic DNA Mini Kit (Invitrogen, Waltham, MA, USA—Catalog number: K182002), following the manufacturer's instructions. The quality of the DNA was assessed using agarose gel electrophoresis, while the quantity was determined using the Qubit 2.0 fluorometer and the Qubit dsDNA BR Assay Kit (Invitrogen, Waltham, MA, USA—Catalog number: Q32850). Subsequently, the DNA samples were divided into three sequencing pools. Two of the pools contained DNA from the normozoospermic individuals, with each pool consisting of DNA from five individuals. The third pool was created by pooling the DNA from teratozoospermic individuals. The DNA within each pool was mixed in equimolar ratios, resulting in a final concentration of 100 ng/ μ L and a total quantity of 2 mg.

After the completion of sample preparation, the DNA samples were shipped to Novogene (Cambridge, UK) for sequencing. Paired-end libraries with 100 bp reads were constructed, and sequencing was performed using an Illumina HiSeq 3000 platform, aiming for an average sequencing coverage of $30 \times$. The quality of the generated FASTQ files was initially assessed using FASTQC [115]. Subsequently, Trimmomatic [116] was used to remove low-quality reads (with a minimum PHRED score of 30) and adapter sequences. Following quality control, the reads were aligned to the GRCh37/hg19 human reference genome obtained from the Ensembl database [117] using the Burrows–Wheeler aligner (BWA) (version 0.7.17) [118]. Duplicate reads resulting from the polymerase chain reaction (PCR) were identified and removed using Picard tools before further analysis. The alignment results were then converted from the SAM to BAM format using SAMtools [119]. Next, the individual BAM files from the two normozoospermic pools were merged into a single file representing normozoospermic individuals while also using SAMtools [119]. A variant calling was performed using freeBayes (version 1.3.6) [120], and the resulting variants were stored in the variant call format (VCF). It should be noted that for BWA and freeBayes, default parameters were used as proposed by the developers. To identify unique variants specific to either normozoospermic or teratozoospermic individuals, the VCF files from both groups were compared using BCFtools [119]. These unique variants, which were not shared between the two groups (teratozoospermic and normozoospermic), were subjected to further analysis, focusing on individuals diagnosed with teratozoospermia. These exclusive variants found in teratozoospermic patients have the potential to contribute to the pathogenic phenotype and provide valuable insights into the molecular mechanisms underlying male infertility, particularly in the context of teratozoospermia. After the detection of unique variants for teratozoospermic individuals, annotation was performed using the VEP tool [121] provided by the Ensembl database. Among the types of annotations performed, these included the genes and the transcripts affected, the type of variants (coding/non-coding), the consequence of the variants (intronic, intergenic, frameshift, missense, synonymous, etc.), and associated minor allele frequencies from the 1000 Genomes Project [122], etc.

4.2. RNA Expression Profiles—Identification of Differentially Expressed lncRNAs between Normozoospermic and Teratozoospermic Men

Data were retrieved from the publication of Zhou and Wang (2020) [27] to identify differentially expressed (DE) lncRNAs between teratozoospermic and normozoospermic individuals. In summary, as described in the publication of Platts et al. (2007) [123], semen samples were collected from thirteen normospermic and eight teratozoospermic men to investigate the RNA expression profiles of human spermatozoa. RNA extraction was performed on purified sperm cells obtained from ejaculate samples, and the extracted RNA was subjected to hybridization using Affymetrix U133 (v2) Microarrays. The data from this

study are available in the GEO database under the accession number GSE6872 [123]. Then, based on the analysis of Zhou and Wang (2020) [27], DE lncRNAs were identified using cut-off criteria with a *p*-value < 0.05 and [logFC] > 2 [27].

4.3. Identification of Exclusive Variants on DE lncRNAs and Variant Prioritization—Investigation of Their Role and Consequence

Following the identification of variants exclusively found in teratozoospermic men through whole-genome sequencing analysis and the identification of differentially expressed (DE) lncRNAs between teratozoospermic and normozoospermic men, these datasets were integrated. Only the exclusive variants found in teratozoospermic men that mapped onto DE lncRNAs were selected for further analysis, as variants occurring in lncRNAs had the potential to influence the expression level, structure, and function of these lncRNAs by interfering with the expression of their corresponding target mRNAs [124]. In this way, they can contribute to the development of complex disorders, such as male infertility [21]. Additionally, these variants hold the potential to serve as valuable biomarkers [124].

Then, to further prioritize the exclusive variants on DE lncRNAs, a series of filters were applied. Specifically, variants with potential functional roles were selected based on information from the 3DSNP [29] and RegulomeDB databases [28]. RegulomeDB integrates data from ENCODE, GEO, and other sources to identify the regulatory roles of non-coding SNPs. Its main function is to assign scores to SNPs, allowing for the differentiation of functional SNPs from a large pool of variants. Each SNP is assigned a rank from 1 to 7, with lower values indicating a higher likelihood of having a regulatory function [28]. Similarly, the 3DSNP database provides information on 3D-interacting genes, enhancer states, promoter states, transcription factor binding sites, altered sequence motifs, and conservation. It calculates a functional score for each SNP, with higher scores indicating a greater likelihood of SNP functionality [29]. Thus, to assess the impact of variants on lncRNA functionality, only variants meeting the criteria of a 3DSNP score > 10 and a RegulomeDB Rank between 1a and 2c were used for further analysis. It is important to note that when evaluating the functionality of variants and prioritizing them, we opted for specific thresholds. These thresholds included a 3DSNP score greater than 10 and a RegulomeDB Rank less than 3. These thresholds were chosen based on previous and similar publications, where they have been associated with a relatively high level of evidence for the potential regulatory functions of SNPs [125–128]. Additionally, the lncRNA structure changes induced by variants were also investigated. The lncRNASNP v3 [33] database utilizes data from RNAsnp [129] to evaluate the effects of variants on lncRNA's secondary structure. Thus, variants were filtered, retaining only those with a *p*-value < 0.2, indicating their influence on lncRNA structure for subsequent analysis. In our analysis, a *p*-value less than 0.2 meant that a variant had a notable effect on lncRNA structure. This threshold aligns with the recommendations from the creators of lncRNASNP v3 [33] and is based on calculations from RNAsnp [129]. In brief, the program computes base-pairing probabilities for both the original (wild-type or WT) and altered (alternate or ALT) sequences containing the SNP. It then assesses the structural distinction between these sequences using metrics like Euclidean distance (d) and Pearson's correlation coefficient (r) for RNA segments spanning at least 50 nucleotides. The most significant structural change, either in terms of the maximum base pairing distance (d_{max}) or minimum correlation coefficient (r_{min}) , is identified. Empirical *p*-values are calculated to gauge the likelihood of this change occurring randomly. If the p-value for d_{max} falls below 0.2, it indicates that the SNP induces a substantial and non-random alteration in RNA structure. Furthermore, considering that lncRNAs interact with miRNAs to regulate gene expression, the lncRNASNP v3 database [33] was employed to identify exclusive variants on teratozoospermic men that result in the gain or loss of miRNA target sites on the aforementioned DE lncRNAs. This specific database was selected as it represents the intersection of results from MiRanda, TargetScan, and Pita, providing the final miRNA targets of lncRNAs. Subsequently, the gene targets of the affected miRNAs were identified using experimental interactions obtained

