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ORIGINAL ARTICLE

Analysis of the finasteride treatment and its withdrawal in the rat hypothalamus and hippocampus at whole-transcriptome level

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Abstract

Purpose As reported in patients treated for androgenetic alopecia with finasteride (i.e., a blocker of the enzyme 5 alpha-reductase) and in an animal model, side effects affecting sexual, psychiatric, neurological, and physical domains, may occur during the treatment and persist with drug suspension. The etiopathogenesis of these side effects has been poorly explored. Therefore, we performed a genome-wide analysis of finasteride effects in the brain of adult male rat.

Methods Animals were treated (i.e., for 20 days) with finasteride (1mg/rat/day). 24 h after the last treatment and 1 month after drug suspension, RNA sequencing analysis was performed in hypothalamus and hippocampus. Data were analyzed by differential expression analysis and Gene-Set Enrichment Analyses (GSEA).

Results Data obtained after finasteride treatment showed that 186 genes (i.e., 171 up- and 15 downregulated) and 19 (i.e., 17 up- and 2 downregulated) were differentially expressed in the hypothalamus and hippocampus, respectively. Differential expression analysis at the drug withdrawal failed to identify dysregulated genes. Several gene-sets were enriched in these brain areas at both time points.

Conclusion Some of the genes reported to be differentially expressed (i.e., *TTR, DIO2, CLDN1, CLDN2, SLC4A5, KCNE2, CROT, HCRT, MARCKSL1, VGF, IRF2BPL*) and GSEA, suggest a potential link with specific side effects previously observed in patients and in the animal model, such as depression, anxiety, disturbance in memory and attention, and sleep disturbance. These data may provide an important background for future experiments aimed at confirming the pathological role of these genes.

Keywords 5 alpha-reductase · Male rat · Post-finasteride syndrome · Side-effects · RNA sequencing analysis

Introduction

Finasteride, a blocker of the 5 alpha-reductase (i.e., the enzyme converting testosterone into dihydrotestosterone and progesterone into dihydroprogesterone) is clinically used for benign prostatic hyperplasia and androgenetic alopecia [1]. Even if the efficacy of this drug is well established in both disorders, several studies have reported important side effects during the treatment, and persistence of them at the drug suspension, with the appearance of the so-called Post-finasteride syndrome (PFS) [1–8]. In particular, PFS patients reported side effects in the sexual domain, such as erectile dysfunction, loss of libido and sexual drive, penile atrophy, and diminished ejaculatory [9–14]. In addition, psychiatric, neurological and physical domains, such as depression, anxiety, panic attacks, reduction in self-confidence, disturbance in memory and attention, sleep disturbance, peripheral neuropathy, genital numbness and paresthesia, muscular atrophy and alteration of fat distribution have been reported [4, 6–8, 12, 13]. To date, the biological basis of these side effects has been poorly explored. Indeed, the observations present in the literature are mainly based on symptoms self-reported by the patients and only a few papers have deeply investigated these aspects. For instance, as demonstrated in PFS patients [13, 15, 16] and in an animal model [17], finasteride treatment is not only able to block the enzyme 5alpha-reductase and consequently the metabolism of testosterone and progesterone, but has a broad

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consequence on the pattern of several other steroids. Indeed, it is able to affect the plasma and brain levels of neuroactive steroids (i.e., a family of steroids, including steroid hormones and neurosteroids, which affects nervous functions). Interestingly, not only their levels but also alterations in their mechanism of action (i.e., via classical and nonclassical steroid receptors) have been reported [17–20]. Accordingly, the important role of neuroactive steroids in regulating nervous functions [21], human and animal PFS studies have ascertained impaired sexual function, depressive symptomatology and alterations in gut microbiota composition and gut–brain axis [12, 13, 22–25]. In particular, in the animal model, depressive-like behavior was associated with increased hippocampal neuroinflammation, altered neurogenesis, and increased reactive astrogliosis [24]. In addition, finasteride is not only an inhibitor of the 5 alpha-reductase but as recently demonstrated it is also able to block the enzyme phenylethanolamine N-methyltransferase, that it is responsible for the conversion of norepinephrine into epinephrine [26]. Thus, finasteride may alter per se this important neurotransmitter system. Recent observations, obtained in penile skin samples by microarray, have shown that 1.446 genes and 2.318 were overexpressed and underexpressed respectively, in PFS patients vs healthy controls [27], suggesting that gene expression differences may be a potential etiology of side effects occurring in these patients. On this basis, by RNA sequencing analysis, we have here evaluated the effect of finasteride chronic treatment (i.e., for 20 days) and its withdrawal (i.e., for 1 month) in two important brain areas of adult male rats, possibly related to the side effects induced by finasteride, such as the hypothalamus and hippocampus.

