THE EFFECT OF FINASTERIDE IN TOURETTE SYNDROME: RESULTS OF A CLINICAL TRIAL

Settore scientifico disciplinari di afferenza
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1. INTRODUCTION

The enzyme steroid 5α reductase (S5αR) catalyzes the conversion of Δ⁴-3-ketosteroid precursors - such as testosterone, progesterone and androstenedione - into their 5α-reduced metabolites. Although the current nomenclature assigns five enzymes to the S5αR family, only the types 1 and 2 appear to play an important role in steroidogenesis, mediating an overlapping set of reactions, albeit with distinct chemical characteristics and anatomical distribution. The discovery that the 5α-reduced metabolite of testosterone, 5α-dihydrotestosterone (DHT), is the most potent androgen and stimulates prostatic growth led to the development of S5αR inhibitors with high efficacy and tolerability. Two of these agents, finasteride and dutasteride, have received official approval for the treatment of benign prostatic hyperplasia and are being tested for prevention of prostate cancer. Finasteride is also approved for male-pattern alopecia and has been shown to induce very limited side effects. Over the last decade, converging lines of evidence have highlighted the role of both 5α-reduced steroids and their precursors in brain neurotransmission and behavioral regulation. Capitalizing on these premises, we and other groups have recently investigated the role of S5αR in neuropsychiatric disorders. Our preliminary data suggest that S5αR inhibitors may elicit therapeutic effects in a number of disorders associated to dopaminergic hyperreactivity, including Tourette syndrome, psychotic disorders and impulse control disorders.

In this thesis I introduce emerging preclinical and clinical evidences related to these effects, including an open label study of efficacy and tolerability of 5-alpha reductase inhibitors in a sample of patients affected by Tourette Syndrome. It further discusses
some of the potential mechanisms underlying the role of S5αR in the pathophysiology of mental disorders

### 1.1 General characteristics of steroid 5α-reductase

Steroid 5α-reductases (S5αRs; 3-oxo-5-α-steroid-4-dehydrogenases; E.C.1.3.99.5) are a family of enzymes catalyzing the saturation of the 4,5 double bond of the A ring of several Δ⁴-3-ketosteroid substrates, including progesterone, deoxycorticosterone, corticosterone, aldosterone, androstenedione and testosterone (Fig.1).

![Scheme of the reaction catalyzed by steroid 5α-reductase (S5αR).](image)

### Table: Substrates and Products

<table>
<thead>
<tr>
<th>SUBSTRATE</th>
<th>(-R_1)</th>
<th>(-R_2)</th>
<th>(-R_3)</th>
<th>PRODUCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOLEST-4-EN-3-ONE</td>
<td>(-H)</td>
<td>(-\text{CH}_3)</td>
<td>(-\text{CHCH}_3\cdot(\text{CH}_2)_n(\text{CH}_3)_2)</td>
<td>5α-CHOLESTAN-3-ONE</td>
</tr>
<tr>
<td>PROGESTERONE</td>
<td>(-H)</td>
<td>(-\text{CH}_3)</td>
<td>(-\text{CO-CH}_3)</td>
<td>5α-DIHYDROPROGESTERONE</td>
</tr>
<tr>
<td>DEOXYCORTICOSTERONE</td>
<td>(-H)</td>
<td>(-\text{CH}_3)</td>
<td>(-\text{CO-CH}_3\cdot\text{OH})</td>
<td>5α-DIHYDRODEOXYCORTICOSTERONE</td>
</tr>
<tr>
<td>CORTICOSTERONE</td>
<td>(-\text{OH})</td>
<td>(-\text{CH}_3)</td>
<td>(-\text{CO-CH}_3\cdot\text{OH})</td>
<td>5α-DIHYDROCORTICOSTERONE</td>
</tr>
<tr>
<td>ALDOSTERONE</td>
<td>(-\text{OH})</td>
<td>(-\text{CHO})</td>
<td>(-\text{CO-CH}_3\cdot\text{OH})</td>
<td>5α-DIHYDROALDOSTERONE</td>
</tr>
<tr>
<td>ANDROSTENEDIONE</td>
<td>(-H)</td>
<td>(-\text{CH}_3)</td>
<td>(=\text{O})</td>
<td>5α-ANDROSTANEDIONE</td>
</tr>
<tr>
<td>TESTOSTERONE</td>
<td>(-H)</td>
<td>(-\text{CH}_3)</td>
<td>(-\text{OH})</td>
<td>5α-DIHYDROTESTOSTERONE</td>
</tr>
</tbody>
</table>

**Fig.1** Scheme of the reaction catalyzed by steroid 5α-reductase (S5αR). NADP+/NADPH: nicotinamide adenine dinucleotide phosphate (in reduced and oxidized form). The atomic numbering (C1-C17) and ring nomenclature (A-D) refer to the cyclopentanoperhydrophenantrene ring system.
This process, which requires nicotinamide adenine dinucleotide phosphate (NADPH) as a co-factor, consists in the formation of an enolate intermediate on the C3 position of the substrate ketosteroid, which consequently results in the protonation of C4 and direct transfer of a hydride ion from NADPH to C5 [1-3]. The reaction catalyzed by S5αR increases the susceptibility of the carbonyl group in C3 to be reduced by 3α-oxysteroid dehydrogenase (3α-HSD) (Fig. 2) or 3β-hydroxysteroid dehydrogenase (3β-HSD), or conjugated with hydrophilic moieties, such as sulfate and glucuronyl groups, in order to facilitate their elimination. In addition to its role in the regulation of steroid catabolism, 5α reduction is the rate-limiting step in the metabolism of non-aromatic steroids, in view of its irreversibility under physiological conditions [4-6].

Several S5αR products play a pivotal role in a large number of physiological processes. For example, the metabolite of testosterone, 5α-dihydrotestosterone (DHT), is the most potent androgen hormone in vivo, and orchestrates the development of male external genitalia and secondary sex traits, as well as the trophism of the prostate [7-9]. The role of S5αR in male hormone metabolism also encompasses the conversion of the adrenal androgen androstenedione into its 5α-reduced metabolite androstanedione, which is further converted into androsterone by 3α-HSD [10] (Fig.2).

The functions of S5αRs are not limited to endocrine regulation. Several 5α-reduced steroids play central roles in the modulation of key physiological functions across different organs and systems. In the eye, the metabolite of cortisol, 5α-dihydrocortisol, plays a role in the production of aqueous humor [11]. In the kidney, 5α-dihydroaldosterone exerts a potent natriuretic function [12]. In the liver, the S5αR-mediated conversion of cholestenone into cholestanone is a fundamental step for the synthesis of cholestanol [13, 14]; moreover, the glucocorticoids 5α-dihydrocorticosterone (3α,5α-THB) and 3α,5α-tetrahydrocorticosterone (3α,5α-THB)
(see Fig.2) are posited to regulate glucose metabolism [15]. Finally, in the brain, the 5α-reduced metabolites of progesterone and deoxycorticosterone, 5α-dihydroprogesterone (DHP) and 5α-dihydrodeoxycorticosterone (5α-DHDOC), are respectively converted by 3α-HSD in 3α,5α-tetrahydroprogesterone (allopregnanolone, AP) and 3α,5α-tetrahydrodeoxycorticosterone (3α,5α-THDOC), which modulate the behavioral reaction to environmental stress by activating the γ-amino-butyric acid (GABA)A receptor [16, 17].
Fig. 2 Schematization of the role of S5αR (steroid 5α-reductase) in steroidogenesis. Metabolic changes in steroid configurations are represented in the same color as the enzymes (boxes) catalyzing the reactions. Enzymes: 3β-HSD: 3β-hydroxysteroid dehydrogenase; 3α-HSD: 3α-hydroxysteroid dehydrogenase; 17β-HSD: 17β-hydroxysteroid dehydrogenase; CYP11B2: Aldosterone synthase; CYP11B1: Steroid 11-β-hydroxylase; Steroid 11-β-hydroxylase; CYP21A2: Steroid 21-hydroxylase; CYP17A1: 17α-hydroxylase/17,20 lyase. Steroids: 5α-DHALdo, 5α-dihydroaldosterone; 3α,5α-THALdo, 3α,5α-tetrahydroaldosterone; 5α-DHB, 5α-dihydrocorticosterone; 3α,5α-THB, 3α,5α-tetrahydrocorticosterone; DOC, deoxycorticosterone; 5α-DHDOC, 5α-dihydro deoxycorticosterone; 3α,5α-THDOC, 3α,5α-tetrahydro deoxycorticosterone; DHP, 5α-dihydroprogesterone; AP, 3α,5α-tetrahydroprogesterone (allopregnanolone); DHEA,
The first functional characterization of 5α-reduction processes was reported in the early 1950s by several independent groups of researchers, in reference to the conversion of deoxycorticosterone and androgenic 3-ketosteroids into their 5α-reduced metabolites [18, 20]. The enzyme responsible for this reaction was therefore termed Δ⁴⁻⁵ α-hydrogenase [21]. In the following years, the identification of similar reactions for other steroids, such as cortisone [22], cholestenone [23] and progesterone [24] led to a plethora of alternative nomenclatures and classifications, based on the conceptual premise that each metabolic process could be catalyzed by a different substrate-specific enzyme [25].