from miRTargetLink 2.0 [36]. To gain further insights into the role of these gene targets and identify deregulated pathways in teratozoospermia resulting from exclusive variants on DE lncRNAs that affect their interaction with miRNAs, Gene Ontology (GO) Enrichment analysis [37,38] and KEGG pathway analysis [39] were conducted using ShinyGO 0.77 [130]. For both GO and KEGG analyses, it is essential to highlight that statistical significance was reported after correcting for the false discovery rate (FDR) in order to account for multiple comparisons. Specifically, we applied an FDR-adjusted *p*-value threshold of <0.05. It is also important to note that only the overlapping gene targets of affected miRNAs were utilized for both the Gene Ontology (GO) Enrichment analysis [37,38] and the KEGG pathways analysis [39].

In summary, the methodology used in this study is presented in Figure 2.





5. Conclusions

In conclusion, this study represents the first comprehensive investigation into the impact of specific variants on lncRNAs' function and structure, utilizing whole-genome

sequencing and RNA expression profiles of patients with teratozoospermia. This research is highly significant as it lays the foundation for future studies by identifying the candidate lncRNAs and variants associated with teratozoospermia for the first time. The prioritized variants found exclusively in teratozoospermic men hold the potential to serve as valuable biomarkers pending further experimentation. Moreover, the identification of a link between male infertility and cancer opens promising avenues for future research. However, the small sample size of recruited patients is a limitation of this study, and therefore, the validation of these findings in a larger sample is recommended. Additionally, functional experiments are essential to confirm the impact of these variants on the structure and function of lncRNAs, as the present study relied on bioinformatics tools. Such validations could strengthen the credibility and applicability of the study's outcomes. However, it should be noted that we made concerted efforts to address the above-mentioned limitations by leveraging multiple databases and implementing a stringent set of criteria and filters during variant analysis.

Therefore, this study makes a significant contribution to our understanding of teratozoospermia by shedding light on important pathways that undergo deregulation due to variants on lncRNAs. The identification of specific lncRNAs and variants serves as a valuable foundation for enhancing teratozoospermia diagnosis, as a diverse range of variants and previously unexplored lncRNAs have been uncovered, holding promise as candidates for future research.

It is worth noting that this study represents a preliminary exploration into SNPs found within differentially expressed lncRNAs and potentially associated with teratozoospermia. Consequently, our findings should be interpreted with caution and validated in larger cohorts. Nevertheless, it is important to recognize that smaller-scale studies, such as the one presented here, can still yield valuable insights and lay the foundation for future research. By identifying specific SNPs and drawing attention to lncRNAs that may contribute to the pathogenesis of male infertility, especially in cases where limited information is available regarding variants affecting lncRNA's function and structure, studies of this nature can offer valuable insights into the genetics of complex diseases and traits, such as teratozoospermia.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms241915002/s1.

Author Contributions: Conceptualization, Z.M. and M.-A.K.; data analysis, M.-A.K. and T.G.; data interpretation, M.-A.K.; data acquisition, A.C. and N.C.; writing—original draft preparation, M.-A.K.; writing—review and editing, Z.M. and T.G.; supervision, Z.M.; funding acquisition, Z.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Spermogene (Fertilaid) project which is co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship, and Innovation, under the call RESEARCH–CREATE–INNOVATE (Grant number T1EΔK-02787).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Medical Faculty of the University of Thessaly (protocol code 20.04/2016 and date of approval 20 April 2016).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: Whole-genome sequencing data presented in this study are available through SRA (BioProject ID PRJNA875412, http://www.ncbi.nlm.nih.gov/bioproject/875412) (accessed on 25 August 2023)) and RNA expression data are also available through GEO (GSE6872, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6872) (accessed on 25 August 2023)).

Conflicts of Interest: The authors declare no conflict of interest.

References

 Mascarenhas, M.N.; Flaxman, S.R.; Boerma, T.; Vanderpoel, S.; Stevens, G.A. National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. *PLoS Med.* 2012, 9, e1001356. [CrossRef] [PubMed]

