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ORIGINAL RESEARCH

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Integrative analysis of GTEx data reveals associations between multi-tissue transcriptomes and human spermatogenic dysfunction

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Abstract

Background: The decline in male sperm production capacity may be related not only to the testes themselves but also may be indirectly influenced by other tissues outside the testes. However, the association between extragonadal tissues and testicular function has not been systematically investigated. **Methods**: Using the public Genotype-Tissue Expression database (GTEx) bulk RNA-seq dataset, we stratified GTEx subjects into testis functional "good" and "poor" groups based on the expression profiles of spermatogenesis-related genes in testicular tissues. To identify the influence of extragonadal tissues on male fertility, we then compared RNA-seq data of various non-testicular tissues between these two groups. **Results**: Our findings reveal significant associations between testicular function and the pituitary gland, adrenal gland, lungs, liver, and key brain regions involved in neuroendocrine regulation, including the amygdala, substantia nigra, anterior cingulate cortex, and frontal cortex. **Conclusions**: These results suggest potential extragonadal pathways that may be associated with male fertility, and provide potential candidate biomarkers for testicular dysfunction diagnosis.

Keywords

Testis; Spermatogenesis; Extragonadal tissues; Brain regions; Lung; Liver; HPT axis; Adrenal gland

1. Introduction

Human spermatogenic capacity exhibits significant interindividual variability, and impaired spermatogenesis, resulting from genetic and environmental factors, can lead to reduced male fertility [1]. It is well established that testicular function can be disrupted by various pathological conditions, including obesity, diabetes, and inflammation, which exert their effects through metabolic, immune, and endocrine dysregulation [2]. However, little is known about which organs or tissues in the human body are more closely associated with testicular spermatogenesis.

From an endocrine perspective, testicular function is regulated by the hypothalamic-pituitary-testicular (HPT) axis. The hypothalamus secretes gonadotropin-releasing hormone (GnRH), which stimulates the pituitary gland to release luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH promotes testosterone production in Leydig cells, while FSH activates Sertoli cells to facilitate sperm maturation. Testosterone exerts negative feedback on the secretion of GnRH and LH, whereas inhibin, produced by Sertoli cells, suppresses FSH levels. Together, testosterone and FSH work synergistically to regulate spermatogenesis. Despite this complex feedback network, it remains unclear whether the efficiency of testicular spermatogenesis correlates with global gene expression patterns in the hypothalamus and pituitary.

Psychological stress has been identified as a significant modulator of male fertility [3], yet the transcriptional mechanisms that connect mental health to spermatogenic dysfunction remain poorly understood. Emerging evidence suggests a bidirectional relationship between brain function and testicular activity. For example, testosterone levels influence the development and reactivity of brain regions such as the hippocampus and amygdala [4, 5]. Notably, the brainstem cell containing locus coeruleus can regulate testosterone synthesis independently of the pituitary pathway [6]. Furthermore, comparative transcriptomic analyses reveal unexpected similarities in gene expression between the brain and testes [7]. Collectively, these findings highlight the importance of investigating brain-testicular interactions, as they may have implications for both neurological disorders and male reproductive health.

In humans, the adrenal gland plays a crucial role in regulating stress responses, metabolism, and blood pressure, while also producing minor sex hormones such as dehydroepiandrosterone. Similarly, the thyroid gland modulates metabolism, growth, and development through the secretion of thyroid hormones (T3 and T4), which exert systemic effects, includ-

ing influences on reproductive function. Although both the adrenal and thyroid glands are known to interact with testicular function, it remains unclear whether their transcriptional dysregulation is linked to variations in spermatogenic capacity.

The Genotype-Tissue Expression database (GTEx) provides a comprehensive repository of transcriptomic data derived from various tissues, including the testis, across a large human cohort [8]. Our previous research has established that testicular transcriptome data from GTEx reliably capture interindividual differences in spermatogenic efficiency [9]. Utilizing this resource, we conducted a natural experiment to examine transcriptomic variations across multiple tissues in individuals stratified by their spermatogenic capacity. To investigate the correlation between extra-testicular tissues and spermatogenesis, we analyzed GTEx subjects with RNA-seq data of both testicular and other tissues, and compared the extra-testicular tissues of subjects with different testicular characteristics. This study elucidates the transcriptional correlates of spermatogenic capacity in non-testicular tissues, providing novel insights into the systemic regulation of male fertility. Our findings may pave the way for improved diagnostic strategies for male infertility and may identify novel therapeutic targets for male subfertility.

2. Materials and methods

2.1 Data collection from GTEx

In this study, all analyses were conducted using publicly accessible data from GTEx. This dataset includes RNA sequencing data from various tissues, as well as metadata for each donor subject (Table 1). The GTEx Analysis V10 dataset was used in this study. Gene expression data and metadata were converted into AnnData format (version 0.11.3) using Jupyter Notebook [10].

2.2 Testis tissue clustering

To perform cluster analysis of the GTEx testis samples, we used bulk RNA-seq data of the testis. Testis clustering was conducted using the Scanpy (version 1.10.4), a tool can be used for clustering analysis of bulk omics data [11]. The gene expression values for each testis, reported as TPM (transcripts per million), were normalized using the sc.pp.normalize_total() function and transformed to log(TPM + 1) using the sc.pp.log1p() method. The ranked genes for each testis cluster were identified using the Wilcoxon method. Additional methods for testis clustering were executed with the default parameters in Scanpy.

2.3 Differential gene expression screening and gene set enrichment analysis

The differentially expressed genes were screened using Diffxpy (v0.7.4). Gene set enrichment analysis in this study was conducted using ShinyGO (version 0.82). The genes exhibiting the most significant differential expression, particularly those involved in critical biological pathways such as cellular metabolism, neuroendocrine regulation, and inflammatory responses, were selected for analysis and presentation.

2.4 Cell type enrichment analysis

To perform cell type enrichment analysis, we first obtained gene expression patterns of different cells in the testis from a testicular scRNA-seq dataset (GSM8037606) [12] using Scanpy. Afterwards, we generated a reference for the cell type enrichment analysis tool xCell [13] using this scRNA-seq data. Then, we performed cell type enrichment analysis on the GTEx testis RNA-seq data to estimate the potential proportions of different testicular cell types within the testis tissue.

2.5 Statistics analysis

The de.test.t_test() method in Diffxpy, which performs Welch's *t*-test (a parametric test to compare the means of two independent groups, without requiring equal variances) for genes expressed in two groups, was utilized for screening differentially expressed genes in testicular data. When the testicular data were analyzed alongside other tissues, as the donor subject number reduced, the de.test.two_sample() method in Diffxpy with the parameter test = "rank" was used, which use Wilcoxon rank sum test (a non-parametric test comparing two independent groups by ranking data to detect distributional differences) to identify the differentially expressed genes between the two groups.

3. Results

3.1 GTEx data collection and testis clustering

The GTEx V10 dataset contains bulk RNA-seq data from various tissues of human subjects. We first extracted the testicular data from GTEx and classify the testicular tissues into two groups, those with good spermatogenic capacity and those with impaired capacity, by comparing the expression of genes related to spermatogenesis and somatic cell proportions within the testis. Then, based on the classification of testicular function (good *vs.* poor), we divided the subjects' extragonadal tissues into two corresponding groups and compare the differences between them. This approach aimed to identify which extragonadal tissues show gene expression patterns that may be associated with testicular function (Fig. 1).