Materials and methods

Animals and treatments

Adult male Sprague–Dawley rats (200-225 g at arrival, Charles River Laboratories, Italy) were used. All procedures were carried out in the animal care facility of the Department of Pharmacological and Biomolecular Sciences (DiSFeB) at the Università degli Studi di Milano, Italy and were approved by the local ethics committee and the Italian Ministry of Health (authorization 1083/2015-PR). All manipulations were performed in accordance with national (D.L. No. 26, March 4, 2014, G.U. No. 61March 14, 2014) and international laws and policies (EEC Council Directive 2010/63, September 22, 2010: Guide for the Care and Use of Laboratory Animals, United States National Research Council, 2011). Rats ($n=24$) were acclimated to the new environment for 1 week. Finasteride (1 mg/rat/day; Sigma-Aldrich, Italy) was dissolved in a vehicle solution of sesame oil and ethanol (5% v/v) and administered subcutaneously for 20 days at a volume of 100 µL/day. Finasteride and vehicle-treated rats were sacrificed at 24 h ($n=4$ for each group) after the last injection and 1 month ($n=4$ for each group) after drug suspension. After sacrifice, hippocampus and hypothalamus were dissected and immediately stored at − 80 °C until the analysis.

RNA extraction

Total RNA from the hippocampus and the hypothalamus was extracted using Trizol (Invitrogen, San Giuliano Milanese, Italy). Briefly, tissues were homogenized with the Tissue Lyzer instrument (Qiagen, Milan, Italy), and chloroform was added to obtain phase separation. RNA was present in the upper aqueous phase, and its separation was obtained with a Directzol™ RNA MiniPrep kit (Zymo Research, Irvine, CA, USA) in accordance with the manufacturer's protocol and as previously reported.

Whole transcriptome sequencing

Total RNA was quantified by NanoDrop™2000 (ThermoFisher scientific, Milano, Italy) and its integrity was verified with the Agilent TapeStation system (Agilent, Santa Clara, USA). RNA integrity number (RIN) > 7.5 was considered sufficient for further analysis. Then, Illumina stranded mRNA prep (Illumina, San Diego, USA) was used according to the manufacturer's protocol to prepare libraries that have been sequenced into a NextSeq 550 instrument (Illumina, San Diego, USA).

Data processing and bioinformatics analysis

Raw sequences were initially tested using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Subsequently, fastq reads were aligned against the reference Rattus Norvegicus genome using the splice-aware aligner Star [28], using the quantMode GeneCounts parameter to perform raw counting at gene level. The Bioconductor package DESeq2 v. 1.30 [29] was applied to perform the differential gene expression analyses. Differential genes were identified by selecting a Benjamini–Hochberg adjusted p-value < 0.1. Bam alignment files were indexed using Samtools [30] generating the bam-associated bai index files. The sorted, indexed bam alignment files, together with bai indexes, were then manually inspected using the Integrative Genomics Viewer [31]. GSEA were carried out using the GSEA tool v. 4.2.1 (https://www.gsea-msigdb.org/gsea/downloads.jsp) by applying 1000 permutations at gene_set level. Gene-sets with a Benjamini–Hochberg adjusted p value < 0.25 were considered statistically significant.

Results

A correlation analysis done at whole-transcriptome level in rat hypothalamus and hippocampus at the two time points in presence vs absence of finasteride showed a very strong correlation for hypothalamus treated or not treated with finasteride after chronic treatment (T0) or at withdrawal (T1) (Pearson's $r = 0.995$) as well as for hippocampus at T0 vs T1 (Pearson's $r = 0.997$), suggesting a similar transcriptional effect of finasteride at the two different time points (Fig. 1A).