- Datta, J.; Palmer, M.J.; Tanton, C.; Gibson, L.J.; Jones, K.G.; Macdowall, W.; Glasier, A.; Sonnenberg, P.; Field, N.; Mercer, C.H.; et al. Prevalence of Infertility and Help Seeking among 15 000 Women and Men. *Hum. Reprod.* 2016, 31, 2108–2118. [CrossRef] [PubMed]
- Pathak, U.I.; Gabrielsen, J.S.; Lipshultz, L.I. Cutting-Edge Evaluation of Male Infertility. Urol. Clin. N. Am. 2020, 47, 129–138. [CrossRef] [PubMed]
- 4. Agarwal, A.; Baskaran, S.; Parekh, N.; Cho, C.L.; Henkel, R.; Vij, S.; Arafa, M.; Panner Selvam, M.K.; Shah, R. Male Infertility. *Lancet* 2021, 397, 319–333. [CrossRef]
- Tü, F.; Werny, F.; Cooper, T.G.; Kliesch, S.; Simoni, M.; Nieschlag, E. Clinical Experience with Azoospermia: Aetiology and Chances for Spermatozoa Detection upon Biopsy. *Wiley Online Libr.* 2011, 34, 291–298. [CrossRef]
- 6. World Health Organization. WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction; Press Concern: Singapore, 1980.
- World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen; Press Concern: Singapore, 2010.
- 8. De Braekeleer, M.; Nguyen, M.H.; Morel, F.; Perrin, A. Genetic Aspects of Monomorphic Teratozoospermia: A Review. J. Assist. *Reprod. Genet.* **2015**, *32*, 615. [CrossRef]
- 9. Coutton, C.; Escoffier, J.; Martinez, G.; Arnoult, C.; Ray, P.F. Teratozoospermia: Spotlight on the Main Genetic Actors in the Human. *Hum. Reprod. Update* **2015**, *21*, 455–485. [CrossRef]
- Jan, S.Z.; Vormer, T.L.; Jongejan, A.; Röling, M.D.; Silber, S.J.; de Rooij, D.G.; Hamer, G.; Repping, S.; van Pelt, A.M.M. Unraveling Transcriptome Dynamics in Human Spermatogenesis. *Development* 2017, 144, 3659–3673. [CrossRef]
- 11. Palazzo, A.F.; Lee, E.S. Non-Coding RNA: What Is Functional and What Is Junk? Front. Genet. 2015, 5, 2. [CrossRef]
- Kyrgiafini, M.A.; Sarafidou, T.; Mamuris, Z. The Role of Long Noncoding RNAs on Male Infertility: A Systematic Review and In Silico Analysis. *Biology* 2022, 11, 1510. [CrossRef]
- 13. Lekka, E.; Hall, J. Noncoding RNAs in Disease. FEBS Lett. 2018, 592, 2884. [CrossRef] [PubMed]
- 14. Bhatti, G.K.; Khullar, N.; Sidhu, I.S.; Navik, U.S.; Reddy, A.P.; Reddy, P.H.; Bhatti, J.S. Emerging Role of Non-coding RNA in Health and Disease. *Metab. Brain Dis.* **2021**, *36*, 1119–1134. [CrossRef] [PubMed]
- 15. Gao, N.; Li, Y.; Li, J.; Gao, Z.; Yang, Z.; Li, Y.; Liu, H.; Fan, T. Long Non-Coding RNAs: The Regulatory Mechanisms, Research Strategies, and Future Directions in Cancers. *Front. Oncol.* **2020**, *10*, 598817. [CrossRef] [PubMed]
- 16. Balas, M.M.; Johnson, A.M. Exploring the Mechanisms behind Long Noncoding RNAs and Cancer. *Non-coding RNA Res.* **2018**, *3*, 108–117. [CrossRef] [PubMed]
- 17. Wang, K.C.; Chang, H.Y. Molecular Mechanisms of Long Noncoding RNAs. Mol. Cell 2011, 43, 904–914. [CrossRef]
- Bolha, L.; Ravnik-Glavač, M.; Glavač, D. Long Noncoding RNAs as Biomarkers in Cancer. *Dis. Markers* 2017, 2017, 7243968. [CrossRef]
- Mukherjee, A.; Koli, S.; Reddy, K.V.R. Regulatory Non-Coding Transcripts in Spermatogenesis: Shedding Light on 'Dark Matter'. Andrology 2014, 2, 360–369. [CrossRef]
- Joshi, M.; Rajender, S. Long Non-Coding RNAs (LncRNAs) in Spermatogenesis and Male Infertility. *Reprod. Biol. Endocrinol.* 2020, 18, 103. [CrossRef]
- Aznaourova, M.; Schmerer, N.; Schmeck, B.; Schulte, L.N. Disease-Causing Mutations and Rearrangements in Long Non-Coding RNA Gene Loci. Front. Genet. 2020, 11, 1485. [CrossRef]
- Hindorff, L.A.; Sethupathy, P.; Junkins, H.A.; Ramos, E.M.; Mehta, J.P.; Collins, F.S.; Manolio, T.A. Potential Etiologic and Functional Implications of Genome-Wide Association Loci for Human Diseases and Traits. *Proc. Natl. Acad. Sci. USA* 2009, 106, 9362–9367. [CrossRef]
- Kulkarni, S.; Lied, A.; Kulkarni, V.; Rucevic, M.; Martin, M.P.; Walker-Sperling, V.; Anderson, S.K.; Ewy, R.; Singh, S.; Nguyen, H.; et al. CCR5AS LncRNA Variation Differentially Regulates CCR5, Influencing HIV Disease Outcome. *Nat. Immunol.* 2019, 20, 824–834. [CrossRef]
- Olazagoitia-Garmendia, A.; Sebastian-delaCruz, M.; Castellanos-Rubio, A. Involvement of LncRNAs in Celiac Disease Pathogenesis. Int. Rev. Cell Mol. Biol. 2021, 358, 241–264. [PubMed]
- 25. Leucci, E.; Vendramin, R.; Spinazzi, M.; Laurette, P.; Fiers, M.; Wouters, J.; Radaelli, E.; Eyckerman, S.; Leonelli, C.; Vanderheyden, K.; et al. Melanoma Addiction to the Long Non-Coding RNA SAMMSON. *Nature* **2016**, *531*, 518–522. [CrossRef] [PubMed]
- 26. Cabianca, D.S.; Casa, V.; Bodega, B.; Xynos, A.; Ginelli, E.; Tanaka, Y.; Gabellini, D. A Long NcRNA Links Copy Number Variation to a Polycomb/Trithorax Epigenetic Switch in FSHD Muscular Dystrophy. *Cell* **2012**, *149*, 819–831. [CrossRef] [PubMed]
- Zhou, Z.; Wang, B. Identification of Male Infertility-Related Long Non-Coding RNAs and Their Functions Based on a Competing Endogenous RNA Network. J. Int. Med. Res. 2020, 48, 0300060520961277. [CrossRef] [PubMed]
- 28. Boyle, A.P.; Hong, E.L.; Hariharan, M.; Cheng, Y.; Schaub, M.A.; Kasowski, M.; Karczewski, K.J.; Park, J.; Hitz, B.C.; Weng, S.; et al. Annotation of Functional Variation in Personal Genomes Using RegulomeDB. *Genome Res.* 2012, 22, 1790–1797. [CrossRef]
- 29. Lu, Y.; Quan, C.; Chen, H.; Bo, X.; Zhang, C. 3DSNP: A Database for Linking Human Noncoding SNPs to Their Three-Dimensional Interacting Genes. *Nucleic Acids Res.* 2017, 45, D643–D649. [CrossRef]
- Martens, L.; Rühle, F.; Witten, A.; Meder, B.; Katus, H.A.; Arbustini, E.; Hasenfuß, G.; Sinner, M.F.; Kääb, S.; Pankuweit, S.; et al. A Genetic Variant Alters the Secondary Structure of the LncRNA H19 and Is Associated with Dilated Cardiomyopathy. *RNA Biol.* 2021, 18, 409. [CrossRef]