As a result, 414 testes from GTEx were collected and clustered into six distinct groups, labeled T1–T6. Within these testicular clusters, T5 and T6 exhibited high expression levels of genes such as the transcription regulator *CRTC1* and the metabolic regulator *ACOT9*. In contrast, clusters T1, T2, T3, and T4 showed elevated expression of genes like *PCYT2* and *S1PR2*. Notably, the *SLC9C2* gene was highly expressed in T3, while the *TDRD15* gene was highly expressed in T4 (Fig. 2A). We then analyzed the gene markers of somatic cells (*VIM* marked all somatic cells, *CD14* marked macrophage cells, *VWF* marked endothelial cells, *DLK1* marked Leydig cells, and *ACTA2* marked myoid cells) and differentiating spermatids (maker genes include *HOOK1*, *TNP2*, *PRM3*, *SPATA18*, *SPATA7* and *SPEM3*) [14] in these testis clusters, and found that somatic cell marker genes were highly expressed in T5 and T6, whereas maturing spermatid

TABLE 1. GTEx datasets and software utilized in this study.

Dataset/Software	URL or Website
GTEx_Analysis_v10_Annotations_SampleAttributesDS.txt	https://storage.googleapis.com/adult-gtex/annotations/v10/metadata-files/GTEx_Analysis_v10_Annotations_SampleAttributesDS.txt
GTEx_Analysis_v10_Annotations_SubjectPhenotypesDS.txt	https://storage.googleapis.com/adult-gtex/annotations/v10/metadata-files/GTEx_Analysis_v10_Annotations_SubjectPhenotypesDS.txt
GTEx_Analysis_v10_RNASeQCv2.4.2_gene_tpm.gct.gz	https://storage.googleapis.com/adult-gtex/bulk-gex/v10/rna-seq/GTEx_Analysis_v10_RNASeQCv2.4.2_gene_tpm.gct.gz
GSM8037606	https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM8037606
Diffxpy v0.7.4	https://github.com/theislab/diffxpy
ShinyGO v0.82	https://bioinformatics.sdstate.edu/go/
Jupyter Notebook	https://jupyter.org/
Scanpy v1.10.4	https://github.com/scverse/scanpy
xCell 2.0	https://github.com/AlmogAngel/xCell2

URL: Uniform Resource Locator.

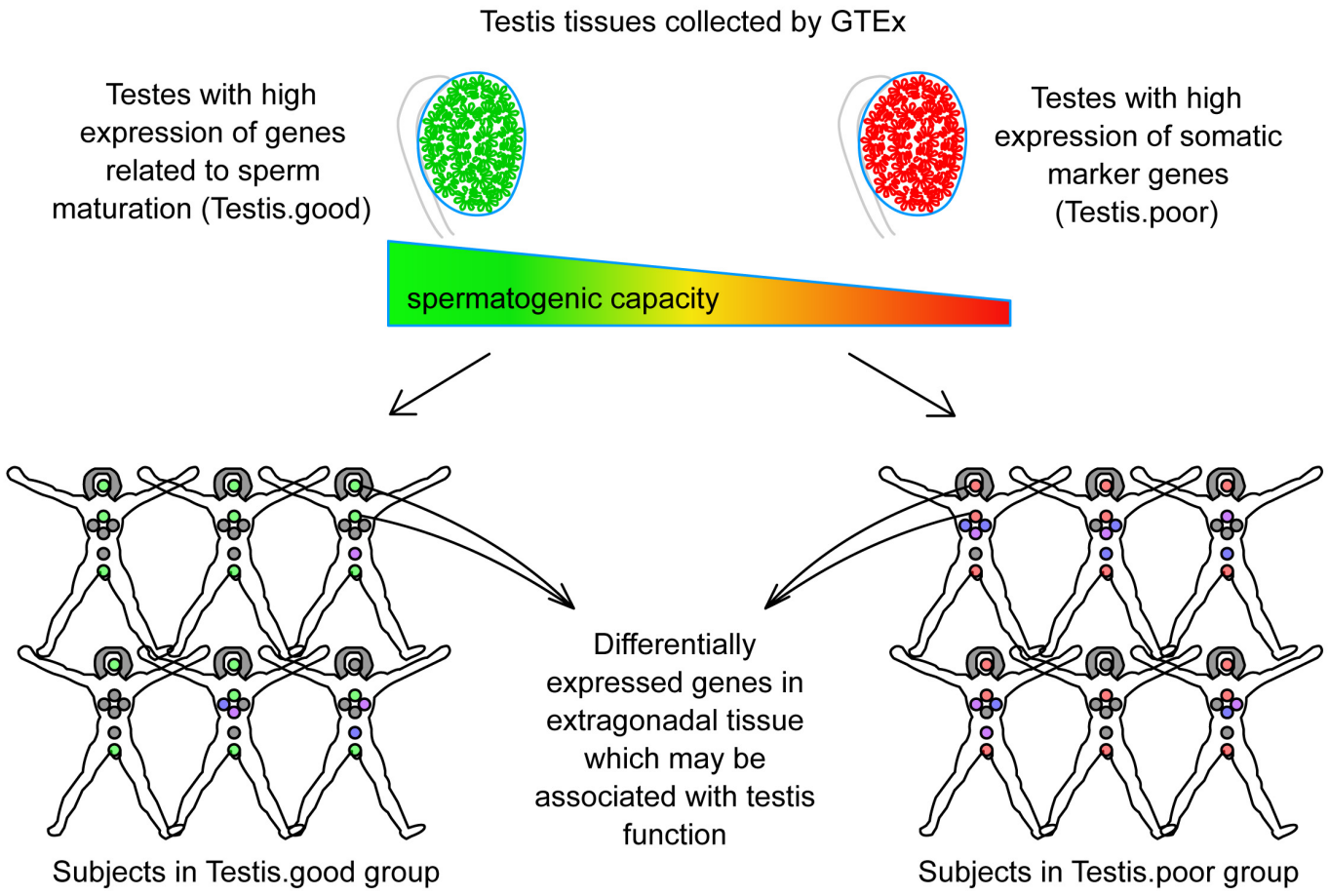


FIGURE 1. The main strategy of this study. The quality of spermatogenic function was first evaluated based on bulk RNA-seq data from the testis. Based on this assessment, the human subjects were divided into two groups (Testis.good and Testis.poor). RNA differences in non-testicular tissues were then compared between these two groups to identify which extra-testicular tissues show gene expression patterns associated with testicular function. GTEx, Genotype-Tissue Expression database.

marker genes were highly expressed in T1 and T2 (Fig. 2B). High expression of maturating spermatid genes in T1 and T2 indicates that the cell ratio of spermatids in testes is high. In contrast, in T5 and T6 testis clusters, high expression of somatic cell markers indicates a high ratio of somatic cells in these testes.

For testicular transcriptome analysis, clusters T1 and T2 were grouped as "Good" (superior spermatogenesis) and clusters T5 and T6 as "Poor" (diminished spermatogenesis) (Fig. 2C). We then screened for differentially expressed genes between the Good and Poor testes using Diffxpy. As a result, in the Poor group, 1846 genes were highly expressed and 2190 genes were lowly expressed (**Supplementary Table 1**). Genes upregulated in Poor testes were primarily linked to chemical stimulus response and cell communication; downregulated genes were linked to spermatogenesis and sperm motility (Fig. 2C). To further analyze the differences between the two groups of testes, we performed cell type enrichment analysis on the RNA-seq data of these testes. The results showed that spermatogonia were more enriched in the Poor group, while late primary spermatocytes/round spermatids and elongating spermatids were more enriched in the Good group. Early primary spermatocytes showed no difference between the two groups (Fig. 2D). For some somatic cells, such as Leydig cells, Sertoli cells, testicular peritubular cells, smooth muscle cells, epithelial cells, and macrophages, these were all more enriched in the Poor group of testes (Fig. 2D). The results of the cell-type enrichment analysis suggest that in the Good group of testes, there are more germ cells at the maturating stage, and the level of inflammation may be lower.