The current genetic, molecular and enzymological evidence on S5αR, however, has greatly challenged this hypothesis; while multiple types of S5αR enzymes have actually been discovered, the two main types subserving a key role in steroidogenesis (named S5αR1 and S5αR2) appear to mediate an overlapping spectrum of reactions [26]. The characterization of different S5αR types proved an extremely difficult task, in view of the numerous technical problems posed by the molecular analysis of S5αR, which requires denaturating detergents for its purification from cell membranes (for a review, see [26]). The identification of S5αR1 and S5αR2 was finally allowed by the cloning of their cDNA [27-30], which paved the way for a number of investigations on the biochemical characteristics and anatomical distribution of these enzymes (for a review, see [26]).

Our knowledge on the morphological, functional and evolutionary relations among different genes and proteins is currently undergoing a general reassessment, in view of novel information provided by high-resolution sequence maps of human genome [31, 32] and the introduction of novel technologies for large-scale, high-throughput cloning [33, 34] and characterization/ modeling of proteins [35, 36]. The employment of these tools has recently allowed to enlarge the family of S5αRs, initially limited to SRD5A1
and \textit{SRD5A2} genes and their products (S5αR1 and S5αR2, respectively), to three additional homologous genes: \textit{SRD5A3} (previously known as \textit{SRD5A2}-like), which encodes for a polypropin reductase conventionally termed S5αR3 [37]; \textit{TECR} (also called synaptic glycoprotein 2 or \textit{GPSN2} in relation to its ortholog first identified in zebrafish), encoding for the enzyme trans-2,3-enoyl-CoA reductase; and \textit{TECR}-like (\textit{TECRL}, alternatively named \textit{GPSN2}-like or \textit{SRD5A2}-like 2).

Although the apparently negligible contribution of S5αR3 and TECR to steroidogenesis has prompted some authors to question the meaning of the current nomenclature [38], phylogenetic analyses have revealed that all the genes in the \textit{SRD5A} family derive from a common ancestor, which underwent duplication in very early evolutionary stages (eukaryotic organisms), leading to the generation of three subfamilies: \textit{SRD5A1/2}, \textit{SRD5A3} and \textit{TECR/TECRL} [39].

In contrast, the differentiation of S5αR1 and S5αR2 (as well as TECR and TECRL) from a common progenitor occurred much later, likely in early chordates [39]. In fact, both enzymes are found in tunicates, fish, amphibians, birds and mammals - including rodents and non-human primates -, but not in nematodes [39-43]. The characteristics of these genes and their products are described below and partially schematized in Tables 1 and 2 for humans and rats, respectively.

\textit{S5αR1}

The first isolated S5αR isoenzyme, S5αR1 is encoded by the gene \textit{SRD5A1}, located in the locus 5p15 of the human genome. The gene is organized in 5 exons and 4 introns, and encodes for a highly lipophilic, non-glycosilated protein, featuring 5 predicted transmembrane helices. Although the enzyme has not been crystallized to date,
mutagenesis studies have ascertained that the specific characteristics of substrate and inhibitor affinity are conferred by an internal segment between amino acids 26 and 29 [44, 45], located in the first transmembrane segment of the molecule. The protein is typically found in the lipidic bilayer of the endoplasmic reticulum membrane, frequently in perinuclear localization [46, 47].

<table>
<thead>
<tr>
<th>Gene</th>
<th>S5αR1</th>
<th>S5αR2</th>
<th>S5αR3</th>
<th>TECR</th>
<th>TECRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>5p15</td>
<td>2p23</td>
<td>4q12</td>
<td>19p13</td>
<td>4q13</td>
</tr>
<tr>
<td>Length (b)</td>
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<td>56,385</td>
<td>25,458</td>
<td>36,411</td>
<td>131,002</td>
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<tr>
<td>Protein size (aa)</td>
<td>259</td>
<td>254</td>
<td>319</td>
<td>308</td>
<td>363</td>
</tr>
<tr>
<td>Transmembrane helices</td>
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<td>4</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Protein weight (Da)</td>
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<td>28,393</td>
<td>36,521</td>
<td>36,034</td>
<td>42,009</td>
</tr>
<tr>
<td>Optimal pH</td>
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<td>5-5.5</td>
<td>6.9</td>
<td>7</td>
<td>?</td>
</tr>
<tr>
<td>Affinity for testosterone</td>
<td>Km = 1.7 μM</td>
<td>Km = 0.2 μM</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Affinity for progesterone</td>
<td>Km = 1.3 μM</td>
<td>Km = 0.2 μM</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Affinity for NADPH</td>
<td>Km = 3-5 μM</td>
<td>Km = 7-10 μM</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

**Table 1.** Characteristics of genes and proteins of the human S5AR family. Molecular and enzymological details unknown to date are indicated with a question mark. The number of transmembrane helices is predicted based on the primary sequence, but not based on actual experimental findings. Data are taken from OMIM, Uniprot, Unigene, Proteobank and BRENDA databases.
S5αR1 is expressed in cells and structures of ectodermal origin, such as epidermal keratynocytes, melanocytes, sebaceous and sweat glands, neurons of central and peripheral nervous systems and adrenal glands [44, 48-51]. In addition, the enzyme has been detected in fibroblasts, hepatocytes, muscle fibers [44, 52, 53] and several other organs, including prostate, lung, colon and kidney [26, 54, 55].

In the adult brain, S5αR1 is the prevalent isoenzyme [26, 42, 56-61], and is therefore conjectured to account for the largest part of the 5α-reduction reactions occurring within neurosteroidogenic pathways [57, 62-64]. Nonetheless, the pattern of localization of S5αR1 in the central nervous system remains partially elusive, in view of divergent results on its cell expression across different species. In mice, the transcript of the gene has been primarily detected in neurons across the mitral cell layer of the olfactory bulb; the pyramidal cells of the layers 2,3 and 5 in the neocortex; the pyramidal and granule cells in CA1, CA3 and dentate gyrus of the hippocampus; the output neurons of

<table>
<thead>
<tr>
<th>Gene</th>
<th>S5αR1</th>
<th>S5αR2</th>
<th>S5αR3</th>
<th>TECR</th>
<th>TECRL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
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<td>6q13</td>
<td>14p11</td>
<td>19q11</td>
<td>14p21</td>
</tr>
<tr>
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<td>15,044</td>
<td>26,688</td>
<td>73,848</td>
</tr>
<tr>
<td>Protein size (aa)</td>
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<td>254</td>
<td>330</td>
<td>308</td>
<td>361</td>
</tr>
<tr>
<td>Homology with human gene</td>
<td>72.5%</td>
<td>77.6%</td>
<td>82.4%</td>
<td>98.7%</td>
<td>85.1%</td>
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<tr>
<td>Transmembrane helices</td>
<td>5</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Protein weight (Da)</td>
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<td>28,772</td>
<td>38,092</td>
<td>36,123</td>
<td>42,057</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of genes and proteins of the S5AR family in rat (Rattus norvegicus). The number of transmembrane helices is predicted based on the primary sequence, but not based on actual experimental findings. Data are taken from OMIM, Uniprot, Unigene, Proteobank and BRENDA databases.
basolateral amygdala; the medium spiny neurons in the striatum; the dorsomedial and reticular nuclei neurons in the thalamus; the Purkinje cells of the cerebellar cortex. Conversely, no expression in glial cells was detected [65]. In preliminary studies, the authors reportedly found a virtually identical pattern of expression in the rat brain. In striking contrast with these findings, other groups of authors found S5αR1 immunoreactivity only in glial and ependymal cells of the rat [48, 66, 67], with high expression in the cortex, thalamus, hypothalamus, circumventricular organs and cerebellum.

Post-mortem studies in humans have revealed the presence of S5αR1 in the temporal cortex, hippocampus, hypothalamus, pons and cerebellum [57, 68]. However, a complete analysis of the total cerebral expression of S5αR enzymes in the human brain is still lacking.

Few data are known on the transcriptional and translational regulation of SRD5A1 and its product. In rats, the promoter of the gene has been identified in the 5′ upstream region of the gene, and consists of a bidirectional sequence with Sp1-dependent activity [69]. In cell cultures, expression of the gene products has been shown to be dependent on a number of factors, such as nerve growth factor [70], stimulation of adrenergic and 5-HT2A serotonin receptors [71, 72], and inhibition of histone deacetylase [73]. Of note, DHT, but not testosterone or progesterone, has been shown to enhance S5αR1 expression [74, 75]. This unusual mechanism of feed-forward stimulation may reflect specific developmental requirements [26], possibly in relation to mechanisms of male sexual maturation.