- Halvorsen, M.; Martin, J.S.; Broadaway, S.; Laederach, A. Disease-Associated Mutations That Alter the RNA Structural Ensemble. PLoS Genet. 2010, 6, e1001074. [CrossRef]
- Wan, Y.; Qu, K.; Zhang, Q.C.; Flynn, R.A.; Manor, O.; Ouyang, Z.; Zhang, J.; Spitale, R.C.; Snyder, M.P.; Segal, E.; et al. Landscape and Variation of RNA Secondary Structure across the Human Transcriptome. *Nature* 2014, 505, 706–709. [CrossRef]
- Yang, Y.; Wang, D.; Miao, Y.R.; Wu, X.; Luo, H.; Cao, W.; Yang, W.; Yang, J.; Guo, A.Y.; Gong, J. LncRNASNP v3: An Updated Database for Functional Variants in Long Non-Coding RNAs. *Nucleic Acids Res.* 2023, 51, D192–D198. [CrossRef]
- 34. Karagkouni, D.; Karavangeli, A.; Paraskevopoulou, M.D.; Hatzigeorgiou, A.G. Characterizing MiRNA-LncRNA Interplay. *Methods Mol. Biol.* **2021**, 2372, 243–262. [PubMed]
- Finotti, A.; Fabbri, E.; Lampronti, I.; Gasparello, J.; Borgatti, M.; Gambari, R. MicroRNAs and Long Non-Coding RNAs in Genetic Diseases. *Mol. Diagn. Ther.* 2019, 23, 155. [CrossRef] [PubMed]
- Kern, F.; Aparicio-Puerta, E.; Li, Y.; Fehlmann, T.; Kehl, T.; Wagner, V.; Ray, K.; Ludwig, N.; Lenhof, H.P.; Meese, E.; et al. MiRTargetLink 2.0—Interactive MiRNA Target Gene and Target Pathway Networks. *Nucleic Acids Res.* 2021, 49, W409–W416. [CrossRef] [PubMed]
- 37. Consortium, T.G.O.; Aleksander, S.A.; Balhoff, J.; Carbon, S.; Cherry, J.M.; Drabkin, H.J.; Ebert, D.; Feuermann, M.; Gaudet, P.; Harris, N.L.; et al. The Gene Ontology Knowledgebase in 2023. *Genetics* **2023**, *224*, iyad031. [CrossRef] [PubMed]
- Ashburner, M.; Ball, C.A.; Blake, J.A.; Botstein, D.; Butler, H.; Cherry, J.M.; Davis, A.P.; Dolinski, K.; Dwight, S.S.; Eppig, J.T.; et al. Gene Ontology: Tool for the Unification of Biology. *Nat. Genet.* 2000, 25, 25–29. [CrossRef]
- Kanehisa, M.; Furumichi, M.; Sato, Y.; Kawashima, M.; Ishiguro-Watanabe, M. KEGG for Taxonomy-Based Analysis of Pathways and Genomes. *Nucleic Acids Res.* 2023, 51, D587–D592. [CrossRef]
- 40. Sparber, P.; Filatova, A.; Khantemirova, M.; Skoblov, M. The Role of Long Non-Coding RNAs in the Pathogenesis of Hereditary Diseases. *BMC Med. Genom.* 2019, 12, 63–78. [CrossRef]
- Landi, M.T.; Bishop, D.T.; MacGregor, S.; Machiela, M.J.; Stratigos, A.J.; Ghiorzo, P.; Brossard, M.; Calista, D.; Choi, J.; Fargnoli, M.C.; et al. Genome-Wide Association Meta-Analyses Combining Multiple Risk Phenotypes Provide Insights into the Genetic Architecture of Cutaneous Melanoma Susceptibility. *Nat. Genet.* 2020, *52*, 494–504. [CrossRef]
- Xu, J.J.; Jiang, L.; Xu, L.J.; Gao, Z.; Zhao, X.Y.; Zhang, Y.; Song, Y.; Liu, R.; Sun, K.; Gao, R.L.; et al. Association of CDKN2B-AS1 Polymorphisms with Premature Triple-Vessel Coronary Disease and Their Sex Specificity in the Chinese Population. *Biomed. Environ. Sci.* 2018, 31, 787–796.
- Wang, Y.; Lu, G.; Xue, X.; Xie, M.; Wang, Z.; Ma, Z.; Feng, Y.; Shao, C.; Duan, H.; Pan, M.; et al. Characterization and Validation of a Ferroptosis-Related LncRNA Signature as a Novel Prognostic Model for Lung Adenocarcinoma in Tumor Microenvironment. *Front. Immunol.* 2022, 13, 903758. [CrossRef]
- Yuan, L.Y.; Qin, X.; Li, L.; Zhou, J.; Zhou, M.; Li, X.; Xu, Y.; Wang, X.J.; Xing, H. The Transcriptome Profiles and Methylation Status Revealed the Potential Cancer-Related LncRNAs in Patients with Cervical Cancer. J. Cell. Physiol. 2019, 234, 9756–9763. [CrossRef] [PubMed]
- Wu, J.; Song, D.; Zhao, G.; Chen, S.; Ren, H.; Zhang, B. Cross-Talk between Necroptosis-Related LncRNAs to Construct a Novel Signature and Predict the Immune Landscape of Lung Adenocarcinoma Patients. *Front. Genet.* 2022, *13*, 966896. [CrossRef] [PubMed]
- Sun, W.; Lei, X.; Lu, Q.; Wu, Q.; Ma, Q.; Huang, D.; Zhang, Y. LncRNA FRMD6-AS1 Promotes Hepatocellular Carcinoma Cell Migration and Stemness by Regulating SENP1/HIF-1α Axis. *Pathol. Res. Pract.* 2023, 243, 154377. [CrossRef]
- 47. Ma, T.; Hu, Y.; Guo, Y.; Yan, B. Tumor-Promoting Activity of Long Noncoding RNA LINC00466 in Lung Adenocarcinoma via MiR-144-Regulated HOXA10 Axis. *Am. J. Pathol.* **2019**, *189*, 2154–2170. [CrossRef]
- Liu, J.; Yu, H.; Cui, H.; Wei, F.; Yan, T.; Li, T.; Liu, Y.; Chu, J. LncRNA LINC000466 Predicts the Prognosis and Promotes the Progression of Triple-Negative Breast Cancer via Modulating MiR-539-5p. *Clin. Breast Cancer* 2022, 22, 374–380. [CrossRef] [PubMed]
- Zhao, M.; Shao, Y.; Xu, J.; Zhang, B.; Li, C.; Gong, J. LINC00466 Impacts Cell Proliferation, Metastasis and Sensitivity to Temozolomide of Glioma by Sponging MiR-137 to Regulate PPP1R14B Expression. *Onco. Targets. Ther.* 2021, 14, 1147–1159. [CrossRef]
- 50. Zimta, A.A.; Tomuleasa, C.; Sahnoune, I.; Calin, G.A.; Berindan-Neagoe, I. Long Non-Coding RNAs in Myeloid Malignancies. *Front. Oncol.* **2019**, *9*, 1048. [CrossRef]
- Wang, Q.; Zhang, C.; Cao, S.; Zhao, H.; Jiang, R.; Li, Y. Tumor-Derived Exosomes Orchestrate the MicroRNA-128-3p/ELF4/CDX2 Axis to Facilitate the Growth and Metastasis of Gastric Cancer via Delivery of LINC01091. *Cell Biol. Toxicol.* 2022, 39, 519–536. [CrossRef]
- 52. Zhou, C.; Duan, S. The Role of Long Non-Coding RNA NNT-AS1 in Neoplastic Disease. Cancers 2020, 12, 3086. [CrossRef]
- Wang, Q.; Yang, L.; Hu, X.; Jiang, Y.; Hu, Y.; Liu, Z.; Liu, J.; Wen, T.; Ma, Y.; An, G.; et al. Upregulated NNT-AS1, a Long Noncoding RNA, Contributes to Proliferation and Migration of Colorectal Cancer Cells in Vitro and in Vivo. *Oncotarget* 2017, *8*, 3441–3453. [CrossRef]
- 54. Ye, H.; Lin, J.; Yao, X.; Li, Y.; Lin, X.; Lu, H. Overexpression of Long Non-Coding RNA NNT-AS1 Correlates with Tumor Progression and Poor Prognosis in Osteosarcoma. *Cell. Physiol. Biochem.* **2018**, *45*, 1904–1914. [CrossRef] [PubMed]