Notably, among these differentially expressed genes, we identified genes associated with cell communication and chemical response, such as *GJA1*, *HIF1A*, and *INSR*, which were highly expressed in Poor testes. Conversely, genes associated with sperm maturation, such as *SPAG6*, *SPEM2*, and *ZPBP*, were expressed at lower levels in Poor testes (Fig. 2E). These differentially expressed genes may not be attributed to the upregulation or downregulation in specific cell types but rather to changes in the cell counts of particular cell populations.

3.2 Hypothalamus-pituitary-testis transcriptional relationships

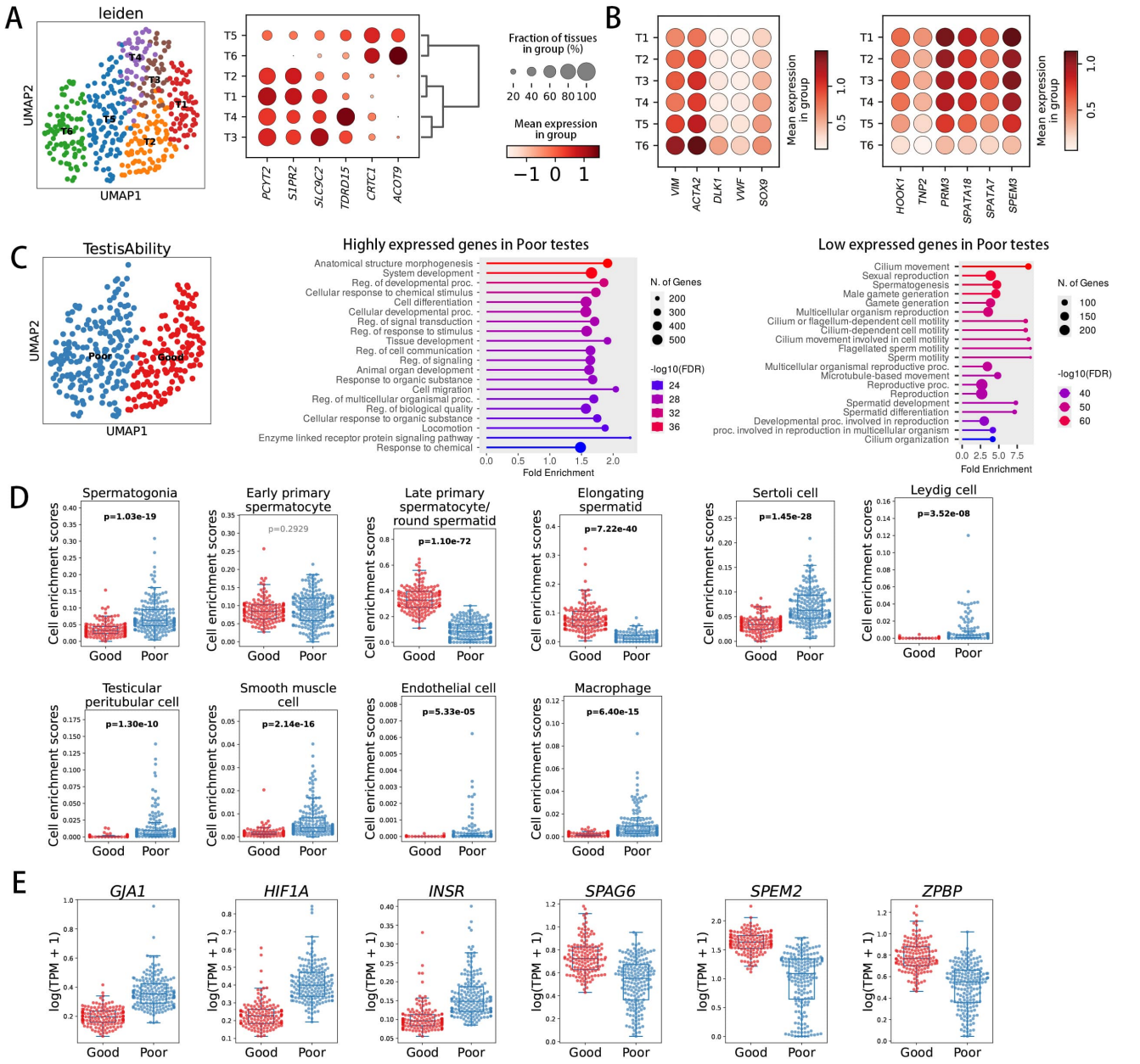
To analyze the changes in gene expression in the hypothalamus and pituitary gland between the Good Testis (T.good) group and the Poor Testis (T.poor) group, we extracted RNA-seq data from human subjects whose testis, hypothalamus, and pituitary gland were all sequenced. A total of 83 subjects were selected, including 45 in the T.good group and 38 in the T.poor group (**Supplementary Table 2**). Using the differentially expressed (DE) gene screening criteria of p-value < 0.01 and mean log2(TPM + 1) >0.01, we identified 20,567 DE genes in the testes, 324 DE genes in the pituitary, and 74 DE genes in the hypothalamus (Fig. 3A and **Supplementary Table 3**). Pituitary DE genes were enriched in copper response and glycolysis pathways (Fig. 3B), while hypothalamic DE genes were enriched in cadmium response and thyroid hormone transport (Fig. 3C). Notably, the pituitary showed ~4.4 times more DE genes than the hypothalamus, suggesting a stronger transcriptional link to testicular function.

Within the HPT axis, hypothalamic GnRH and pituitary FSH/LH play critical endocrine roles in regulating testicular spermatogenesis. Interestingly, our analysis revealed no significant expression change in the core neuroendocrine genes *GNRH1* (hypothalamus) or *FSHB*/*LHB*/*CGA* (pituitary) between the T.good and T.poor groups (Fig. 3D), suggesting that spermatogenic impairment may involve alternative regulatory mechanisms. The homeobox protein gene *HMX3* is essential for the development of the human inner ear and the hypothalamic-pituitary axis [15]. The expression of glucokinase in the pituitary may function as a glucose sensor to regulate pituitary secretion [16], while the glucokinase regulatory protein encoded by *GCKR* can inhibit the activity of glucokinase [17]. In the T.poor group, the *HMX3* gene was found to be downregulated in the pituitary, whereas the *GCKR* gene was upregulated (Fig. 3E). Neuromedin-S peptides are believed to be involved in the regulation of the hypothalamic-pituitary-adrenal axis [18]. The vasoconstrictor endothelin-1, expressed in the hypothalamus, can stimulate the secretion of GnRH [19]. In subjects from the T.poor group, the neuromedin-S coding gene *NMS* was downregulated, while the endothelin-1 coding gene *EDN1* was upregulated in the hypothalamus (Fig. 3E). In the brain, the angiopoietin-like 4 protein, encoded by *ANGPTL4*, primarily functions in metabolic crosstalk between glia cells and neurons [20]. The voltage-dependent calcium channel gamma coding genes *CACNG1–8* are associated with schizophrenia [21]. In the T.poor group, the *ANGPTL4* gene was found to be upregulated in both the hypothalamus ($p < 0.01$) and pituitary ($p < 0.05$), whereas *CACNG6* was downregulated (Fig. 3F). All these data suggest that the metabolism and secretion of the hypothalamus and pituitary may be associated with testicular function in T.poor subjects.