Our current insight into the specific functions of S5αR1 is limited. Two different polymorphisms of the SRD5A1 gene associated with lower enzymatic activity have
been linked to higher risk of peripheral arterial disease [76] and polycystic ovary syndrome [77]. Nevertheless, no pathological phenotype has been associated to a nonsense mutation of this gene in humans. Indeed, the congenital deficiency of S5αR1 may not result in major abnormalities, as suggested by the lack of overt physical and sexual impairments in transgenic male mice with a knockout (KO) mutation for the Srd5a1 gene [41]. Female mice with this mutation, however, were reported to display parturition defects and reduced litter size, plausibly due to their high expression of estrogens, which may have caused deficits in the ripening process of the cervix during late pregnancy [78, 79]. Interestingly, recent preclinical evidence has highlighted a key role for S5αR1 in behavioral regulation and stress response. Short-term stressors have been shown to enhance the synthesis of neurosteroids, such as AP and 3α,5α-THDOC [80-84]. This phenomenon may be partially linked to the increases in brain 5AR1 observed following acute stress [85-87] or as a long-term consequence of prenatal stress [88]. Conversely, prolonged, inescapable stress has been shown to induce a reduction in brain S5αR1 expression [89-91]. These findings suggest that 5AR1 activity may play a physiological role in stress response through regulation of neurosteroid synthesis. In consideration of the role of AP and 3α,5α-THDOC in the modulation of the signaling of (GABA)A receptor [16, 17], it is possible that changes in S5αR1 may have profound repercussions on the functional regulation of this receptor and its broad set of function in emotional regulation. Accordingly, changes in neurosteroid levels in the brain of rats subjected to stress have been associated with plastic changes in GABA_A receptors activity [92]. In relation to this concept, it is worth noting that S5αR1 KO male mice fail to exhibit a reduction in anxiety- and depression-like behavior following administration of progesterone [93]; likewise, they display no aggressive responses to testosterone [93].
The gene *SRD5A2*, which encodes for S5αR2, is located in chromosome 2p23 and results from the duplication of a common ancestor with *SRD5A1*, as denoted by the common exon-intron organization (5 exons and 4 introns) and a relatively high level of identity shared by both genes [39]. This similarity is also reflected in other matching aspects of S5αR1 and S5αR2, such as the common localization in the membrane of endoplasmic reticulum and the overlapping enzymatic actions.

The elements of divergence between the two isoenzymes are related to their biochemical and physiological characteristics, such as optimal pH, substrate specificity and localization [26, 28, 30, 42, 94-96]. For example, S5αR2 has a higher affinity for testosterone and progesterone than S5αR1 (see Table 1), and is responsible for the synthesis of about two thirds of circulating DHT [53]. The substrate specificity for S5αR2 has been linked to the amino acid sequence 15-17 [45], probably located in the first of the four predicted transmembrane helices. Another key element that has been extensively used to differentiate the two isoenzymes is their pH optimum in vitro (see Table 1). This criterion, however, may not reflect different properties of the enzymes in their native states; in fact, several lines of immunocytochemical and enzymological evidence suggest that both enzymes may have neutral pH optima [28, 29, 42, 57].

The pattern of distribution of S5αR2 is also remarkably distinct from that of S5αR1. In general, S5αR2 is predominantly expressed in tissues and organs of the male urogenital tract (including prostate, epididymis, testicles and seminal vesicles), as well as genital skin, hair follicles and liver [53]. In the brain, S5αR2 transcript has been mainly found in early developmental stages [97]. In the adults, S5αR2 expression is posited to be dependent on androgen signaling [98]. Although a complete
characterization of S5αR2 expression in brain regions has never been reported to date, the enzyme (or its transcript) has been detected in several regions, including hypothalamus, prefrontal cortex and nucleus accumbens [91, 99, 100].

The main physiological function of SRD5A2 has been linked to the development of male genitalia. Indeed, the congenital deficiency of SRD5A2 results in pseudovaginal perineoscrotal hypospadias, a rare form of male pseudohermaphroditism characterized by normal urogenital tracts but underdeveloped external male genitalia [101] and prostate. In mice, the knockout of this enzyme resulted in a different phenotypical presentation, with fully formed internal and external genitalia, but a reduced size of the prostate and seminal vesicle [102]. This interesting finding underscores the relative importance of S5αR1 and 2 in the synthesis and metabolism of androgens is likely to be variable across different species, and advocates caution on the translational value of research on S5αR isoforms in murine models (a thorough analysis of this important concept can be found [26]).

Further evidence about the role of SRD5A2 gene comes from association studies between some of its polymorphic variants and the risk for certain disorders. Of the 28 different polymorphic mutations of this gene, one of the most prevalent is the substitution of valine with leucine in position 89 (V89L). This haplotype (presumably resulting in higher enzymatic activity) has been associated with higher vulnerability for polycystic ovary syndrome as well as higher risk (and/or aggressiveness) of several malignant tumors, including prostate, ovarian and early-onset breast cancer [103-107]. Furthermore, this polymorphic variant has been linked to increased sperm motility, as well as reduced risk of hypospadias [109].
The substitution of alanine with threonine at codon 49 (A49T) has also been associated with clinical conditions typically linked to higher enzyme activity, such as increased risk of prostate cancer [69, 110, 111] and increased sperm concentration [108]. However, the same mutation has also been found in association to alterations indicative of reduced enzymatic function, such as low-severity hypospadia and decreased risk of vertex balding [112]. Finally, recent evidence suggests that this polymorphic variant may also be associated with higher risk of autism in boys [113].

S5αR3

S5αR3 was discovered in 2007 through the analysis of the genome-wide gene expression profile of hormone-refractory prostate cancer cells [114]. While this enzyme is reportedly able to slowly convert testosterone into 5α–dihydrotestosterone [115], its main physiological function has been revealed to be linked to the reduction of polyprenol to dolichol [116], a key process that enables the N-glycosylation of proteins. The localization of S5αR3 has been described as ubiquitous throughout the organism [116], but very little is currently known on the cell- and tissue-specificity of its expression.

In mice, SDR5A3 knockout has been shown to lead to embryonic death by day E12.5. The mutation is often conducive to cardiac and neural malformations [116]. In humans, the congenital deficiency of this enzyme results in a complex syndrome featuring mental retardation, ocular abnormalities (such as optic disc atrophy and coloboma, with loss of vision), cerebellar malformations and coagulation deficits [116, 117]. Nevertheless, these patients do not feature apparent alterations in their steroid levels, suggesting that this enzyme may only play an accessory role in steroidogenesis [116].
TECR and TECRL

TECR is posited to catalyze the last step for the elongation of fatty acids, consisting in the reduction of the double bond of a trans-enoyl coA [118]. A second, shorter isoform of TECR (157 aa) has been reported, featuring the absence of the sequence between aa 20 and 170, but its functional significance remains unknown. While it is likely that TECRL may catalyze a similar enzymatic reaction, its substrate remains unknown to date. No information is available on the ability of these enzymes to process the 5α reduction of ketosteroids.

To the best of our knowledge, the phenotype resulting from the deficiencies of TECR and TECRL enzymes remains unknown. Should such mutations be viable, it is logical to envision that their phenotypic consequences may be akin to those observed in other syndromes related to the deficiencies of other enzymes implicated in the fatty acid synthesis cascade, such as the Acyl-Co A dehydrogenases. These conditions typically result in hypoglycemia, muscle and heart problems and are often lethal [119-122].

1.2 S5αR inhibitors

The development of inhibitors for S5αR was prompted by the discoveries that DHT regulates prostate trophism and S5αR2 deficiency resulted in a lower prevalence of male-pattern alopecia. The first class of inhibitors developed for clinical use, 4-azasteroids, featured a cyclopentanoperhydrophenanthrene nucleus with the substitution of the carbon atom in C4 position with nitrogen. Among these compounds, the compound 4-MA (17β-N,N-diethylacarbamoyl-4-methyl-4-aza-5α-androstan-3-one) was initially selected in view of its high potency and affinity for both S5αR1 and S5αR2
However, the hepatotoxicity, micromolar affinity for the androgen receptor, and lack of selectivity over 3β-HSD [124] of this agent led to its rapid withdrawal from human studies. Subsequent research on the characteristics of 4-azasteroids paved the way for the development of finasteride [MK906; 17β-(N-tert-butylcarbamoyl)-4-aza-5α-androst-1-en-3-one] (Fig.3), an unsaturated derivative of 4-MA, with relatively high selectivity for S5αR2 over S5αR1 [125, 126]. This compound proved to be highly effective in the control of DHT levels and the progression of benign prostatic hyperplasia (BPH), and was therefore approved by the US Food and Drug Administration (FDA) for this disorder in 1992. Five years later, the same agency approved low-dose finasteride for the therapy of male-pattern hair loss.