- Zheng, Y.; Yuan, M.; Su, L.; Ge, M.; Dong, F. ZNF252P-AS1 Enhances Myeloid-Derived Suppressor Cell Expansion and Tumor Glycolysis to Accelerate Laryngeal Squamous Cell Carcinoma Development via the MiR-377-3p/Lactate Dehydrogenase a Axis. *Mol. Cell. Toxicol.* 2023, 1–11. [CrossRef]
- 56. Wu, H.; Li, A.; Zheng, Q.; Gu, J.; Zhou, W. LncRNA LZTS1-AS1 Induces Proliferation, Metastasis and Inhibits Autophagy of Pancreatic Cancer Cells through the MiR-532/TWIST1 Signaling Pathway. *Cancer Cell Int.* **2023**, *23*, 130. [CrossRef] [PubMed]
- 57. WANG, W.; CHENG, X.; ZHU, J. Long Non-Coding RNA OTUD6B-AS1 Overexpression Inhibits the Proliferation, Invasion and Migration of Colorectal Cancer Cells via Downregulation of MicroRNA-3171. *Oncol. Lett.* **2021**, *21*, 193. [CrossRef] [PubMed]
- 58. Kong, S.; Xue, H.; Li, Y.; Li, P.; Ma, F.; Liu, M.; Li, W. The Long Noncoding RNA OTUD6B-AS1 Enhances Cell Proliferation and the Invasion of Hepatocellular Carcinoma Cells through Modulating GSKIP/Wnt/β-Catenin Signalling via the Sequestration of MiR-664b-3p. *Exp. Cell Res.* 2020, 395, 112180. [CrossRef]
- Ferreux, L.; Boumerdassi, Y.; Dulioust, E.; Bertagna, X.; Roucher-Boulez, F.; Bourdon, M.; Thiounn, N.; Patrat, C. Testicular Impairment in Primary Adrenal Insufficiency Caused by Nicotinamide Nucleotide Transhydrogenase (NNT) Deficiency—A Case Report: Implication of Oxidative Stress and Importance of Fertility Preservation. *Basic Clin. Androl.* 2023, 33, 17. [CrossRef]
- Roucher-Boulez, F.; Mallet-Motak, D.; Samara-Boustani, D.; Jilani, H.; Ladjouze, A.; Souchon, P.F.; Simon, D.; Nivot, S.; Heinrichs, C.; Ronze, M.; et al. NNT Mutations: A Cause of Primary Adrenal Insufficiency, Oxidative Stress and Extra-Adrenal Defects. *Eur. J. Endocrinol.* 2016, 175, 73–84. [CrossRef]
- 61. Ramírez-Bello, J.; Jiménez-Morales, M. Functional Implications of Single Nucleotide Polymorphisms (SNPs) in Protein-Coding and Non-Coding RNA Genes in Multifactorial Diseases. *Gac. Med. Mex.* **2017**, *153*, 238–250.
- Baytak, E.; Gong, Q.; Akman, B.; Yuan, H.; Chan, W.C.; Küçük, C. Whole Transcriptome Analysis Reveals Dysregulated Oncogenic LncRNAs in Natural Killer/T-Cell Lymphoma and Establishes MIR155HG as a Target of PRDM1. *Tumour Biol.* 2017, 39, 1010428317701648. [CrossRef]
- Kim, S.; Günesdogan, U.; Zylicz, J.J.; Hackett, J.A.; Cougot, D.; Bao, S.; Lee, C.; Dietmann, S.; Allen, G.E.; Sengupta, R.; et al. PRMT5 Protects Genomic Integrity during Global DNA Demethylation in Primordial Germ Cells and Preimplantation Embryos. *Mol. Cell* 2014, *56*, 564–579. [CrossRef] [PubMed]
- Dong, F.; Chen, M.; Chen, M.; Jiang, L.; Shen, Z.; Ma, L.; Han, C.; Guo, X.; Gao, F. PRMT5 Is Involved in Spermatogonial Stem Cells Maintenance by Regulating Plzf Expression via Modulation of Lysine Histone Modifications. *Front. Cell Dev. Biol.* 2021, 9, 673258. [CrossRef]
- Deng, Y.; Guo, K.; Tang, Z.; Feng, Y.; Cai, S.; Ye, J.; Xi, Y.; Li, J.; Liu, R.; Cai, C.; et al. Identification and Experimental Validation of a Tumor-Infiltrating Lymphocytes-Related Long Noncoding RNA Signature for Prognosis of Clear Cell Renal Cell Carcinoma. *Front. Immunol.* 2022, *13*, 1046790. [CrossRef] [PubMed]