3.3 Transcriptional associations between the adrenal and thyroid glands and testicular function

Beyond the HPT axis, we investigated two other major endocrine regulators, the adrenal and thyroid glands, for their potential associations with testicular function. From GTEx, we identified 85 subjects with RNA-seq data for testes, adrenal, and thyroid glands: 36 with high spermatogenic capacity (T.good) and 59 with impaired spermatogenesis (T.poor) (**Supplementary Table 2**). Using stringent criteria ($q < 0.05$; mean log2(TPM + 1) >0.01), we observed tissue-specific patterns: testes showed extensive alterations (20,972 DE genes), adrenal glands moderate changes (260 DE genes), and thyroid glands minimal variation (21 DE genes) (Fig. 4A and **Supplementary Table 4**). The magnitude of transcriptional changes revealed a striking difference in endocrine-testis associations, with adrenal glands exhibiting 12-fold more DE genes than thyroid glands. This indicates that the adrenal gland has a closer link with testicular status than that of the thyroid gland.

In the T.poor group, we found the immune response-related genes, such as the attractin coding gene *ATRN* and the C3/C4 complement encoding genes *C3* and *C4B*, were upregulated



F I G U R E 2. Classification of spermatogenic capacity based on testicular transcriptomes. (A) Clustering of testes according to their gene expression profiles. (B) The expression patterns of somatic cell marker genes and sperm marker genes in the testis clusters. (C) Gene set enrichment analysis of differentially expressed genes in testes exhibiting reduced spermatogenic ability. The testes were categorized into "good" and "poor" groups based on their spermatogenic capacity. (D) Cell type enrichment analysis of the two groups of testes. (E) Genes that are either upregulated or downregulated in testes with diminished spermatogenic ability. *GJA1*, gap junction alpha-1; *HIF1A*, hypoxia-inducible factor 1-alpha; *INSR*, insulin receptor; *SPAG6*, sperm-associated antigen 6; *SPEM2*, SPEM family member 2; *ZPBP*, zona pellucida binding protein.

in the adrenal gland. Additionally, only *C4B* was upregulated in the thyroid gland (Fig. 4B). This suggests that increased inflammatory activity in both adrenal and thyroid tissues may be linked to impaired spermatogenic function in the testes. Among the DE genes, we identified several genes exhibiting consistent expression patterns between the adrenal and thyroid glands. For instance, the *RAMP2* gene, which encodes a protein that regulates the activity of receptor for adrenomedullin, calcitonin, and amylin [22–24], along with the membrane protein coding gene *KANTR*, were downregulated in both the adrenal and thyroid glands, but were upregulated in the testis of the T.poor group (Fig. 4C). Another gene, *NPFF*, which encodes a morphine-modulating peptide, was found to be downregulated in both the adrenal and thyroid glands of the T.poor group, but showed no significant change in the testes (Fig. 4D). These data indicate that the inflammatory state and endocrine regulation in both the adrenal and thyroid glands may be associated with the spermatogenic capacity of the testes.

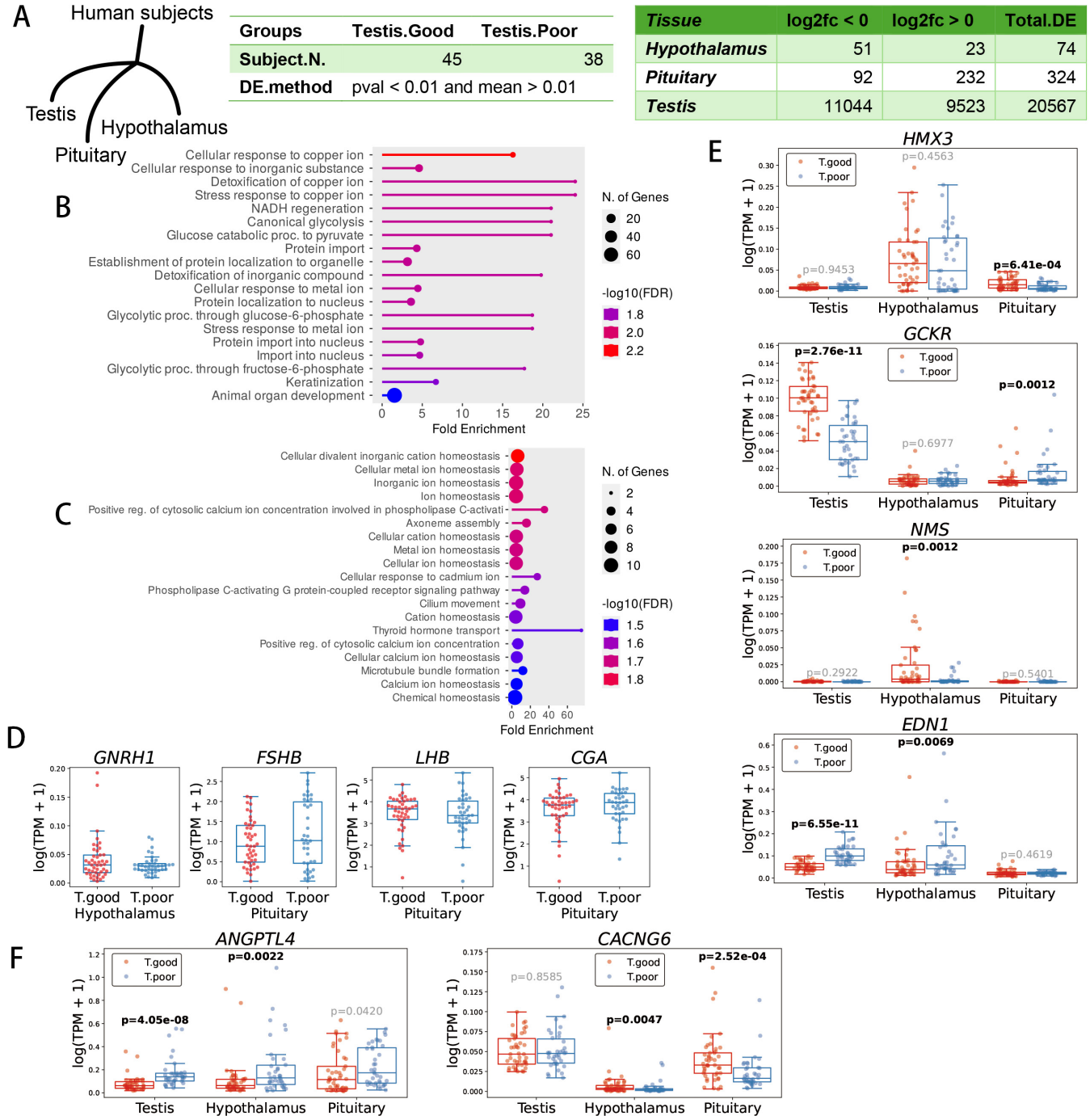
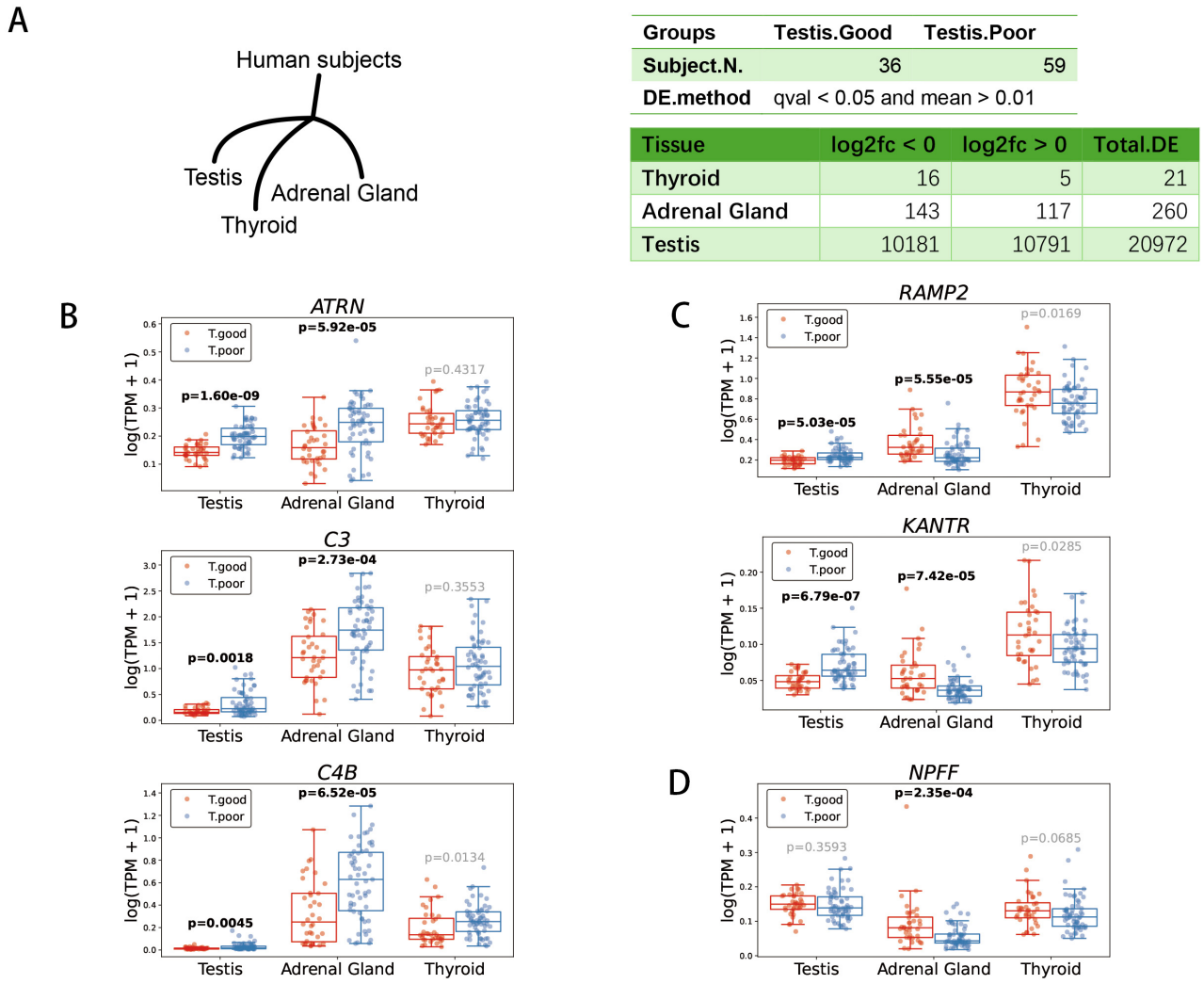