To isolate the transcriptional programs associated with finasteride treatment in the hypothalamus at T0, we initially performed a differential expression analysis, which revealed 186 differentially expressed genes. Among these, 171 and 15 genes were up- and downregulated, respectively (Supplementary Table 1). In particular, we reported altered genes, such as Transthyretin (*TTR*), Iodothyronine Deiodinase 2 (*DIO2*), Claudin 2 (*CLDN2*) and 1 (*CLDN1*), Solute Carrier Family 4 Member 5 (*SLC4A5*), Potassium Voltage-Gated Channel Subfamily E Regulatory Subunit 2 (*KCNE2*), carnitine octanoyltransferase (*CROT*), Hypocretin Neuropeptide Precursor (*HCRT*), myristoylated alanin-rich C-kinase (*MARCKSL1*), Interferon Regulatory Factor 2 Binding Protein Like (*IRF2BPL*), and nerve growth factor inducible (*VGF*), that may be possibly related with side effects reported after finasteride treatment (Fig. 1B).

To investigate the transcriptional programs modulated by finasteride in hypothalamus at T0, we carried out Gene-Set Enrichment Analyses (GSEA) using the classical GSEA hallmarks as reference gene-sets. Using this approach we identified the hallmark WNT_BETA_CATENIN_SIGNALING as significantly enriched in finasteride-treated hypothalamus at T0 (Fig. 1 C,D; Normalized Enrichment Score (NES) 1.40; $p_{adj} = 0.24$). Differential expression analysis performed in the hippocampus at T1 failed to identify dysregulated genes (Supplementary Table 2), which suggests a modest transcriptional effect of finasteride at this timepoint. However, GSEA performed at T1 revealed a significant positive enrichment (Fig. 1E,F; NES 1.36; $p_{adj} = 0.23$) of the hallmark IL6_JAK_STAT3_SIGNALING.

Data obtained in the hippocampus after chronic treatment with the drug showed that 19 genes were significantly affected, of them 17 were up and 2 downregulated (Supplementary Table 3). GSEA performed at T0 in the hippocampus revealed that, like in the case of hypothalamus (Fig. 1 C,D), the hallmark WNT_BETA_CATENIN_SIGNALING was significantly enriched (Fig. 2 A,B; NES 1.58; $p_{adj} = 0.052$). On the contrary, others hallmarks, such as OXIDATIVE_PHOSPHORYLATION (NES −1.59; $p_{adj} = 0.037$), MYC_TARGETS_V1 (NES −1.43; $p_{adj} = 0.13$), INTERFERON_ALPHA_RESPONSE (NES −1.37; $p_{adj} = 0.088$), E2F_TARGETS (NES −1.32; $p_{adj} = 0.10$), and FATTY_ACID_METABOLISM (NES −1.39; $p_{adj} = 0.10$) were significantly decreased (Fig. 2A,B).

Differential expression analysis performed in the hippocampus at T1 failed to identify dysregulated genes (Supplementary Table 4), however, GSEA revealed a decrease in the INTERFERON_ALPHA_RESPONSE (NES -1.73; $p_{adj} = 0.005$) and INTERFERON_GAMMA_RESPONSE hallmark (NES −1.57; $p_{adj} = 0.028$). Notably, MYC_TARGETS_V1 (NES −1.48; padj = 0.028), OXIDATIVE_PHOSPHORYLATION (NES −1.49; $p_{adj} = 0.029$) and FATTY_ACID_METABOLISM (NES −1.38; $p_{adj} = 0.069$) hallmarks were also downmodulated not only at T0 (Fig. 2A,B) but also at T1 (Fig. 3A,B). Interestingly, a significant enrichment of the WNT_BETA_CATENIN_SIGNALING hallmark present in this brain area at T0 (Fig. 1C,D) was still present at T1 (Supplementary Fig. 1; NES 1.43; $p_{adj} = 0.11$). In addition, an enrichment in hallmarks such as HP_CENTRAL_SLEEP_APNEA (Fig. 3A,B; NES 1.74; $p_{adj} = 0.028$), REACTOME_CIRCADIAN_CLOCK (Fig. 3A,B; NES 1.62; $p_{adj} = 0.051$) and GOBP_CIRCADIAN_SLEEP_WAKE_CYCLE (Fig. 3A,B; NES 1.22; $p_{adj} = 0.23$) was also observed.