- 66. Xiang, X.; Guo, Y.; Chen, Z.; Zhang, F.; Qin, Y. Accurate Prognostic Prediction for Patients with Clear Cell Renal Cell Carcinoma Using a Ferroptosis-Related Long Non-Coding RNA Risk Model. *Cancer Biomark.* **2023**, *37*, 95–107. [CrossRef] [PubMed]
- 67. Chen, C.; Zheng, H. LncRNA LINC00944 Promotes Tumorigenesis but Suppresses Akt Phosphorylation in Renal Cell Carcinoma. *Front. Mol. Biosci.* **2021**, *8*, 697962. [CrossRef]
- McKay, J.D.; Hung, R.J.; Han, Y.; Zong, X.; Carreras-Torres, R.; Christiani, D.C.; Caporaso, N.E.; Johansson, M.; Xiao, X.; Li, Y.; et al. Large-Scale Association Analysis Identifies New Lung Cancer Susceptibility Loci and Heterogeneity in Genetic Susceptibility across Histological Subtypes. *Nat. Genet.* 2017, 49, 1126–1132. [CrossRef]
- 69. Tan, C.; Cao, J.; Chen, L.; Xi, X.; Wang, S.; Zhu, Y.; Yang, L.; Ma, L.; Wang, D.; Yin, J.; et al. Noncoding RNAs Serve as Diagnosis and Prognosis Biomarkers for Hepatocellular Carcinoma. *Clin. Chem.* **2019**, *65*, 905–915. [CrossRef]
- Huang, X.; Liu, X.; Du, B.; Liu, X.; Xue, M.; Yan, Q.; Wang, X.; Wang, Q. LncRNA LINC01305 Promotes Cervical Cancer Progression through KHSRP and Exosome-Mediated Transfer. *Aging* 2021, 13, 19230–19242. [CrossRef]
- Duan, C. LncRNA SLC16A1-AS1 Contributes to the Progression of Hepatocellular Carcinoma Cells by Modulating MiR-411/MITD1 Axis. J. Clin. Lab. Anal. 2022, 36, e24344. [CrossRef]
- Logotheti, S.; Marquardt, S.; Gupta, S.K.; Richter, C.; Edelhäuser, B.A.H.; Engelmann, D.; Brenmoehl, J.; Söhnchen, C.; Murr, N.; Alpers, M.; et al. LncRNA-SLC16A1-AS1 Induces Metabolic Reprogramming during Bladder Cancer Progression as Target and Co-Activator of E2F1. *Theranostics* 2020, 10, 9620–9643. [CrossRef]
- Zhou, J.; Liu, T.; Xu, H.; Wang, Y.; Yang, T.; Liu, L. LncRNA FIRRE Promotes the Proliferation and Metastasis of Hepatocellular Carcinoma by Regulating the Expression of PXN through Interacting with MBNL3. *Biochem. Biophys. Res. Commun.* 2022, 625, 188–195. [CrossRef] [PubMed]
- 74. Wang, Y.; Li, Z.; Xu, S.; Li, W.; Chen, M.; Jiang, M.; Fan, X. LncRNA FIRRE Functions as a Tumor Promoter by Interaction with PTBP1 to Stabilize BECN1 MRNA and Facilitate Autophagy. *Cell Death Dis.* **2022**, *13*, 98. [CrossRef] [PubMed]
- 75. Lu, H.; Xu, D.; Wang, P.; Sun, W.; Xue, X.; Hu, Y.; Xie, C.; Ma, Y. RNA-Sequencing and Bioinformatics Analysis of Long Noncoding RNAs and MRNAs in the Asthenozoospermia. *Biosci. Rep.* **2020**, *40*, BSR20194041. [CrossRef] [PubMed]
- 76. Sun, T.C.; Zhang, Y.; Yu, K.; Li, Y.; Yu, H.; Zhou, S.J.; Wang, Y.P.; Deng, S.L.; Tian, L. LncRNAs Induce Oxidative Stress and Spermatogenesis by Regulating Endoplasmic Reticulum Genes and Pathways. *Aging* 2021, 13, 13764–13787. [CrossRef] [PubMed]
- 77. Kui, F.; Ye, H.; Chen, X.L.; Zhang, J. Microarray Meta-Analysis Identifies Candidate Genes for Human Spermatogenic Arrest. *Andrologia* **2019**, *51*, e13301. [CrossRef]