The inhibition of S5αR mediated by finasteride is based on the transfer of a hydride group from NADPH to the Δ^1-double bond of the drug. This process results in the formation of a covalent NADP-dihydrofinasteride adduct, which potently binds to the free S5αR enzyme, competing with its endogenous substrates [127]. The extremely low dissociation constant of this compound for S5αR2 ($K_i = 3–5$ nM), however, results in a very slow turnover of the enzyme-adduct complex ($T_{1/2} \approx 30$ days), rendering the inhibition virtually irreversible [26]. In the 1990s, the quest for novel S5αR inhibitors led to the development of dutasteride (GG745; (5α,17β)-N-(2,5-bis(trifluoromethyl)-phenyl)-3-oxo-4-azaandrost-1-ene-17-carboxamide) [128; 129] (Fig.3), a 4-azasteroid with much higher potency than finasteride in inhibiting both S5αR1 and S5αR2 (100 and 3 times greater, respectively). Dutasteride has been approved for BPH in 2002 and has been shown to induce therapeutic outcomes comparable to finasteride in this disorder [130, 131].
Although finasteride and dutasteride are the only approved S5αR inhibitors, novel promising classes of drugs are emerging, such as the steroidal 3-carboxylic acid epristeride, a potent, irreversible inhibitor of S5αR2 [43]; the selective S5αR1 inhibitor MK-386 [132, 133]; novel non-steroidal molecules, such as FR146687 [134] and FK-143 [135] (for a complete review on the topic see [136]).

In addition, the extract of the berries from the Saw palmetto (*Serenoa repens*), a palm tree native to the Southern Atlantic coast, has recently emerged as a popular treatment for prostate enlargement both in the United States and Europe. The extract is a purified mixture of fatty acids (such as lauric, oleic, myristic, palmitic and linoleic acid) and sterols. As certain polyunsaturated fatty acid have been shown to inhibit S5αRs [137], it has been hypothesized that the therapeutic properties of the extract may be contributed by a blockade of these enzymes [10, 138].
**Therapeutic indications.**

A large body of data from randomized clinical trials has demonstrated the efficacy of S5αR inhibitors in the treatment of BPH. Several clinical trials have shown the long-term efficacy of finasteride (5 mg/day) in alleviating symptoms, improving urinary flow rates, reducing prostate volume and decreasing the risk of acute urinary retention and the need for surgical intervention [139-141].

Additionally, finasteride has been shown to significantly reduce the risk of prostate cancer (24.4% vs 18.8% in placebo group), irrespective of age, ethnicity, family history and baseline PSA levels [142, 143]. It is worth noting that a higher incidence of high-grade prostate cancer (Gleason scores 7-10) (37% vs 22.2% with placebo) has also been found in finasteride-treated patients [144], although it is currently unknown whether such an increase may reflect confounding factors rather than an actual biological phenomenon. In view of the well-established growth stimulatory potential of DHT for the prostate, as well as the documented role of S5αR1 overexpression in the progression of prostate cancer [145], the dual inhibition mediated by dutasteride is expected to elicit greater beneficial effects than those observed with finasteride on prostate cancer, by slowing its progression or even causing its regression [141, 146]; this putative advantage, albeit supported by preclinical data [145], has yet to be corroborated by conclusive clinical evidence based on large-scale, long-term comparative studies.

Lower doses of finasteride are also indicated for the treatment of androgenetic alopecia, both orally (1 mg/day) or locally (by gel). The drug significantly diminishes the progression of the baldness and often stimulates new growth. The mechanism of action is based on the reduction of DHT levels, which play a key role in male-pattern hair loss
Interestingly, however, the drug has shown no beneficial effect in treating the disorder in postmenopausal women. Discontinuation of finasteride results in loss of any positive effects on hair growth within 12 months [149]. As increased S5αR activity is also conducive to female hirsutism [150], finasteride therapy has been proposed for this condition. The results have been promising, with efficacy comparable to antiandrogen agents, but no appreciable side effects [140, 151, 152]. It should be noted that finasteride is contraindicated in pregnant women, in view of the high risk of birth defects. Although no data are currently available on the excretion of S5αR inhibitors in milk, lactating mothers should also refrain from using finasteride, to avoid potential side effects on the sex development of their children.

Tolerability and peripheral side effect

Since its development and first clinical studies, finasteride was reported to elicit very limited side effects in volunteers and patients [153,154]. The high tolerability and safety of finasteride has been confirmed by several randomized clinical trials over very long follow-up periods [142, 155-157]. The most common side effects induced by finasteride, at the daily dose of 5 mg (recommended dosage for BPH) include decreased libido, ejaculatory disorders and erectile dysfunction, and are ascribed to the reduction in DHT levels as well as the increased estrogen synthesis (due to enhanced availability of testosterone and androstenedione for aromatization). The reported incidence of these adverse effects is variable across different studies, but is generally low (around 5%) and mainly reported during the initial stages of treatment. None of these side effects has been found to significantly interfere with therapeutic compliance, as indicated by comparable rates of treatment withdrawal between finasteride- and placebo-treated groups [149, 158]. Of note, in the PLESS (Proscar Long-Term Efficacy and Safety) trial
(on 3040 male BPH patients over 4 years), the drop-out rate was higher in the placebo group (42%) in comparison with the patients treated with finasteride (34%) [140].

In a small percentage of patients, finasteride has been shown to induce gynecomastia and breast tenderness [159, 160], likely due to enhancements in estrogen levels. The time of onset of gynecomastia (2-4 months) observed in the most of finasteride users is generally delayed relative to other sexual-related adverse events. Interestingly, gynecomastia tends to be more frequently unilateral in individuals treated with low doses of finasteride [161, 162]. Unlike other side effects, breast enlargement has reported to be long-lasting in several cases, even following finasteride withdrawal [159].

Other very rare side effects include hypersensitivity reactions, such as itch, skin rash, lips swelling or lid edema [163]. The cutaneous rash may be linked to the increased photosensitivity produced by certain steroids (such as 5β-reduced steroids), which would be disproportionately produced in the liver as a result of S5αR inhibition [164]. No significant side effects have been reported on other steroid-regulated metabolic processes, such as bone turnover, lipoprotein formation or hemoglobin production. Similarly, finasteride treatment does not result in any increase in the rate of serious adverse events [155].

Despite its higher potency and efficacy in blocking both S5αR1 and S5αR2 isotypes, dutasteride has been shown to induce a spectrum of side effects strikingly akin to those observed with finasteride. A 2-year study of dutasteride for BPH treatment revealed low rates of impotence, decreased libido, ejaculation disorder, and gynecomastia. Similarly to finasteride, these side effects were transient, with no statistically significant difference noted at 2 years [130]. The tolerability of dutasteride appears comparable to that of finasteride during long-term treatments [142, 155, 165-167].
Psychological effects.

In addition to its endocrine and metabolic effects, inhibition of S5αR results in a broad array of effects in emotional and cognitive regulation in rodents, which stem from the ensuing alterations in the profile of neuroactive steroids within cortical and limbic brain regions. While only few clinical studies have analyzed the psychotropic effects of S5αR inhibitors, numerous investigations in experimental animals have focused on the behavioral implication of finasteride treatment.

The best-characterized and most frequently investigated behavioral effect of pharmacological S5αR blockade in rodents is the suppression of the synthesis of AP and 3α,5α-THDOC, which leads to perturbed modulation of GABA<sub>A</sub> receptor function and altered responsiveness to environmental stimuli and stress [168-170]. Indeed, both AP and 3α,5α-THDOC have been shown to elicit anxiolytic-like actions in different animal models [171-173]. Furthermore, AP has been shown to regulate aggression and fear responses in male mice [174-177].

Systemic administration of finasteride has been shown to increase indices of anxiety-like behaviors in the open field and elevated plus maze paradigms and depression-like behavior in the forced swim test [178, 179]. Several brain regions, including hippocampus and amygdala, have been shown to play a role in these effects [178, 180, 181].

Finasteride may exert its effects on anxiety and mood by decreasing the levels of other 5α-reduced neurosteroids, such as androsterone and 3αdiol, which have been shown to exert anxiolytic and antidepressant properties [182-185].
The translational value of the preclinical investigations on finasteride's behavioral effects is partially limited by the fact that, in rats, finasteride has a high affinity for both S5αR1 and S5αR2 [44, 186]. This characteristic is strikingly at variance with the relative selectivity of finasteride for S5αR2 in humans, suggesting possible differences in the neuropsychological outcomes of this drug. Accordingly, several studies have even reported potential beneficial effects of finasteride on anxiety and mood in BPH patients, but this outcome is arguably influenced by their enhancement in quality of life due to the reduction of urinary symptoms [187].

Few studies have actually shown an association between finasteride administration and increased anxiety and depression in patients with BPH and androgenetic alopecia [188, 189]. In line with this concept, several studies have reported an inverse relationship between AP levels and anxiety and depression symptoms in patients [52, 190]; furthermore, antidepressant administration has been shown to significantly increase the low levels of AP found in patients with major depression [191-195].

The role of neurosteroids in mediating antidepressant effects, however, has been recently challenged [196, 197] in consideration of the lack of changes in neurosteroid concentrations in relation to the mood-enhancing effects of non-pharmacological antidepressant treatments, such as electroconvulsive therapy or repetitive transcranial magnetic stimulation[198-200].
1.3. **S5αR inhibitors as putative therapeutic agents for some neuropsychiatric disorders.**

Multiple lines of evidence suggest the implication of steroidal mediators in the pathophysiology of schizophrenia and other psychiatric disorders.