FIGURE 3. Testis-associated genes in the pituitary and hypothalamus. (A) Differentially expressed (DE) genes in the pituitary and hypothalamus of subjects with varying testicular spermatogenesis abilities. (B) Pathways enriched by DE genes in the pituitary. (C) Pathways enriched by DE genes in the hypothalamus. (D) No significant changes in the coding genes for GnRH, LH, and FSH in the hypothalamus and pituitary of subjects with poorer testicular function. (E) Genes that are differentially expressed in either the pituitary or hypothalamus. (F) Genes that are differentially expressed in both the pituitary and hypothalamus. *GNRH1*, progonadoliberin-1; *FSHB*, follitropin subunit beta; *LHB*, lutropin subunit beta; *CGA*, glycoprotein hormones alpha chain; *ANGPTL4*, angiopoietin-related protein 4; *CACNG6*, voltage-dependent calcium channel gamma-6 subunit; *HMX3*, homeobox protein HMX3; *GCKR*, glucokinase regulatory protein gene; *NMS*, neuromedin-S; *EDN1*, endothelin-1.



Groups	Testis.Good	Testis.Poor
Subject.N.	36	59
DE.method	qval < 0.05 and mean > 0.01	

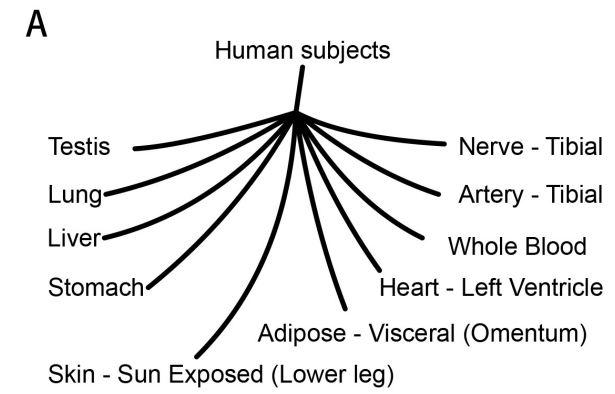
Tissue	log2fc < 0	log2fc > 0	Total.DE
Thyroid	16	5	21
Adrenal Gland	143	117	260
Testis	10181	10791	20972

FIGURE 4. Differentially expressed genes in the adrenal and thyroid glands associated with testicular function. (A) Differentially expressed (DE) genes in the adrenal and thyroid glands of subjects with varying testicular spermatogenic abilities. (B) Immune response genes were upregulated in the testis, adrenal gland, and thyroid of subjects in the T.poor group. (C) Genes that were downregulated in both the adrenal gland and thyroid but upregulated in the testis. (D) The gene *NPFF* was downregulated in both the adrenal gland and thyroid, with no significant change observed in the testis. *ATRN*, attractin; *C3*, complement C3; *C4B*, complement C4-B; *RAMP2*, receptor activity-modifying protein 2; *KANTR*, KANTR integral membrane protein gene; *NPFF*, pro-FMRFamide-related neuropeptide FF.

3.4 Multi-tissue transcriptomic associations with testicular function

To investigate systemic associations with spermatogenic capacity, we analyzed bulk RNA-seq data from various somatic tissues in addition to testes, including the lung, liver, stomach, skin (sun-exposed), visceral adipose tissue, left ventricle of the heart, whole blood, tibial artery, and tibial nerve. To control for potential confounding effects of mechanical ventilation on pulmonary transcriptomes, we excluded subjects with a history of ventilator use. This stringent filtering resulted in 11 eligible subjects (6 T.good subjects and 5 T.poor subjects; **Supplementary Table 2**). Differential expression analysis (p-value < 0.01; mean log2(TPM + 1) >0.01) revealed 6595 DE genes in the testes, 189 DE genes in the lung, 90 DE genes in the liver and fewer than 90 DE genes in each of the other tissues (Fig. 5A and **Supplementary Table 5**). These data suggest that the lung and liver may be more closely related to testicular function compared to the other tissues or organs.

In the lungs of the T.poor group, the *PNMT* gene, which encodes a methyltransferase that converts norepinephrine into epinephrine, and acts on phenylethanolamine and octopamine, was downregulated only in the lungs. Conversely, the *CCR1* gene, which regulates immune responses, was upregulated in both the lungs and testis (Fig. 5B). In the liver of the T.poor group, the *SLC2A8* gene, which encodes a membrane protein responsible for the transport of glucose and fructose, was downregulated in both the liver and testis. Additionally, the *GABRE* gene, which encodes the gamma-aminobutyric acid receptor subunit epsilon, was upregulated in the liver and testis (Fig. 5C). These data suggest that immune responses, metabolism, and endocrine regulation in the lung or liver may be associated with the spermatogenic capacity of the testis.