Discussion

Data here obtained by RNA sequencing showed that chronic treatment (i.e., for 20 days) with finasteride affects the expression of hypothalamic and hippocampal rat genes. As we reported, the most affected brain area is the hypothalamus, with 15 genes downregulated and 171 genes upregulated. Among the downregulated genes, we will here discuss those that, based on the literature available, could be associated with the side effects reported by the patients during the treatment and observed in the experimental model. For instance, *TTR* encodes for a carrier protein involved in the transport of thyroxine (T4) and retinol. Besides its role as a carrier protein, downregulation of this gene induces learning and memory impairment, aggressive behavior, and neurodegeneration [32–35]. In the context of the effects of thyroid hormones in the nervous system, it is important to highlight that we also observed a significant decrease in the gene *DIO2*. This gene encodes for the enzyme responsible for the conversion of prohormone T4 into the biologically active thyroid hormone, triiodothyronine (T3). Therefore, impairment in this enzymatic conversion may affect the important role exerted by T3 in brain functionality (e.g., on synaptic plasticity, oxidative stress, inflammation, mood, and neurotransmitter regulation) by genomic and nongenomic mechanisms [36–41]. Indeed, as demonstrated in adult mice

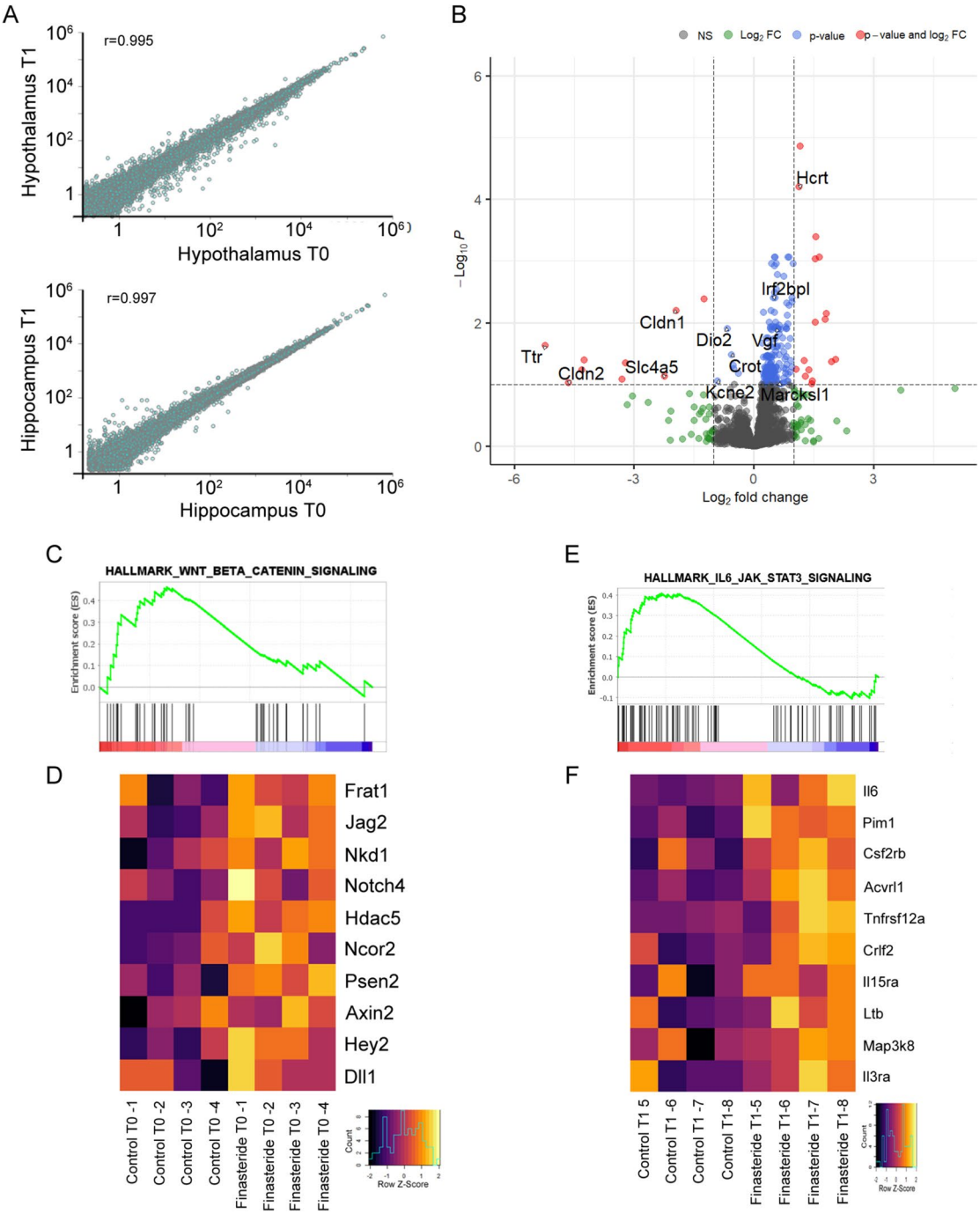

Fig. 1 **A** Pearson correlation analysis of whole-transcriptome case/control Log_2-FoldChange ratios in Hypothalamus (upper panel) at T0 (*x* axis) vs T1 (*y* axis) and in Hippocampus (lower panel) at T0 (*x* axis) vs T1 (*y* axis). **B** Volcano plot showing the whole-transcriptome case/control Log_2-FoldChange ratios (*x* axis) and the associated $Colog_{10}$ transformed *p* value in Hypothalamus at T0. Grey dots highlight genes non-significant and with absolute Log_2-FoldChange ≤ 1; green dots genes with absolute Log_2-FoldChange > 1 and -Log10 *p*-value < 1; blue dots genes with absolute Log_2-FoldChange < 1 and -Log10 *p*-value > 1; red dots genes with absolute Log_2-FoldChange > 1 and -Log10 *p*-value > 1. **C** GSEA plot of the WNT-beta-catenin and **D** associated heatmap in Hypothalamus at T0 in control and Finasteride-treated rats. **E** GSEA plot of the IL6-JAK-STAT3 signaling and **F** associated heatmap in Hypothalamus at T1 in control and Finasteride-treated rats.$n = 4$ for each experimental group