The conceptual premises for the employment of S5αR inhibitors in schizophrenia lie in several lines of preclinical and clinical evidence highlighting a role for neuroactive steroids in psychotic disorders. One of the most compelling evidence in favor of this link is the observation that the well-characterized gender differences in schizophrenia may be supported by different hormonal profiles between males and females [201-203]. In men, the onset of psychotic symptoms occurs generally earlier (in late adolescence / young adulthood), and with a more insidious presentation than in women; furthermore, men tend to display higher severity of psychotic manifestations, higher rate of negative symptoms and worse clinical outcome and functioning [201, 204-208]. This phenomenon has been largely ascribed to the protective role of estrogens.

Accordingly, women have been shown to have a higher vulnerability to psychotic manifestations during estrogen withdrawal, such as after the delivery of babies [209] or during menopause. In addition, several lines of evidence have recently shown that androgen precursors, such as testosterone and dehydroepiandrosterone (DHEA), may modulate the expression of psychotic phenomena. Despite a number of initial contradictory findings on the efficacy of these steroids as adjunctive therapies to standard antipsychotic drugs (see [203]), recent studies have highlighted both androgens as potential targets for schizophrenia therapy. Following preliminary studies reporting low levels of testosterone in patients with predominantly negative symptoms [210], recent clinical evidence has shown that serum total and free testosterone levels
are inversely correlated with the negative score of the Positive and Negative Syndrome Scale (PANSS) [211, 212]. In addition, testosterone augmentation has been documented to induce improvements in negative symptoms in a recent placebo-controlled, double blind trial on schizophrenia male patients [213].

High levels of DHEA have often been reported in schizophrenia [214], and have been highlighted as a potential predictor for poor cognitive function [215]. Of note, cortisol/DHEA ratio has been associated with a number of cognitive functions [216] and may serve as an index for responsiveness to antipsychotic treatment [217]. Moreover, DHEA was found to significantly attenuate antipsychotic-induced extrapyramidal symptoms and negative symptoms, as assessed through the Scale for the Assessment of Negative Symptoms (SANS), but not the PANSS scale [215, 218, 219]. Another promising neurosteroid target is pregnenolone (as well as its derivative pregnenolone sulfate). These molecules have been repeatedly proposed for the management of psychotic symptoms [220-227]. Indeed, in a recent double-blind, randomized study on 58 schizophrenia and schizoaffective disorder patients, addition of low doses of pregnenolone (30 mg/day) to standard antipsychotic treatments was found to significantly ameliorate positive symptoms, as assessed by the PANSS. In contrast, higher doses of pregnenolone (200 mg/day) did not elicit any significant effect vs placebo [228]. Both pregnenolone and DHEA levels were found to be higher in the cingulate and parietal cortex of subject with schizophrenia [229]. The effects of pregnenolone and DHEA (and their sulfates) may be related to their effects on neuronal excitability and synaptic plasticity, as well as neuroprotection (reviewed in [228, 230]).

In contrast with these findings, levels of the 5α-reduced neurosteroid AP were found significantly decreased in post-mortem samples from parietal cortex as compared to control subjects [229]. This complex scenario, featuring increased levels of certain
steroid precursors and possibly reduced concentrations of some of their metabolites may signify that certain steroidogenic enzymes are functionally altered in schizophrenia and other psychotic disorders. In line with this hypothesis, environmental stress, a major factor in the etiology of psychosis [231] has been shown to affect the synthesis and metabolism of several neurosteroids [80, 88, 232]. As stated above, for example, cogent preclinical evidence has documented that S5αR expression and function are affected by short-term and chronic stress. Neuroactive steroids have also been shown to modulate the responses of all the key neurobiological targets implicated in schizophrenia, such as dopamine, GABA, as well as the glutamate N-methyl-d-aspartate (NMDA) and σ receptors [16, 85, 233, 234].

Preclinical evidences.

Based on these premises it has been hypothesized that, in view of the central role of S5αR in the metabolism of testosterone as well as the synthesis of AP and other neurosteroids, the blockade of this enzyme may elicit therapeutic effects in schizophrenia. In particular, inhibition of S5αR may have resulted in decreased levels of 5α-reduced steroids and potential increase in the concentrations of their precursors, which may elicit beneficial effects in psychotic disorders. Furthermore, it has been speculated that, by increasing the availability of testosterone and androstenedione for aromatization, the effects of S5αR blockade may also include the neuroprotective and beneficial action of estrogens in psychosis [235-237].

The first studies on S5αR inhibitors were performed in rat models of schizophrenia, based on the stimulation of dopamine receptors with direct and indirect agonists. In addition to hyperlocomotion and stereotyped behavior (two well-validated assays to measure behavioral outcomes of dopaminergic activation, with high predictive validity
for typical and atypical antipsychotics), it has been focused on the model of prepulse inhibition (PPI) of the acoustic startle reflex, and its disruption mediated by dopamine agonists and NMDA antagonists [238].

PPI is the normal reduction in startle amplitude occurring when a startling stimulus is preceded by a weaker prepulse and functions as a measure of preattentional sensorimotor gating. This endophenotype has been extensively employed to model the well-documented gating deficits in schizophrenia and other deficits, also in view of the highly remarkable finding that psychotic patients display deficits in PPI of the blink reflex (reviewed in [239]). The high face and predictive validity of PPI as a tool for the assessment of neurobehavioral deficits related to schizophrenia is highlighted by the fact that, in animals, administration of dopaminergic agonists as well as other psychotomimetic agents significantly reduces this index, in a fashion sensitive to typical and atypical antipsychotic drugs [240].

In addition to schizophrenia, PPI deficits have also been found in other neuropsychiatric disorders related to dopamine dysregulation, such as mania [241] and Tourette syndrome [242, 243].

Recent results of preclinical studies showed that S5αR inhibitors such as finasteride and dutasteride, prevented the PPI deficits and other behavioral changes induced in rats by both d-amphetamine, a dopamine release enhancer, and apomorphine, a potent dopamine receptor agonist [238].

The potent antidopaminergic properties of S5αR inhibitors were not accompanied by the extrapyramidal symptoms commonly triggered by neuroleptics, such as haloperidol
In fact, even very high doses (1000 mg/kg) failed to produce cataleptic reactions in either the bar or the paw tests.

All the behavioral effects mediated by finasteride and other S5αR inhibitors were observed within a short time after administration, suggesting that the NSs responsible may function through non-genomic interactions. Indeed, many neuroactive steroids are known to influence behavioral and cognitive functions through fast-acting interplay with numerous neurotransmitter systems [244].

Subsequent studies showed that the effects of finasteride were observed in both male and female rats (Fig 4) and were not affected by orchiectomy in adult males (Fig 5 and Fig.6), suggesting that the mechanisms mediating the antipsychotic-like effects did not depend on gonadal androgens [Soc. Neurosci. 2010, abstract n° 665.23].
**Fig. 5.** Effects of systemic finasteride on startle reflex (A) and PPI (B) in sham-operated (SHAM) and orchiectomized (ORX) rats. Orchiectomy did non alter the ability of systemic FIN to prevent the PPI deficits induced by the dopamine (DA) receptor agonists apomorphine (Fig. 5a). Finasteride doses are indicated in mg/kg (I.P.). V, vehicle of finasteride; SAL, saline; APO, apomorphine (0.25 mg/kg, S.C.). Values are expressed as mean ± S.E.M. ***, P<0.001 vs rats treated with vehicle and saline (pre-treatment x treatment interaction); ###, P<0.001 vs rats treated with vehicle and APO. Main statistical effects are not indicated.

**Fig. 6.** Effects of systemic finasteride on startle reflex (A) and PPI (B) in sham-operated (SHAM) and orchiectomized (ORX) rats. Orchiectomy did non alter the ability of systemic FIN to prevent the PPI deficits induced by the dopamine (DA) receptor agonists d-amphetamine. VEH, vehicle of finasteride; SAL, saline; AMPH, d-amphetamine (2.5 mg/kg, S.C.). Values are expressed as mean ± S.E.M. **, P<0.01 vs rats treated with vehicle and saline (pre-treatment x treatment interaction); ###, P<0.001 vs rats treated with vehicle and AMPH. Main statistical effects are not indicated.
In view of the important contributions of the dopaminergic system in psychotic disorders, it has been investigated whether antipsychotic-like effects of finasteride were mediated by this system. This research revealed that local injection of finasteride into nucleus accumbens e medial prefrontal cortex - two key terminals of the mesolimbic and mesocortical pathways of the dopaminergic system - attenuated apomorphine induced PPI deficit. (fig 7) [Devoto et al submitted].