Groups	Testis.Good	Testis.Poor
Subject.N.	6	5
DE.method	pval < 0.01 and mean > 0.01	

Tissue	log2fc < 0	log2fc > 0	Total.DE
Stomach	2	3	5
Heart - Left Ventricle	7	0	7
Whole Blood	1	14	15
Adipose - Visceral (Omentum)	14	13	27
Artery - Tibial	5	22	27
Nerve - Tibial	22	18	40
Skin - Sun Exposed (Lower leg)	0	72	72
Liver	5	85	90
Lung	169	20	189
Testis	2494	4101	6595

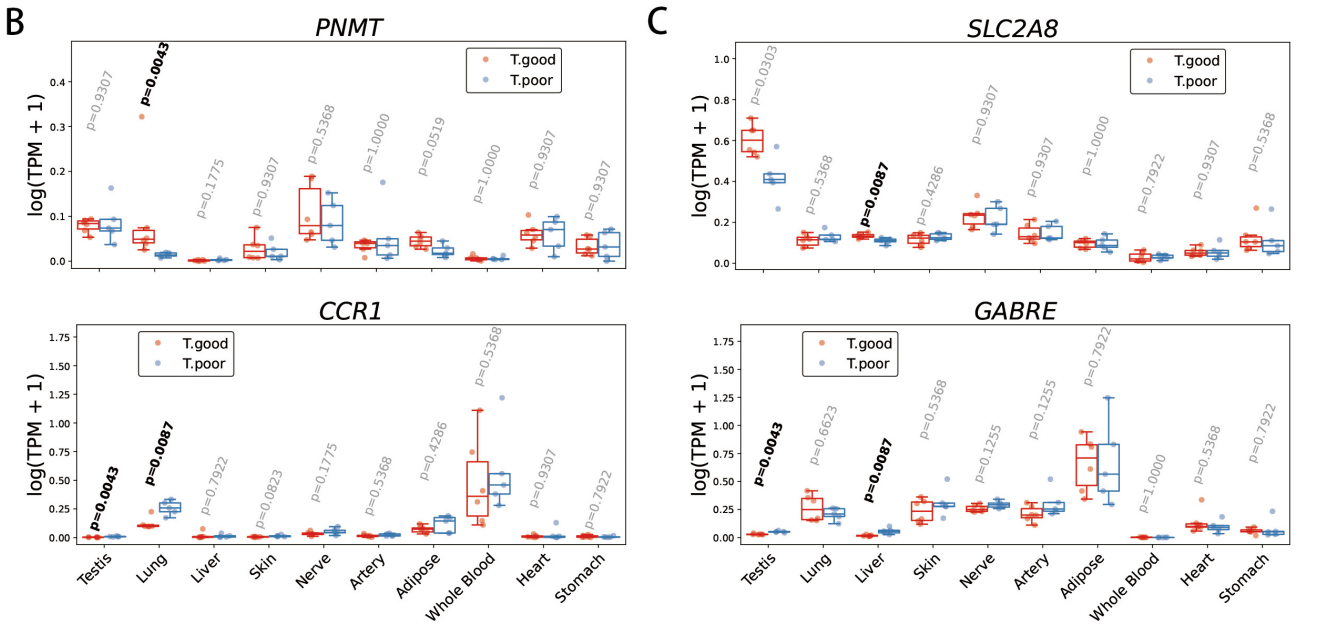


FIGURE 5. Genes in lung and other non-testicular tissues associated with testicular function. (A) Differentially expressed (DE) genes in various tissues/organs based on differing testicular spermatogenesis abilities. (B) Genes that are differentially expressed in the lung. (C) Genes that are differentially expressed in the liver. *PNMT*, phenylethanolamine N-methyltransferase; *CCR1*, C-C chemokine receptor type 1; *SLC2A8*, solute carrier family 2, facilitated glucose transporter member 8; *GABRE*, gamma-aminobutyric acid receptor subunit epsilon.

3.5 Transcriptomic associations between testicular function and multiple brain regions

Beyond the hypothalamic-pituitary axis, our study reveals significant correlations between testicular function and various brain regions involved in emotional and cognitive processing. We analyzed bulk RNA-seq data from testes and 11 distinct brain regions in 14 human subjects (6 T.good, 8 T.poor; **Supplementary Table 2**), identifying tissue-specific patterns of differential gene expression (p-value < 0.01; mean log2(TPM + 1) >0.01): 5965 DE genes in the testes, 163 DE genes in the amygdala, 139 DE genes in the substantia nigra, 133 DE genes in the anterior cingulate cortex, 124 DE genes in the frontal cortex, and fewer than 100 DE genes in each of the other brain regions (**Supplementary Table 6** and Fig. 6A).

In these brain regions, the amygdala is involved in emotional processes such as fear. The substantia nigra is associated with voluntary movement and reward processing in humans, and the loss of dopaminergic neurons in the substantia nigra may lead to Parkinson's disease [25]. The anterior cingulate cortex and frontal cortex also play a role in regulating emotional functions [26]. In the amygdala, we found that the *IRX3* gene was upregulated in the T.poor group, while the *ALDH1A1* gene was downregulated (Fig. 6B). The 5-hydroxytryptamine receptor gene *HTR1E* was downregulated in the substantia nigra of the T.poor group, and the neuronal acetylcholine receptor gene *CHRNA2* was downregulated in the anterior cingulate cortex and amygdala of the T.poor group (Fig. 6C). In both the anterior cingulate cortex and frontal cortex, we found that the gamma-aminobutyric acid receptor gene *GABRG1* and the peroxisomal acyl-coenzyme A oxidase gene *ACOX2* were upregulated in the T.poor group (Fig. 6D). These data suggest that testicular function may be associated with brain regions involved in emotional processing.

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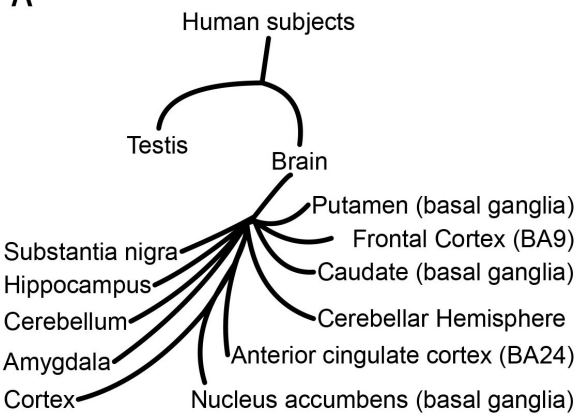

Groups	Testis.Good	Testis.Poor
Subject.N.	6	8
DE.method	pval < 0.01 and mean > 0.01	

Tissue	log2fc < 0	log2fc > 0	Total.DE
Brain - Putamen (basal ganglia)	3	5	8
Brain - Hippocampus	13	1	14
Brain - Nucleus accumbens (basal ganglia)	16	5	21
Brain - Cerebellar Hemisphere	19	14	33
Brain - Cerebellum	29	17	46
Brain - Cortex	17	78	95
Brain - Caudate (basal ganglia)	1	95	96
Brain - Frontal Cortex (BA9)	1	123	124
Brain - Anterior cingulate cortex (BA24)	117	16	133
Brain - Substantia nigra	139	0	139
Brain - Amygdala	155	8	163
Testis	2275	3690	5965