- Yang, F.; Deng, X.; Ma, W.; Berletch, J.B.; Rabaia, N.; Wei, G.; Moore, J.M.; Filippova, G.N.; Xu, J.; Liu, Y.; et al. The LncRNA Firre Anchors the Inactive X Chromosome to the Nucleolus by Binding CTCF and Maintains H3K27me3 Methylation. *Genome Biol.* 2015, 16, 52. [CrossRef]
- 79. Hatok, J.; Racay, P. Bcl-2 Family Proteins: Master Regulators of Cell Survival. Biomol. Concepts 2016, 7, 259–270. [CrossRef]
- 80. Mostafa, T.; Rashed, L.; Nabil, N.; Amin, R. Seminal BAX and BCL2 Gene and Protein Expressions in Infertile Men With Varicocele. *Urology* **2014**, *84*, 590–595. [CrossRef]
- 81. Chen, H.; Lam Fok, K.; Jiang, X.; Chan, H.C. New Insights into Germ Cell Migration and Survival/Apoptosis in Spermatogenesis. Spermatogenesis 2012, 2, 264–272. [CrossRef]
- Eisenberg, M.L.; Li, S.; Brooks, J.D.; Cullen, M.R.; Baker, L.C. Increased Risk of Cancer in Infertile Men: Analysis of U.S. Claims Data. J. Urol. 2015, 193, 1596–1601. [CrossRef]
- Anderson, R.E.; Hanson, H.A.; Lowrance, W.T.; Redshaw, J.; Oottamasathien, S.; Schaeffer, A.; Johnstone, E.; Aston, K.I.; Carrell, D.T.; Cartwright, P.; et al. Childhood Cancer Risk in the Siblings and Cousins of Men with Poor Semen Quality. J. Urol. 2017, 197, 898–905. [CrossRef] [PubMed]
- Eisenberg, M.L.; Betts, P.; Herder, D.; Lamb, D.J.; Lipshultz, L.I. Increased Risk of Cancer among Azoospermic Men. *Fertil. Steril.* 2013, 100, 681–685. [CrossRef] [PubMed]
- Hotaling, J.M.; Walsh, T.J. Male Infertility: A Risk Factor for Testicular Cancer. *Nat. Rev. Urol.* 2009, 6, 550–556. [CrossRef] [PubMed]
- Nagirnaja, L.; Aston, K.I.; Conrad, D.F. Genetic Intersection of Male Infertility and Cancer. *Fertil. Steril.* 2018, 109, 20–26. [CrossRef]
- 87. Tvrda, E.; Agarwal, A.; Alkuhaimi, N. Male Reproductive Cancers and Infertility: A Mutual Relationship. *Int. J. Mol. Sci.* 2015, 16, 7230–7260. [CrossRef]
- Neto, F.T.L.; Bach, P.V.; Najari, B.B.; Li, P.S.; Goldstein, M. Spermatogenesis in Humans and Its Affecting Factors. Semin. Cell Dev. Biol. 2016, 59, 10–26. [CrossRef]
- 89. Link, W. Introduction to FOXO Biology. Methods Mol. Biol. 2019, 1890, 1-9.
- Zhang, X.; Tang, N.; Hadden, T.J.; Rishi, A.K. Akt, FoxO and Regulation of Apoptosis. *Biochim. Biophys. Acta-Mol. Cell Res.* 2011, 1813, 1978–1986. [CrossRef]
- Luo, D.; He, Z.; Yu, C.; Guan, Q. Role of P38 MAPK Signalling in Testis Development and Male Fertility. Oxid. Med. Cell. Longev. 2022, 2022, 6891897. [CrossRef]
- Ni, F.D.; Hao, S.L.; Yang, W.X. Multiple Signaling Pathways in Sertoli Cells: Recent Findings in Spermatogenesis. *Cell Death Dis.* 2019, 10, 541. [CrossRef]
- 93. Li, M.W.M.; Mruk, D.D.; Cheng, C.Y. Mitogen-Activated Protein Kinases in Male Reproductive Function. *Trends Mol. Med.* 2009, 15, 159–168. [CrossRef] [PubMed]
- Deng, C.-Y.; Lv, M.; Luo, B.-H.; Zhao, S.-Z.; Mo, Z.-C.; Xie, Y.-J. The Role of the PI3K/AKT/MTOR Signalling Pathway in Male Reproduction. *Curr. Mol. Med.* 2021, 21, 539–548.
- Yao, P.L.; Lin, Y.C.; Richburg, J.H. TNF Alpha-Mediated Disruption of Spermatogenesis in Response to Sertoli Cell Injury in Rodents Is Partially Regulated by MMP2. *Biol. Reprod.* 2009, *80*, 581. [CrossRef] [PubMed]
- 96. Masola, V.; Greco, N.; Tozzo, P.; Caenazzo, L.; Onisto, M. The Role of SPATA2 in TNF Signaling, Cancer, and Spermatogenesis. *Cell Death Dis.* **2022**, *13*, 977. [CrossRef] [PubMed]
- Kierszenbaum, A.L. Tyrosine Protein Kinases and Spermatogenesis: Truncation Matters. Mol. Reprod. Dev. 2006, 73, 399–403. [CrossRef]
- Jenardhanan, P.; Mathur, P.P. Kinases as Targets for Chemical Modulators: Structural Aspects and Their Role in Spermatogenesis. Spermatogenesis 2015, 4, e979113. [CrossRef]
- 99. Rothzerg, E.; Ho, X.D.; Xu, J.; Wood, D.; Märtson, A.; Kõks, S. Upregulation of 15 Antisense Long Non-Coding RNAs in Osteosarcoma. *Genes* **2021**, *12*, 1132. [CrossRef]
- Liu, L.; Li, X.; Wu, H.; Tang, Y.; Li, X.; Shi, Y. The COX10-AS1/MiR-641/E2F6 Feedback Loop Is Involved in the Progression of Glioma. Front. Oncol. 2021, 11, 648152. [CrossRef]
- 101. Zhou, C.; Jiang, X.; Liang, A.; Zhu, R.; Yang, Y.; Zhong, L.; Wan, D. COX10-AS1 Facilitates Cell Proliferation and Inhibits Cell Apoptosis in Glioblastoma Cells at Post-Transcription Level. *Neurochem. Res.* **2020**, *45*, 2196–2203. [CrossRef]
- 102. Xing, C.; Cai, Z.; Gong, J.; Zhou, J.; Xu, J.; Guo, F. Identification of Potential Biomarkers Involved in Gastric Cancer Through Integrated Analysis of Non-Coding RNA Associated Competing Endogenous RNAs Network. *Clin. Lab.* 2018, 64, 1661–1669. [CrossRef]
- Xu, H.; Jia, J. Immune-Related Hub Genes and the Competitive Endogenous RNA Network in Alzheimer's Disease. J. Alzheimers. Dis. 2020, 77, 1255–1265. [CrossRef]
- 104. Xu, Y.; Yu, X.; Zhang, M.; Zheng, Q.; Sun, Z.; He, Y.; Guo, W. Promising Advances in LINC01116 Related to Cancer. Front. cell Dev. Biol. 2021, 9, 736927. [CrossRef]
- 105. Zhang, E.; He, X.; Zhang, C.; Su, J.; Lu, X.; Si, X.; Chen, J.; Yin, D.; Han, L.; De, W. A Novel Long Noncoding RNA HOXC-AS3 Mediates Tumorigenesis of Gastric Cancer by Binding to YBX1. *Genome Biol.* 2018, 19, 154. [CrossRef] [PubMed]
- 106. Su, C.; Wang, W.; Mo, J.; Liu, F.; Zhang, H.; Liu, Y.; Chen, X.; Liao, Z.; Zhang, B.; Zhu, P. Long Noncoding RNA HOXC-AS3 Interacts with CDK2 to Promote Proliferation in Hepatocellular Carcinoma. *Biomark. Res.* **2022**, *10*, 65. [CrossRef]