These findings are in agreement with previous data, showing that both regions may be central in the behavioral actions of finasteride and S5αR products [178, 245-247]. Moreover, both the nucleus accumbens and the medial prefrontal cortex are key substrates for the dopaminergic regulation of PPI (reviewed in [243]) and play a well-established role in the pathophysiology of schizophrenia. The impact of S5αR on the dopaminergic modulation of sensorimotor gating, however, does not appear to involve the direct participation of other forebrain regions, such as the dorsal caudate, basolateral amygdala and ventral hippocampus (fig 7) [Devoto et al submitted].
Fig. 7 APO-induced PPI deficits were reversed by local lateral FIN injections in medial prefrontal cortex (mPFC) and nucleus accumbens shell (nAcS), but not other regions involved in gating regulation, such as dorsal caudate, ventral hippocampus and basolateral amygdala. Effects of local finasteride (FIN) on startle reflex and PPI across medial prefrontal cortex (mPFC) (A-C), nucleus accumbens shell (NAcS) (D-F), dorsal caudate (DCau) (G-I), basolateral amygdala (J-L) and ventral hippocampus (VHip) (M-O). Localization of the cannulae is schematized in the left column. APO, apomorphine (0.25 mg/kg, S.C.). + and – refer to the presence of a drug or its vehicle (Ringer solution-DMSO for FIN, saline for APO). Values are expressed as mean ± S.E.M. *, P<0.05; **, P<0.01; ***, P<0.001 vs rats treated with Ringer-DMSO and saline (pre-treatment x treatment interaction); #, P<0.05; ##, P<0.01 vs rats treated with vehicle and APO; °, P<0.05 vs rats treated with FIN and saline.
Intracerebral administration of finasteride was not accompanied by changes in extracellular concentrations of dopamine in either region (Fig 8). These results suggest that the antipsychotic-like properties of finasteride are mainly related to post-synaptic processes [Devoto et al. submitted].

**Fig.8.** Time-related effects of finasteride (FIN, 0.5 µg/0.5 µl/side) injections in the medial prefrontal cortex (mPFC; left column) and nucleus accumbens shell (NAcS; right column) on local dopamine (DA) concentrations, by itself (A-B) or in combination with apomorphine (APO, 0.25 mg/kg, S.C.) . VEH, FIN’s vehicle (DMSO). The period corresponding to PPI testing is indicated by a bar alongside the time axis. Each time point represents the content of DA in the sample relative to the baseline. ***, P<0.05 vs baseline (Main effect for time; APO 0’ vs APO 40’).** Values are expressed as mean ± S.E.M.
Clinical evidences.

To date, there are only few reports on the effect of 5-alpha reductase inhibitors in neurological and psychiatric disorders. In a recent case report finasteride was tested in a male patient affected by chronic schizophrenia and unresponsive to standard antipsychotic therapies [248]. Over the first 24 days of experimental treatment with finasteride (5 mg/day), the patient exhibited a mild improvement in positive and negative symptoms, with no reported side effects. Following discontinuation of the medication, the patient experienced a precipitation of his psychotic symptoms, which led him to request the continuation of finasteride regimen [248].

Another recently documented case of a male adult affected by benign essential blepharospasm (BEB) responded poorly to botulinum toxin but exhibited a significant improvement of his dystonic symptoms in response to finasteride (5 mg/day) prescribed for concomitant BPH [249]. Nevertheless, the same therapy failed to induce noticeable improvements in other patients, underscoring that S5αR inhibition may address only a select subset of BEB patients [249]. Interestingly, this possibility mirrors the equivocal role of dopamine in this disorder, as activation of its receptors has been shown to either exacerbate or attenuate the severity of blepharospasm [250-255].

Finasteride-mediated effects on alcohol response were investigated in a placebo-controlled study on male and female “social drinkers” (i.e. reportedly drinking at least three times a week and three times on one occasion during the month prior the testing) found that a high dose of finasteride (200 mg over 24 h) reduced several self-reported stimulant effects of alcohol, as measured by the Central-Stimulant and Dynamic-Peripheral Subscales of the Alcohol Sensation Scale [256]. The biological mechanisms involved in these clinical implications are currently unknown.
Potential therapeutic role of S5αR inhibitors has been found also in impulse-control disorders as pathological gambling [Bortolato et al, submitted] occurring in response to dopamine-replacement therapies in Parkinson’s disease (PD). [257; 258]. Interestingly, both the prefrontal cortex and the ventral striatum have been shown to play a key role in pathological gambling [259] and in the behavioral outcomes of dopamine-replacement therapy [260].

**Finasteride in Gilles de La Tourette Syndrome**

Tourette syndrome (TS) is a chronic neuropsychiatric disorder, characterized by motor and one or more phonic tics although not necessarily concurrently, lasting longer than 1 year [261, 262]. It affects between 1% and 3% of the general population [263]. The onset of TS manifestations occurs typically in childhood (6-7 years of age), and the severity and frequency of tics typically tends to diminish during adolescence in most patients [264; 265; 266]. Tics are defined as sudden, rapid, recurrent, non-rhythmic, stereotyped movement or vocalization [267] that wax and wane in severity over weeks and months [268]. The motor patterns of tics can involve individual muscles or small groups of muscles (simple tics: eye blinking, nose twitching, mouth opening, sniffing) or more muscle acting in a coordinated pattern to produce movements that can resemble purposeful voluntary movements (complex tics: head shaking, scratching, touching, hitting, gestures or uttering phrases). Extreme forms of this disorder present as paroxysms or continuous orchestrated displays of simple and complex motor tics, such as self-injurious motor tics, hitting or biting, and socially unacceptable coprolalic utterances – e.g., shouting obscenities, racial slurs– and gestures. [269].
The majority of adult TS patients also display comorbid psychiatric disorders, such as mood and personality and anxiety disorders, including obsessive-compulsive disorder (OCD) [270, 271, 266].

The only medications currently approved for TS therapy are haloperidol and pimozide; however, the number of side effects induced by these medications, such as extrapyramidal symptoms [272, 268, 273] has led to the quest for alternative therapies. Atypical antipsychotics and α-adrenergic agonists are interesting options for TS therapy, but they also often lead to adverse metabolic and cardiovascular effects [274, 271].

While rich evidence has shown that hyperactivation of dopamine signaling plays a central role in the pathophysiology of TS [275, 276] several findings point to androgens in the pathophysiology and clinical course of TS [277-280].

In contrast with evidence supporting an autosomal dominant transmission for TS, this disorder affects men much more commonly than women [281]. This background indicates that androgens may facilitate the phenotypical expression of TS in men. Accordingly, TS symptoms are exacerbated by exogenous androgens [282]. Furthermore, the severity of tic symptoms in children affected by TS is correlated to the degree of preference for masculine play, suggesting that the disorder might be underpinned by elevations in androgen levels [280]. Finally, TS features a number of symptoms strongly reminiscent of androgen-mediated behaviors, such as impulsivity, aggressiveness, rage, increased sex drive and premature erotic urges [283, 284].

The hypothesis that androgens might precipitate TS symptoms prompted researchers to test the effects of androgen receptor antagonists, such as flutamide and cyproterone, in TS patients [285-287]. Although both compounds were shown to attenuate TS symptoms, two main factors discourage their use in TS: first, their therapeutic effects
seem to be modest and short-lived; second, they induce a number of severe side effects, including liver toxicity, dysmetabolic symptoms, cardiovascular and gastrointestinal disorders and severe sexual dysfunctions [288].

Compelling evidence suggests that TS is underpinned by alterations in dopaminergic signaling. Specifically, while dopamine agonists exacerbate tics, dopaminergic blockade has been previously shown to suppress tic severity [289]. Notably, PPI deficits mediated by dopaminergic agonists are likely the best validated model for the gating impairments in TS [290]. Indeed, TS patients typically report that tics are accompanied by an inner tension that intensifies until the discomfort becomes so great that they feel compelled to enact the tic and are preceded by the perception of intrusive, distressing stimuli in their perceptual domain [264; 291-293]. These gating disturbances termed “premonitory urges” are reflected in the PPI deficits exhibited by TS patients [241-243; 294].

A recent evidence shows that finasteride exerted significant therapeutic properties in a severe, treatment-refractory case of TS [295]. It was a severe case of male TS patient with explosive vocalizations, stereotyped coprolalic utterances, ritual behaviors, self-injuring motor tics, aggressive and contamination-theme obsessions and excessive sexual drive. Previous therapeutic attempts with typical antipsychotics (pimozide - 4 mg/day - clomipramine - 37.5 mg/day- chlorpromazine - 25 mg/day), had resulted in transient improvements, but the high rate of extrapyramidal and cognitive side effects had led him to repeated withdrawals. Finasteride (5 mg/day) led to a gradual improvement of his motor and vocal tics, as assessed by standardized scales with no reported side effects. The discontinuation of the regimen after 18 weeks, however, resulted in an abrupt, dramatic exacerbation of the symptoms, which was countered by reinstatement of the S5αR inhibitor. Over the last 2 years of follow-up treatment, the patient has shown a stable improvement of his tics, with a marked reduction in severity.
of his vocal and motor tics (about 30% and 50% of his pre-treatment scores, respectively). The treatment has not led to any overt sign of toxicity or side effects; while a reduction in sex drive was reported, this effect was described by the patient as “beneficial”, in consideration of his excessive libido before initiation of the therapy [295].