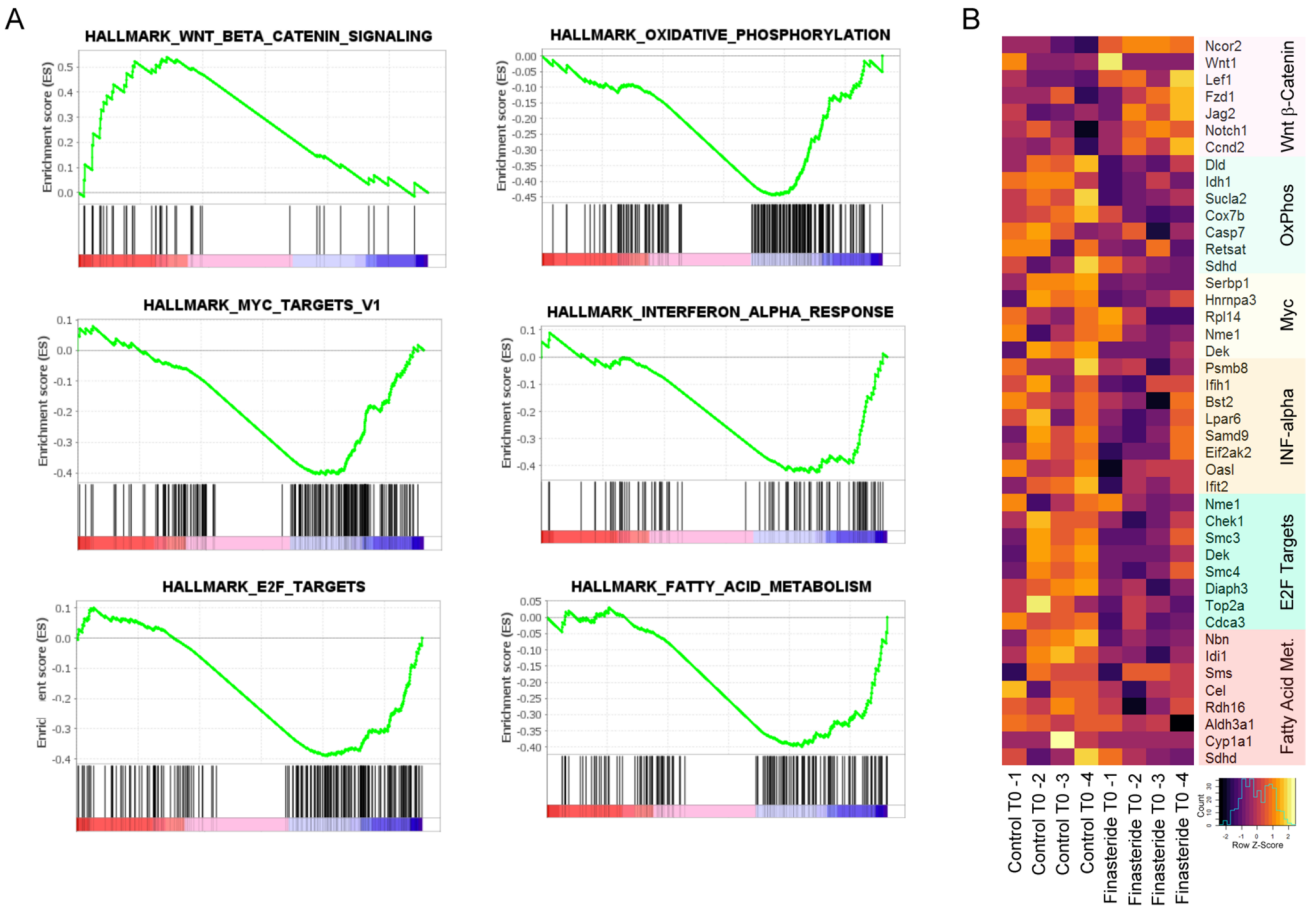

Fig. 2 **A** GSEA plots showing positive and negative enrichment of specific gene-sets in Hippocampus at T0. **B** Heatmap reporting the leading genes associated with the GSEA shown in panel A sets in Hippocampus at T0 in control and Finasteride-treated rats. $n=4$ for each experimental group

lacking *DIO2*, reduced expression of several target genes of tyroid hormones [42, 43], altered motor ability [44], emotional alteration with increased anxiety-like behavior as well as enhanced fear memory was observed [45].

Other genes downregulated in the hypothalamus of rats chronically treated with finasteride are *CLDN2* and *CLDN1*. Claudin proteins are functional and structural components of tight junctions [46] that in the nervous system, apart from maintaining blood–brain barriers, also play important roles in maintaining the synaptic and neuronal structure and function. In line with these observations, alteration of these genes is related to neuropathological events [47]. Other genes downregulated are *SLC4A5* and *KCNE2*, also known to exert key roles in the nervous system. For instance, *SLC4A5* encodes Na+/HCO_3-cotransporter 4, a membrane protein that plays a critical role in maintaining pH and ion balance in cells by transporting sodium and bicarbonate ions [48, 49]. Multiple defects were observed in the nervous system of *SLC4A5* deficient mice, such as decreased volume of lateral brain ventricles, decreased intracranial pressure, changes in the choroid plexus epithelium cell morphology and changes in cerebrospinal fluid composition [50]. Mice lacking *KCNE2* showed increased behavioral responsiveness to stress and seizure susceptibility [51]. *CROT* is also downregulated by finasteride treatment in the hypothalamus. The encoded protein converts 4,8-dimethylnonanoyl-CoA to its corresponding carnitine ester. This transesterification occurs in the peroxisome and is necessary for transport of medium- and long-chain acyl-CoA molecules out of the peroxisome to the cytosol and mitochondria [52]. Therefore, the protein plays a role in lipid metabolism and fatty acid beta-oxidation. As demonstrated, at least in a model of hepatic cells, knockdown of *CROT* has an important impact on fatty acid profile, with increase in the amount of medium chain saturated fatty acid and unsaturated C24 [52]. Therefore these data may suggest a role for this gene in regulating the peroxisomal oxidative pathway. In the brain, peroxisomes are mainly located in astrocytes and oligodendrocytes [53]. Dysfunction of peroxisomal