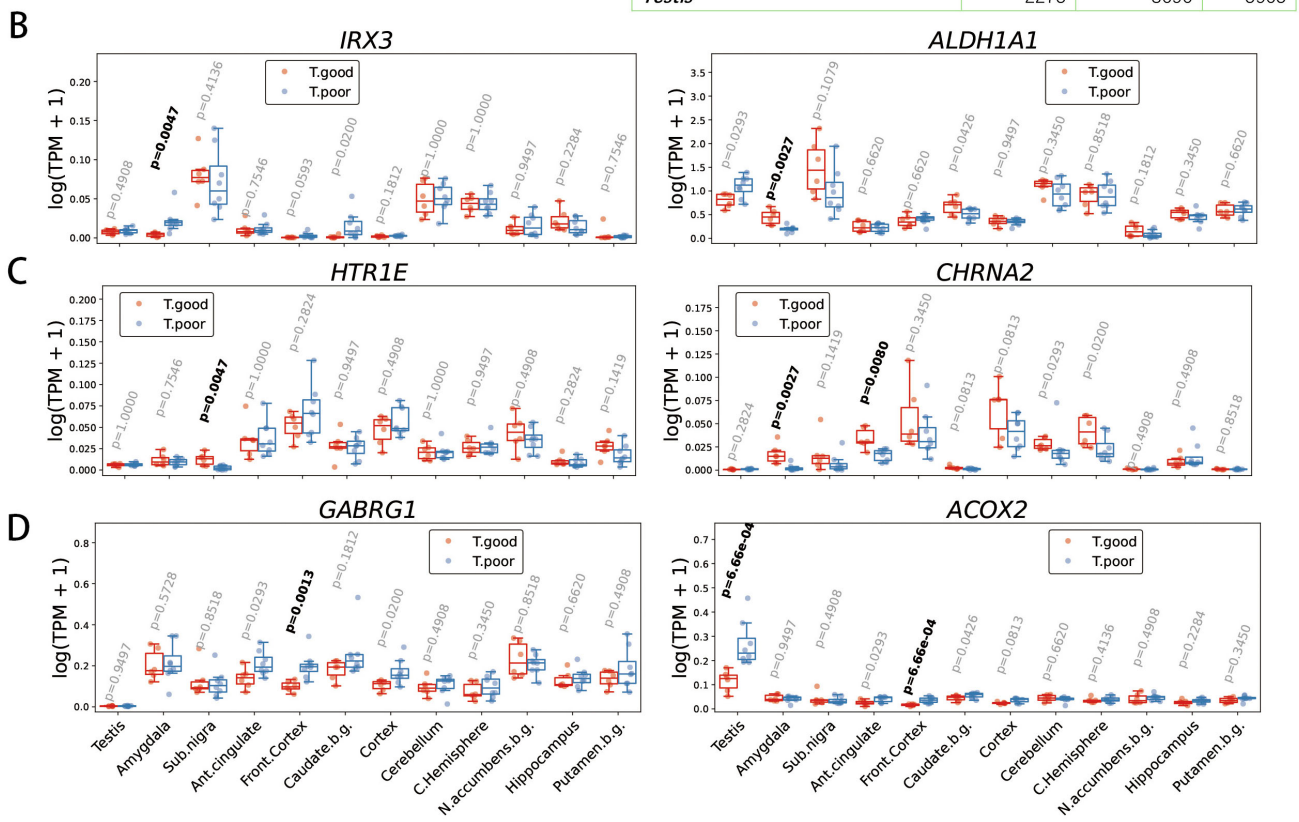

FIGURE 6. Brain regions associated with testicular spermatogenesis. (A) Differentially expressed (DE) genes in brain regions based on varying abilities of testicular spermatogenesis. (B) Genes that are differentially expressed in the amygdala. (C) Genes that are differentially expressed in the substantia nigra. (D) Genes that are differentially expressed in the anterior cingulate cortex and frontal cortex. *IRX3*, iroquois-class homeodomain protein IRX-3; *ALDH1A1*, aldehyde dehydrogenase 1A1; *HTR1E*, 5-hydroxytryptamine receptor 1E; *CHRNA2*, neuronal acetylcholine receptor subunit alpha-2; *GABRG1*, gamma-aminobutyric acid receptor subunit gamma-1; *ACOX2*, peroxisomal acyl-coenzyme A oxidase 2.

In the brain, the activation, inhibition, and communication between neurons rely on neurotransmitters that function at synapses. We analyzed additional neurotransmitter receptors that may be associated with testicular function (**Supplementary Table 7**). As a result, we found that the dopamine receptor gene *DRD4* was downregulated in the hippocampus of the T.poor group (Fig. 7A), while the glutamate receptor gene *GRIN2D* was downregulated in the amygdala of the T.poor group (Fig. 7B). In the substantia nigra, in addition to *HTR1E*, the acetylcholine receptor gene *CHRM4*, the glycine receptor gene *GLRB*, the gamma-aminobutyric acid receptor genes *GABRA2* and *GABRB3*, as well as the glutamate receptor genes *GRIK2* and *GRM1* were all downregulated in samples of T.poor group (Fig. 7C). These data indicate a significant correlation between testicular function and neuronal communication in specific brain regions, particularly in the substantia nigra.