In line with these clinical and preclinical premises, we investigate in this report the therapeutic potential effect of finasteride, the prototypical inhibitor of steroid 5-α-reductase (5AR), in a population of unresponsive severe male adult TS patients.
2 AIMS OF THE STUDY

The pharmacological armamentarium for Tourette syndrome (TS) is still extremely limited and unsatisfactory, in view of the suboptimal therapeutic efficacy and the serious side effects of the currently available medications.

We report here the first prospective, open-label study on treatment response, safety and tolerability of finasteride (FIN) in 10 adult males with TS.

The aim of this study was to investigate the efficacy and tolerability of FIN as a treatment for tics in adult males with TS.
3  METHODS

3.1  Subjects.

The trial was a prospective open-label study with 10 male adult TS patients, recruited through the Tourette Syndrome Center at the University of Cagliari, referrals from local professionals and the AST-SIT Association (Associazione Sindrome di Tourette “Siamo in tanti”).

Recruitment of subjects occurred according to the following inclusion criteria: (1) Age included between 18–and 43 years at the time of release of informed consent; (2) male gender; (3) full diagnostic criteria for TS as established by the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DSM-IV-TR) (American Psychiatric Association 2000), on examination by a neurologist and/or neuropsychiatrist investigator; (4) previous failure to respond or inability to tolerate antipsychotic or other medications, as determined by the investigators; (5) no changes in TS drug therapy over the last two months prior to the beginning of the trial; (6) significant distress or impairment induced by tics, as reported by patients; (7) Yale Global Severity Scale (YGTSS) [296] score ≥ 50 (mild-moderate) at the beginning of the trial; (8) no significant abnormalities in hematochemical, endocrine, and urinalytical parameters (9) normal intelligence and lack of concomitant neurological disorders; (10) ability to swallow pills.
Subjects were allowed to maintain previous medications, but their doses were left unmodified throughout the study. All patients provided written informed consent for use of FIN. The study protocol was approved by the Institutional Review Board (IRB) of the University of Cagliari.

### 3.2 Procedures.

All subjects underwent medical, neurological and psychiatric assessment at baseline. All laboratory data (including serum hematology, chemistry evaluation, urinalysis and testosterone and progesterone serum levels) were within normal limits before initiation of treatment. FIN was always administered at 5 mg/day, as the common dosage used for benign prostatic hyperplasia (BPH) and this regimen was held constant throughout the trial.

TS symptoms and tic severity were assessed and monitored at pretreatment (baseline) and at 2, 6, 12 and 18 weeks of treatment, both with physical and neuropsychiatric examinations and using the Yale Global Tic Severity Scale (YGTSS) [296]. At the baseline and at the end of the trial (18th week of treatment), obsessive-compulsive symptoms were assessed with the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) [297].

For the safety analysis, investigators assessed patient well-being, incidence of adverse events and treatment compliance through an open-ended interview at each visit. An
adverse event was defined as any unexpected and unfavourable change in the patient's health during the study period, whether considered to be related to treatment or not.

3.3 Data analysis.

Data were expressed as means ± SEM (analyzed as absolute values and in relation to the baseline). Normality was tested by Lilliefors’ test for each time point. Analyses of the temporal changes in YGTSS scores was performed by Friedman’s test for repeated measures, followed by Dunn’s post-hoc analyses. Effect size was assessed by Kendall’s W coefficient. Y-BOCS scores were evaluated by Wilcoxon pair-matched test, followed by Dunn's multiple comparisons test for post-hoc analysis.
4 RESULTS

4.1 Description of sample.

Sociodemographic characteristics of the study sample are described in Table 3. Psychiatric comorbidity was observed in seven patients that met criteria of DSM-IV TR; four patients met criteria for Obsessive Compulsive Disorder (OCD); two patients for Obsessive Compulsive Personality Disorder (OCP-D) generalized anxiety disorder; one patient for Major Depressive Disorder (MDD).

Four subjects took other psychotropic medications: one subject was under aripiprazole and clordemetildiazepam; one subject was under amitriptyline and clotiapine; one subject was under pimozide; one subject was under sertraline) for their comorbid psychiatric disorders during the study; the dosages of these medications remained stable during the study.

4.2 Dosing, range, and compliance.

Treatment duration was approximately 18 weeks. No dosing titration occurred. In all the sample population, 2 subjects were treated with FIN while taking their previous tic medications (one patient with aripiprazole and another one with pimozide respectively).
The remaining 8 subjects were on no medication and received FIN during the trial. In addition to assuming FIN, the MDD patient assumed amitriptyline 40 mg/day and clotiapine 15 mg/day, instead one patient with comorbid OCD was treated with sertraline 50 mg/day through the three last weeks of the trial (See Table 3).
<table>
<thead>
<tr>
<th>Patients</th>
<th>Age of TS onset (years)</th>
<th>TS duration (years)</th>
<th>Comorbid disorders axis I (DSM-IV TR)</th>
<th>Previous medications to FIN</th>
<th>Reasons for therapeutic failure of previous medications</th>
<th>Concomitant medications to FIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14 y</td>
<td>22y</td>
<td>None</td>
<td>Olanzapine 5 mg/die; Risperidone 2 mg/die; Tetrabenazine 25 mg/die; Clonazepam 0.6 mg/die.</td>
<td>Drowsiness; muscle pain; restlessness</td>
<td>none</td>
</tr>
<tr>
<td>2</td>
<td>15y</td>
<td>10y</td>
<td>None</td>
<td>Haloperidol 2mg/die</td>
<td>No side effects; ineffectiveness</td>
<td>none</td>
</tr>
<tr>
<td>3</td>
<td>11y</td>
<td>24y</td>
<td>None</td>
<td>Thioridazine chloridrate 10 mg/die; Haloperidol 2 mg/die</td>
<td>Drowsiness; muscle pain and fatigue</td>
<td>none</td>
</tr>
<tr>
<td>4</td>
<td>11y</td>
<td>8y</td>
<td>OCD</td>
<td>Haloperidol 1 mg/die; Risperidone 1 mg/die; Pimozide 8 mg/die</td>
<td>Drowsiness; confusion and decreased concentration; muscle pain ; ineffectiveness</td>
<td>Pimozide 4 mg/die</td>
</tr>
<tr>
<td>5</td>
<td>15y</td>
<td>31y</td>
<td>OCD</td>
<td>Lorazepam 1.5 mg/die</td>
<td>Ineffectiveness</td>
<td>none</td>
</tr>
<tr>
<td>6</td>
<td>28y</td>
<td>3y</td>
<td>OCD</td>
<td>Alprazolam 1 mg/die</td>
<td>Drowsiness</td>
<td>Sertraline 50 mg/die</td>
</tr>
<tr>
<td>7</td>
<td>6y</td>
<td>19y</td>
<td>OCP-D</td>
<td>Diazepam 5 mg/die; Clonazepam 3 mg/die Alprazolam 0.5 mg/die; Paroxetine 10 mg/die</td>
<td>Ineffectiveness</td>
<td>none</td>
</tr>
<tr>
<td>8</td>
<td>8y</td>
<td>21y</td>
<td>OCD</td>
<td>Haloperidol 1.5 mg/die; Aripiprazole 15 mg/die; Pimozide 2 mg/die; Sertraline 50 mg/die</td>
<td>Drowsiness; sleep disorders; confusion; akathisia; tremors</td>
<td>Aripiprazole 15 mg/die , Clordemetildiazepam 1.5 mg/die</td>
</tr>
<tr>
<td>9</td>
<td>12 y</td>
<td>21y</td>
<td>MDD</td>
<td>Risperidone 4-6 mg/die</td>
<td>Restlessness; muscle tension; increased salivation and appetite; insomnia.</td>
<td>Amitriptyline 40 mg/die; Clotiapine 15 mg/die</td>
</tr>
<tr>
<td>10</td>
<td>7 y</td>
<td>14y</td>
<td>OCP-D</td>
<td>Haloperidol 2 mg/die; Risperidone 2.5 mg/die; Olanzapine 10 mg/die; Clordemetildiazepam 0.5 mg/die</td>
<td>Drowsiness; decreased concentration.</td>
<td>none</td>
</tr>
</tbody>
</table>

**Table 3. Characteristics of study population (n = 10)**

4.3 Effects of FIN on TS and tic severity.

Preliminary evaluations by Lilliefors’ test for YGTSS scores found significant deviations from normality for global severity (at 6 weeks; \( p < 0.05 \)) and motor tics (at 12 weeks; \( p < 0.01 \)) (data not shown); thus, calculation of the effects of FIN was performed with Friedman’s test.

This analysis revealed a significant, time-dependent decline in YGTSS global severity score (\( \chi^2(4) = 31.12, p < 0.0001 \)). Post-hoc comparisons (performed by Dunn’s test) revealed that the severity was significantly reduced at 6, 12 and 18 weeks of treatment (Fig. 9 A). The effect size, estimated by the Kendall’s coefficient of concordance, was \( W = 0.78 \).