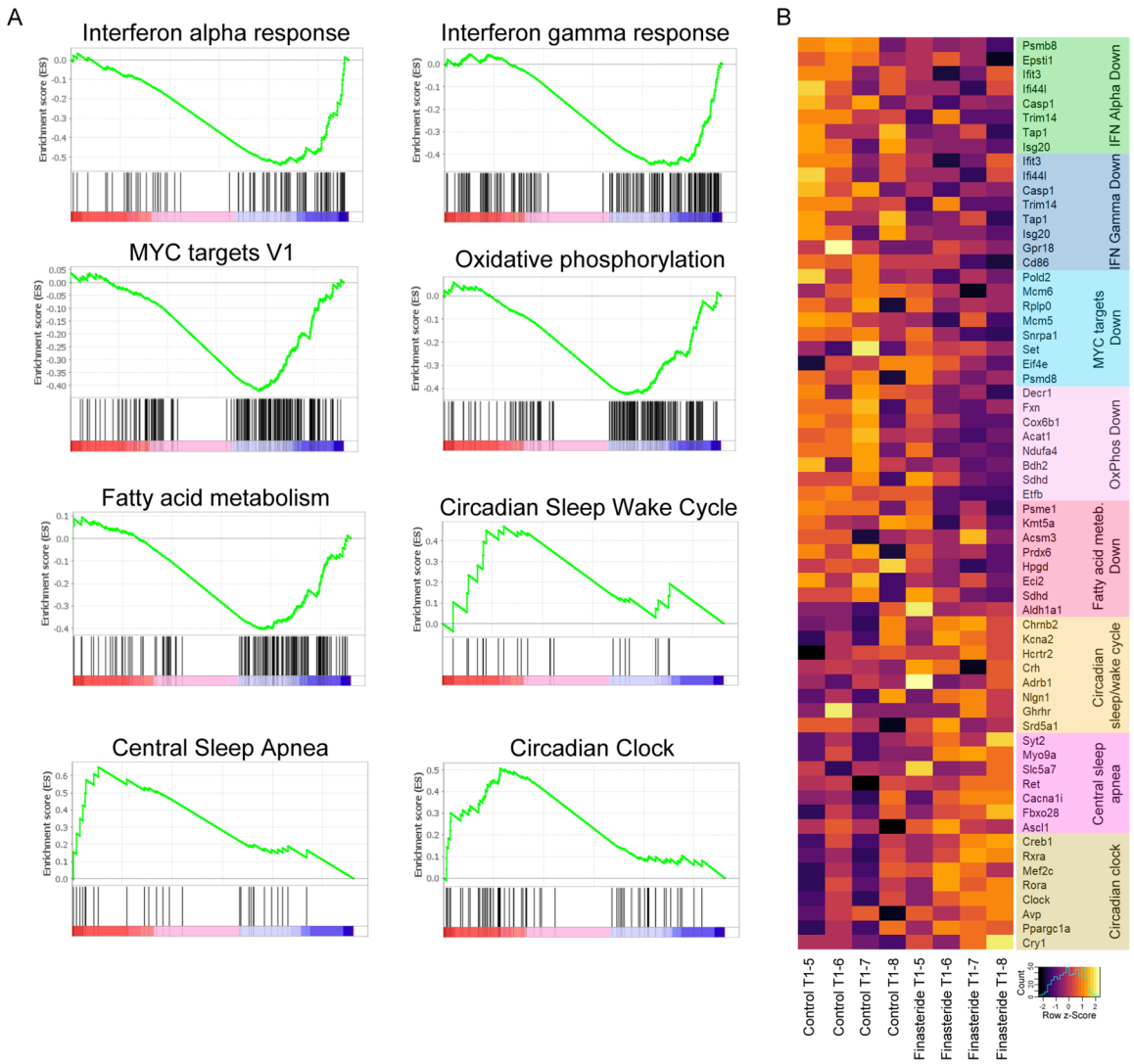

Fig. 3 **A** GSEA plots showing positive and negative enrichment of specific gene-sets in Hippocampus at T1. **B** Heatmap reporting the leading genes associated with the GSEA shown in panel A sets in Hippocampus at T1 in control and Finasteride-treated rats. $n = 4$ for each experimental group

mechanisms has been linked to alterations in the nervous system, such as demyelination, oxidative stress, and neuroinflammation [54].