- 107. Cai, S.; Zhou, Y.; Pan, Y.; Liu, P.; Yu, K.; Chen, S. Long Non-Coding RNA A1BG-AS1 Promotes Tumorigenesis in Breast Cancer by Sponging MicroRNA-485-5p and Consequently Increasing Expression of FLOT1 Expression. *Hum. Cell* 2021, 34, 1517–1531. [CrossRef]
- 108. Bai, J.; Yao, B.; Wang, L.; Sun, L.; Chen, T.; Liu, R.; Yin, G.; Xu, Q.; Yang, W. LncRNA A1BG-AS1 Suppresses Proliferation and Invasion of Hepatocellular Carcinoma Cells by Targeting MiR-216a-5p. *J. Cell. Biochem.* **2019**, *120*, 10310–10322. [CrossRef]
- Yao, J.; Chen, X.; Liu, X.; Li, R.; Zhou, X.; Qu, Y. Characterization of a Ferroptosis and Iron-Metabolism Related LncRNA Signature in Lung Adenocarcinoma. *Cancer Cell Int.* 2021, 21, 340. [CrossRef] [PubMed]
- 110. Chen, C.; Zhao, J.; Liu, J.N.; Sun, C. Mechanism and Role of the Neuropeptide LGI1 Receptor ADAM23 in Regulating Biomarkers of Ferroptosis and Progression of Esophageal Cancer. *Dis. Markers* 2021, 2021, 9227897. [CrossRef] [PubMed]
- Zhi, Y.; Du, J.; Qian, M.; Song, N. Long Non-Coding RNA RP11-342L8.2, Derived from RNA Sequencing and Validated via RT-QPCR, Is Upregulated and Correlates with Disease Severity in Psoriasis Patients. *Ir. J. Med. Sci.* 2022, 191, 2643–2649. [CrossRef]
- 112. Yuan, H.; Ren, Q.; Du, Y.; Ma, Y.; Gu, L.; Zhou, J.; Tian, W.; Deng, D. LncRNA MiR663AHG Represses the Development of Colon Cancer in a MiR663a-Dependent Manner. *Cell Death Discov.* **2023**, *9*, 220. [CrossRef]
- 113. Liu, H.; Zhang, Q.; Lou, Q.; Zhang, X.; Cui, Y.; Wang, P.; Yang, F.; Wu, F.; Wang, J.; Fan, T.; et al. Differential Analysis of LncRNA, MiRNA and MRNA Expression Profiles and the Prognostic Value of LncRNA in Esophageal Cancer. *Pathol. Oncol. Res.* 2020, 26, 1029–1039. [CrossRef] [PubMed]
- 114. Li, X.; Zhu, J.; Zhong, Y.; Liu, C.; Yao, M.; Sun, Y.; Yao, W.; Ni, X.; Zhou, F.; Yao, J.; et al. Targeting Long Noncoding RNA-AQP4-AS1 for the Treatment of Retinal Neurovascular Dysfunction in Diabetes Mellitus. *EBioMedicine* 2022, 77, 103857. [CrossRef] [PubMed]
- 115. Andrew, S. FastQC: A Quality Control Tool for High Throughput Sequence Data. 2010. Available online: https://www. bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed on 25 August 2023).
- Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A Flexible Trimmer for Illumina Sequence Data. *Bioinformatics* 2014, 30, 2114–2120. [CrossRef] [PubMed]
- Cunningham, F.; Allen, J.E.; Allen, J.; Alvarez-Jarreta, J.; Amode, M.R.; Armean, I.M.; Austine-Orimoloye, O.; Azov, A.G.; Barnes, I.; Bennett, R.; et al. Ensembl 2022. *Nucleic Acids Res.* 2022, 50, D988–D995. [CrossRef] [PubMed]
- 118. Li, H.; Durbin, R. Fast and Accurate Short Read Alignment with Burrows-Wheeler Transform. *Bioinformatics* 2009, 25, 1754–1760. [CrossRef] [PubMed]
- 119. Danecek, P.; Bonfield, J.K.; Liddle, J.; Marshall, J.; Ohan, V.; Pollard, M.O.; Whitwham, A.; Keane, T.; McCarthy, S.A.; Davies, R.M.; et al. Twelve Years of SAMtools and BCFtools. *Gigascience* **2021**, *10*, giab008. [CrossRef]
- 120. Garrison, E.; Marth, G. Haplotype-Based Variant Detection from Short-Read Sequencing. 2012. Available online: https://arxiv. org/abs/1207.3907 (accessed on 25 August 2023).
- McLaren, W.; Gil, L.; Hunt, S.E.; Riat, H.S.; Ritchie, G.R.S.; Thormann, A.; Flicek, P.; Cunningham, F. The Ensembl Variant Effect Predictor. *Genome Biol.* 2016, 17, 122. [CrossRef]
- 122. Auton, A.; Abecasis, G.R.; Altshuler, D.M.; Durbin, R.M.; Bentley, D.R.; Chakravarti, A.; Clark, A.G.; Donnelly, P.; Eichler, E.E.; Flicek, P.; et al. A Global Reference for Human Genetic Variation. *Nature* **2015**, *526*, 68–74.
- 123. Platts, A.E.; Dix, D.J.; Chemes, H.E.; Thompson, K.E.; Goodrich, R.; Rockett, J.C.; Rawe, V.Y.; Quintana, S.; Diamond, M.P.; Strader, L.F.; et al. Success and Failure in Human Spermatogenesis as Revealed by Teratozoospermic RNAs. *Hum. Mol. Genet.* 2007, 16, 763–773. [CrossRef]
- 124. Srinivasan, S.; Clements, J.A.; Batra, J. Single Nucleotide Polymorphisms in Clinics: Fantasy or Reality for Cancer? *Crit. Rev. Clin. Lab. Sci.* 2016, 53, 29–39. [CrossRef]
- 125. Kyrgiafini, M.A.; Sarafidou, T.; Giannoulis, T.; Chatziparasidou, A.; Christoforidis, N.; Mamuris, Z. Gene-by-Sex Interactions: Genome-Wide Association Study Reveals Five SNPs Associated with Obesity and Overweight in a Male Population. *Genes* 2023, 14, 799. [CrossRef] [PubMed]
- 126. Han, Z.; Huang, H.; Gao, Y.; Huang, Q. Functional Annotation of Alzheimer's Disease Associated Loci Revealed by GWASs. PLoS ONE 2017, 12, e0179677. [CrossRef]
- 127. Shahin, M.H.; Sá, A.C.; Webb, A.; Gong, Y.; Langaee, T.; McDonough, C.W.; Riva, A.; Beitleshees, A.L.; Chapman, A.B.; Gums, J.G.; et al. Genome-Wide Prioritization and Transcriptomics Reveal Novel Signatures Associated with Thiazide Diuretics Blood Pressure Response. *Circ. Cardiovasc. Genet.* 2017, 10, e001404. [CrossRef] [PubMed]
- 128. Mesbah-Uddin, M.; Elango, R.; Banaganapalli, B.; Shaik, N.A.; Al-Abbasi, F.A. In-Silico Analysis of Inflammatory Bowel Disease (IBD) GWAS Loci to Novel Connections. *PLoS ONE* **2015**, *10*, e0119420. [CrossRef] [PubMed]
- Sabarinathan, R.; Tafer, H.; Seemann, S.E.; Hofacker, I.L.; Stadler, P.F.; Gorodkin, J. RNAsnp: Efficient Detection of Local RNA Secondary Structure Changes Induced by SNPs. *Hum. Mutat.* 2013, 34, 546–556. [CrossRef] [PubMed]
- Ge, S.X.; Jung, D.; Jung, D.; Yao, R. ShinyGO: A Graphical Gene-Set Enrichment Tool for Animals and Plants. *Bioinformatics* 2020, 36, 2628–2629. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.