A significant, time-dependent reduction was also found in total tic score (\( \chi^2(4) = 34.16, p < 0.0001 \)). Post-hoc comparisons (performed by Dunn’s test) revealed that the severity was significantly reduced at 12 and 18 weeks of treatment in comparison to baseline values (Fig. 9B). Kendall’s \( W \) coefficient of concordance for these parameters was 0.85.

The analyses of motor and phonic tics confirmed a dramatic reduction in both scores (motor: \( \chi^2(4) = 34.16, p < 0.0001 \); phonic: \( \chi^2(4) = 34.62, p < 0.0001 \); Friedman’s test) (Fig. 9 C-D), with effect sizes of 0.76 and 0.87, respectively (Kendall’s \( W \) coefficient).
Fig. 9. Effects of finasteride on tic severity and tic in Tourette patients (n=10).

*** p < 0.0001; ** p < 0.001; * p < 0.05 vs baseline YGTSS scores. YGTSS, Yale Global Tic Severity Scale. For further details see text.
4.4 Effects of FIN on obsessive-compulsive symptoms.

Exploratory analyses were conducted to assess effects of FIN on obsessive and compulsive traits (Y-BOCS) in the 6 patients with these features (Fig. 10). There was a significant reduction in Y-BOCS total scores ($Z=2.20 \ p<0.05$, Wilcoxon matched-pairs test; effect size: $r=0.90$) and Y-BOCS Compulsion subscale scores ($Z=2.20 \ p<0.05$, Wilcoxon matched-pairs test; effect size: $r=0.90$) but no significant reduction in Y-BOCS Obsession scores ($Z=1.83$, NS, Wilcoxon matched-pairs test; effect size: $r=0.75$).

**Fig. 10. Effects Of Finasteride on OCD Severity in Tourette (n= 10).**

* $p < 0.05$ vs baseline Y-BOCS Total and Compulsive scores. Y-BOCS, Yale Brown Obsessive Compulsive Scale. For further details see text.
4.5 Adverse effects.

The majority of subjects tolerated FIN well; no patients of this sample dropped out before 18 weeks. Most common adverse effects were reported to be mild and included decreased libido in two patients of the sample and occasional difficulty in achieving erection in one of them. No depressive symptoms worsened in MDD patient or severe anxious features occurred in the sample, despite FIN has been recently reported to induce depression in a subset of patients [304].

No significant differences in body weight, vital signs or other clinical and lab parameters were found between baseline and end point.
5. DISCUSSION

Results of this prospective, open-label, safety and tolerability study suggest the efficacy of FIN (5 mg/day) in reducing global severity, total and motor tics within 6 weeks from treatment initiation in a small sample of male adults with TS who had failed to respond or had been unable to tolerate previous tic treatment. Our exploratory analysis also revealed a mild, yet significantly beneficial effect on compulsive, but not obsessive symptoms.

To the best of our knowledge, this is the first open-label report of therapeutic effects of FIN in TS. The present results confirm and extend our previous finding on the therapeutic effects of FIN in a severe case of TS, refractory to antipsychotic treatment [295]. Of note, the patient described in that original report (not included in the present open label study) is still under FIN treatment and has reported a stable improvement of its symptoms over the last 3 years, with a general improvement of his quality of life and no noticeable adverse effects. This premise suggests that FIN therapy may be a viable long-term therapeutic option for TS male adult patients unresponsive to antipsychotics or other treatments. Furthermore, our present findings complement previous reports documenting the therapeutic potential of 5AR in chronic schizophrenia [248] and perseverative and idiosyncratic personality traits in adult male patients [249; Bortolato et al. submitted].

The enzyme 5AR catalyzes numerous processes, including the conversion of testosterone into the potent psychotropic androgen dihydrotestosterone (DHT), as well as the synthesis of neurosteroids from progesterone [16;17].
The mechanism of FIN-induced reduction in TS symptoms may reflect its effects on metabolism of testosterone, consisting in inhibiting DHT synthesis and favouring the aromatase-mediated conversion of testosterone in β-estradiol. Accordingly, several lines of evidence point to androgens in the pathophysiology and clinical course of TS [see 277- 280]. For example, this disorder affects men more commonly than women [281] and its symptoms are exacerbated by exogenous anabolic androgens [282] TS features a number of symptoms strongly reminiscent of androgen-mediated behaviors, such as impulsivity, aggressiveness, rage and increased sex drive [283].

Previous trials have found that androgen receptor antagonist, such as flutamide and cyproterone, attenuate TS symptoms [285, 286, 287]. However, the therapeutic effects of these compounds were reportedly modest, short-lived, and countered by important side effects, including liver toxicity, dysmetabolic symptoms, cardiovascular and gastrointestinal disorders and sexual dysfunctions [288].

The theoretical framework supporting these trials was based on the idea that TS symptoms may be exacerbated by the direct signaling of testosterone.

Our current results suggest that tic severity may be compounded not by this steroid, but rather by its 5-alpha-reduced androgenic metabolites, such as DHT, which are not susceptible to aromatization. Indeed, FIN may exert protective effects in TS by increasing testosterone’s availability for its conversion into β-estradiol. This premise suggests that alterations in the brain-based metabolic fates of testosterone (and/or other androgen precursors, such as androstenedione) may play a role in the pathogenesis of TS. Indeed, previous studies have reported low LH and testosterone in some TS patients [298, 299]. It is possible that an exaggerated conversion of
testosterone into its androgenic (rather than estrogenic) metabolites in the brain may underpin some of the neurochemical imbalances featured in TS. Alternatively, FIN may have elicited its effect by inhibiting the conversion of progesterone in neurosteroids, thereby enhancing its brain levels [300]. Interestingly, progesterone acts as a potent antagonist for σ1 receptors, which have been shown to modulate dopaminergic signaling [233, 301].

In preclinical studies, FIN and other 5AR inhibitors exerted profound antipsychotic-like effects in animal models of TS [238]. These effects appear to be supported by the prefrontal cortex and ventral striatum [Devoto et al, submitted], two dopaminergic regions that play a central role in the pathophysiology of TS [302] as well as in the behavioural outcomes of dopamine-replacement therapy [258-260, Bortolato et al. submitted] Further studies are warranted to investigate the possible interactions between steroids and dopamine receptors in relation to the psychotropic effects of FIN in TS and related disorders. Preclinical studies are in progress in our laboratory to identify which S5αAR substrate or metabolite is primarily involved in FIN antipsychotic-like action.

Extensive clinical literature shows that FIN is very well tolerated and induces limited side effects [303, 304]. Accordingly, FIN was very well tolerated in our study, and adverse effects were mild and manageable [305].

Despite the neutral side-effect profile of FIN in this and other studies, the generalizability of our findings to other groups of TS patients is obviously limited by the inapplicability of this therapy to children (who represent the greatest target population in this disorder) and pre-menopausal female adults (in view of the teratogenic effects of FIN) [306, 307]. Nevertheless, the identification of the neurobiological underpinnings of the effects of FIN
and other 5AR inhibitors may open to novel, well-tolerated therapeutic options for this disorder.

Some limitations of this study design must be taken into account. All subjects in our sample were treatment-refractory; thus, we cannot rule out that the observed effects may reflect some unique mechanism not observed in the majority of TS patients. Furthermore, the open-label nature of the study and the lack of treatment-blinding in the design pose important limits in the value of these results, in view of the possibility of placebo effects, as well as ascertainment and observer bias. Finally, the presence of concomitant medications implies that some of the observed effects may be related to drug interaction and pharmacodynamic synergisms, rather than to the inherent effects of FIN. Future controlled, comparative studies are warranted to properly evaluate the effectiveness of the therapeutic action of FIN in adult males with TS.
6. CONCLUSION

The therapeutic effects of finasteride as an adjunctive treatment in TS have been confirmed in a first open-label study with ten adult male TS patients. In these patients, finasteride was found to elicit significant reduction of the severity of tics and associated compulsive (but not obsessive) manifestations within six weeks from initiation of the therapy.

S5αR is the key enzyme in the synthesis of androgens and neurosteroids, and its regulation is fundamental in the management of steroid signaling in the brain. By means of several complementary approaches, research has provided a great deal of knowledge about the function of S5αR isoenzymes and their role in the hormonal balance in periphery. Preliminary information on the role of S5αR in several mental disorders is paving the way to a new series of future investigations which will further our understanding of the role of these enzymes in the pathophysiology of Tourette Syndrome and other mental disorders, and examine the specific role of neurosteroids in the behavioral outcomes of S5αR inhibition. S5αR inhibitors have many desirable characteristics for the pharmacotherapy of mental illnesses: they are clinically approved, have been extensively characterized in patients and are very well-tolerated so might provide a useful complement to the current pharmacological armamentarium of novel agents for some of the most challenging psychiatric disorders, such as Tourette Syndrome, schizophrenia, impulse-control disorders, as well as alcohol- and substance-related disorders. Despite promising clinical results, it is important to stress that efficacy of finasteride for treatment of TS and other neuropsychiatric disorders cannot be evaluated from case reports and observations, but double-blind, placebo-controlled
clinical trials should confirm the actual therapeutic potential of S5αR blockers in these disorders.
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