Notably, upregulated genes were also identified upon treatment with finasteride. Among these, it is interesting to discuss *HCRT*. This gene encodes a hypothalamic neuropeptide precursor protein that gives rise to two mature neuropeptides, orexin A and orexin B. These two molecules play a significant role in the regulation of sleep-wakefulness [55]. Indeed, orexin system deficiency is associated with narcolepsy in animal models [56, 57] and in human [58–60]. Accordingly, treatment with orexin caused wakefulness and suppressed sleep in animal models [61–63]. In addition, alteration in orexin system is also associated with psychiatric disorders. For instance, hyperactivity of the system is related to acute stress reactions, depression, and anxiety-like behavior [55]. In this context, we also reported upregulation of myristoylated alanin-rich C-kinase (*MARCKSL1*). As demonstrated in transgenic mice, overexpression of this gene is associated with anxiety-like behavior [64]. In addition, other genes upregulated after finasteride treatment in the hypothalamus, like *VGF* and *IRF2BPL*, are associated with neurological disorders. The protein encoded by *VGF* is exclusively synthesized in neuronal and neuroendocrine cells [65, 66]. Mice overexpressing *VGF* showed behavioral abnormalities, such as hyperactivity, memory impairment, lower sociality, and higher depressive state, as well as morphological alterations, like smaller brain weight, expansion of the lateral ventricle, striatal morphological abnormalities [67]. Alterations in *IRF2BPL* levels has been associated with

neurological phenotypes [68, 69] and with major depressive disorder [70]. Altogether, these data indicate that genes modulated by treatment with finasteride in the rat brain are potentially linked to some of the side effects observed in patients during the drug treatment. In particular, the closer relationship seem to be with psychiatric and neurological domains (i.e., depression, anxiety, disturbance in memory and attention, sleep disturbance). This is further confirmed by the GSEA we performed in the hypothalamus and hippocampus. As reported here, the WNT_BETA_CATENIN_SIGNALING hallmark is significantly enriched by the finasteride treatment in both brain areas considered. An increase in WNT/β-catenin signaling has been reported to be associated with disturbance in circadian rhythms and sleep [71]. Moreover, in the hippocampus, after finasteride treatment we also observed a significant decrease in GSEA hallmarks, such as the OXIDATIVE_PHOSPHORYLATION, MYC_TARGETS_V1, INTERFERON_ALPHA_RESPONSE, E2F_TARGETS, and FATTY_ACID_METABOLISM, suggesting mitochondrial dysfunction, oxidative stress, neuroinflammation and impairment in synaptic plasticity that are important features of neurodegeneration and mood disorders [72–75]. Interestingly, a decrease in the hallmarks OXIDATIVE_PHOSPHORYLATION, MYC_TARGETS_V1, INTERFERON_ALPHA_RESPONSE, and FATTY_ACID_METABOLISM was still present at finasteride withdrawal, suggesting persistence of the side effects induced by the drug. Dysregulated neuroinflammation, impaired synaptic plasticity, as well as altered microglial activation, may be also suggested by a decrease in the INTERFERON_GAMMA_RESPONSE hallmark that was observed in the hippocampus upon withdrawal of finasteride [76–79]. Interestingly, in this brain area we also reported an enrichment in HP_CENTRAL_SLEEP_APNEA, REACTOME_CIRCADIAN_CLOCK, and GOBP_CIRCADIAN_SLEEP_WAKE_CYCLE hallmarks further suggesting a dysregulation of gene networks involved in sleep and mood disorders, as well as in cognitive processes [80, 81].

In conclusion, the data obtained here suggest interesting gene targets that could be related to some of the side effects observed during finasteride treatment and withdrawal. Therefore, these data may provide an interesting background for future experiments addressed to confirm the pathological role of these genes in this experimental model, exploring the impact in their signaling pathways, and evaluating possible therapeutic strategy able to counteract their pathological effects.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s40618-024-02345-y.

Author contribution The study was designed by SG and RCM. SG, LC, and SD contributed to data acquisition and interpretation, and conducted the experiments. RP was the biostatistician that performed and supervised the statistical analysis. The manuscript was written by SG, RP, and RCM. All the authors approved the final version of the manuscript before submission.

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Data availability Datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval The study procedure was approved by the Ethics Committee of Università degli Studi di Milano, Italy (authorization 1083/2015-PR).

Informed consent For this type of study, consent is not required.

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