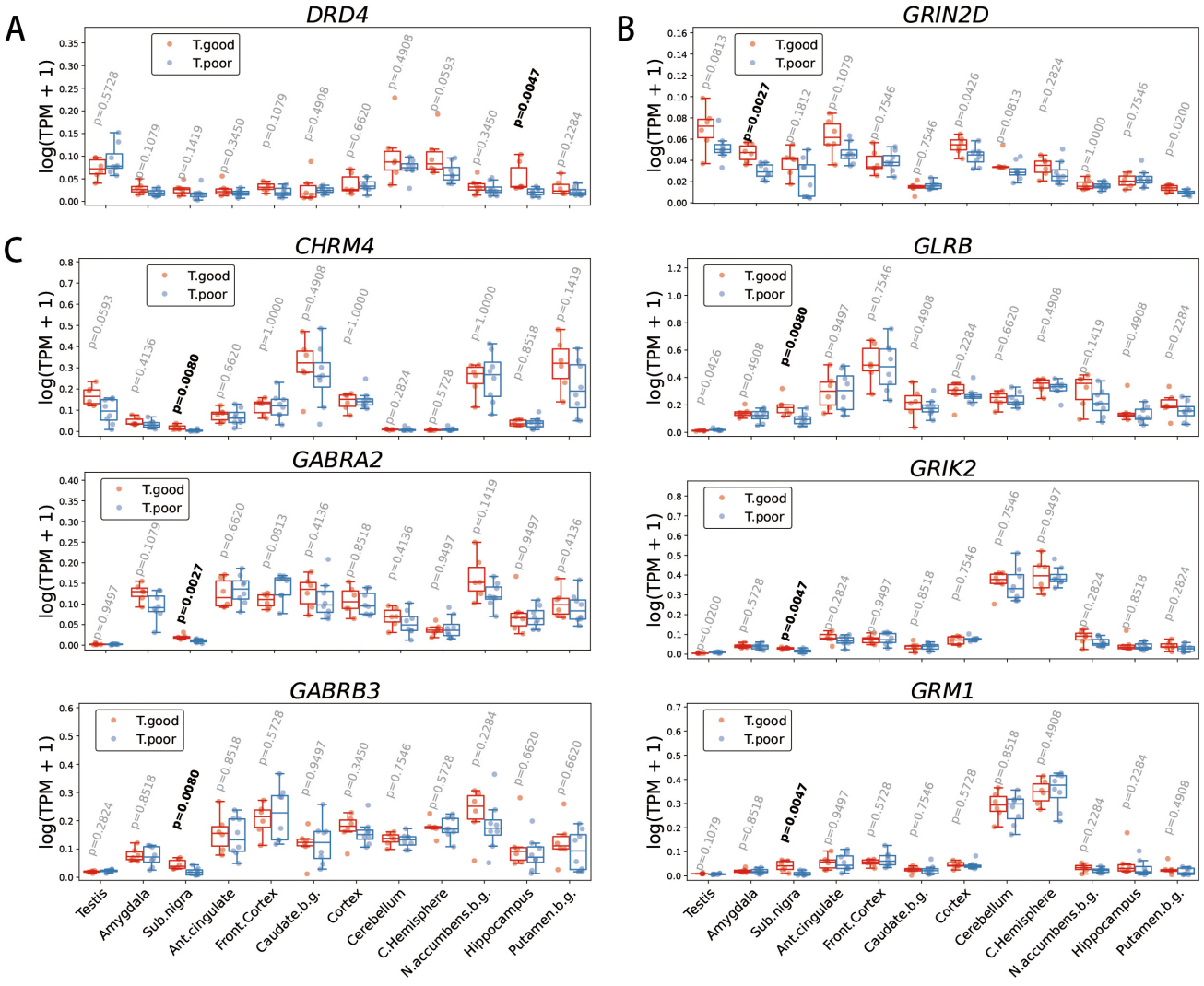

FIGURE 7. Differentially expressed brain neurotransmitter receptors associated with testis function. (A) The dopamine receptor gene *DRD4* was downregulated in the hippocampus of subjects in the T.poor group. (B) The glutamate receptor gene *GRIN2D* was downregulated in the amygdala of T.poor subjects. (C) Several neurotransmitter receptor genes were downregulated in the substantia nigra of T.poor subjects. *DRD4*, D(4) dopamine receptor; *GRIN2D*, glutamate receptor ionotropic; *CHRM4*, muscarinic acetylcholine receptor M4; *GLRB*, glycine receptor subunit beta; *GABRA2*, gamma-aminobutyric acid receptor subunit alpha-2; *GRIK2*, glutamate receptor ionotropic, kainate 2; *GABRB3*, gamma-aminobutyric acid receptor subunit beta-3; *GRM1*, metabotropic glutamate receptor 1.

4. Discussion

Understanding the relationship between testicular spermatogenic capacity and systemic tissues is crucial for elucidating the interplay between reproductive health and overall physiological homeostasis. Using data from GTEx, we stratified human subjects into groups with higher and lower testicular spermatogenic capacity and analyzed the transcriptomic differences in systemic tissues between the two groups. In addition to the HPT axis and adrenal gland, we found that the lung and liver exhibit a stronger association with testicular spermatogenic capacity compared to tissues such as blood and stomach. Furthermore, in the brain, regions associated with emotional regulation, including the amygdala, substantia nigra, anterior cingulate cortex, and frontal cortex, also demonstrate associations with testicular function. These findings suggest that the development of drugs targeting the testis should not only be approached from a multi-omics perspective [27–30], but also consider the potential interactions across different tissues.

Male infertility can result from either monogenic variants [31] or the combined effects of environmental factors and polygenic susceptibility [1, 32]. In the adrenal gland, high expression of inflammatory factors is associated with decreased spermatogenic capability in the testes. The lungs are directly exposed to airborne exogenous substances (*e.g.*, pathogens, pollutants), making them highly susceptible to triggering immune responses [33]. The liver's metabolic capacity is intrinsically linked to immune regulation through the detoxification of immunomodulatory substances, synthesis of complement and clotting factors, and antigen presentation by Kupffer cells and hepatocytes [34]. In testes with lower spermatogenetic

capability, inflammation-associated genes such as *C3*, *C4B*, and *ATRN* are also upregulated. These findings suggest that the systemic inflammation elevated by abnormalities in the lungs or liver might be associated with testicular function.

Lifestyle modification is considered a potential approach to addressing male infertility caused by testicular failure [35]. In this study, we found that inflammation and neuroendocrine factors correlate with testicular function. The UK Biobank data has shown that environmental particulate matter pollution elevates lung inflammation [36]. Air pollution has been considered an inconclusive risk factor of male infertility [37]; therefore, we speculate that air pollution may lead to a decline in testicular function by inducing inflammatory responses in the lungs, and reducing exposure to air pollution might potentially help improve male fertility. However, whether environmental factors affect testicular function through the lungs still requires support from animal studies and epidemiological investigations.

In the brain, the substantia nigra primarily regulates motor control and reward processing through the production of dopamine. In this study, we identified a significant relationship between the testes and the differentially expressed neurotransmitter receptors in the substantia nigra. Additionally, it has been reported that testosterone can modulate the expression of dopamine-related genes in this region [38]. These findings suggest that disorders in the testes may be associated with changes in human behavior through the substantia nigra and/or other brain regions. The connection between the testes and mental health may be unidirectional or bidirectional. However, whether it is the impact of the testes on mental health or the influence of mental health on the testes, as well as the potential application of psychotropic drugs in reproductive disease, all warrant further research in the future.

In this study, we identified differential expression patterns of bioactive peptide-encoding genes: *NPFF* was downregulated in the adrenal gland and thyroid, while *NMS* showed downregulation, and *EDN1* exhibited upregulation in the hypothalamus. These neuropeptides may serve as potential biomarkers for both detecting and investigating the underlying causes of male subfertility, or as potential therapeutic targets for indirectly improving testicular spermatogenic function.

In summary, this study provides a global exploration of the potential associations between extra-testicular tissues and testicular function. Our findings suggest that specific genes in endocrine organs, respiratory and digestive systems, as well as brain tissues, may be linked to testicular function. These genes are largely involved in cellular responses to inflammation, as well as hormonal and metabolic regulation. In the future, it would be valuable to conduct more comprehensive association analyses from an epidemiological perspective, examining relationships between male fertility and phenotypes related to inflammation, neuroendocrine function, and metabolism. From a clinical perspective, there may be value in considering systemic factors when assessing male spermatogenic capacity. These factors include mental health status, the functional state of organs such as the liver and lungs, and blood-based markers (*e.g.*, peptides and inflammatory factors) originating from non-testicular tissues.

Last but not least, this study has some primary limitations. First, we analyzed the relationships between the testes and various tissues rather than the causality. Consequently, we cannot ascertain whether changes in these tissues or organs affect testicular spermatogenic capacity, whether differences in spermatogenic capacity influence other tissues or organs, or whether an underlying abnormality simultaneously impacts both the testes and a specific tissue. Second, the sample size is limited when comparing the strength of associations between various tissues and testicular function, which may increase the potential errors due to sampling bias. For comparative studies with small sample sizes, more experimental evidence is needed. Third, the GTEx version 10 dataset, collected from postmortem donor specimens, may yield under- or over-estimated functional assessments of specific genes in certain tissues or organs due to potential inconsistencies in RNA-seq protocols, including tissue procurement, RNA extraction, and library preparation for sequencing, across different individuals. Fourth, in this study, we utilized tools designed for single-cell transcriptomics to analyze bulk RNA-seq data, which may lead to omissions in certain details. And finally, it should be noted that the observed mRNA expression differences in this study may not necessarily reflect corresponding protein levels in tissues, and since individual genetic variations were not incorporated into our analysis, the upregulation or downregulation of mRNA alone may not accurately represent functional changes in protein activity.

5. Conclusions

In conclusion, this study analyzed the correlation between testicular transcriptomic profile stratified by spermatogenic capacity and transcriptomes of non-testicular tissues, and found that many non-testicular tissues and organs, such as brain tissue, lung, liver, and endocrine organs, may have potential correlations with testicular spermatogenic capacity. Although the causal relationships underlying these correlations require further exploration, they may be useful for improving diagnosis and treatment of male fertility.

AVAILABILITY OF DATA AND MATERIALS

The GTEx V10 open-access data (https://www.gtexportal.org/) was utilized in this study. The scripts used in this research have been uploaded to GitHub (https://github.com/MaJYatGZ/GTEx_testis_Analysis).

AUTHOR CONTRIBUTIONS

JYM, SQC and YYC—contributed the central idea. JYM, SMW and XA—analyzed most of the data. JYM, XA, FYX, SQC and YYC—wrote the paper. SMW and JYM—revised the paper. All authors contributed to refining the ideas and finalizing this paper.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found, in the online version, at https://oss.jomh.org/files/article/2060283140180459520/attachment/Supplementary%20material.xlsx.

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