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# Identification of HMGCR, PPGARG and prohibitin as potential druggable targets of dihydrotestosterone for treatment against traumatic brain injury using system pharmacology

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ABSTRACT

*Background:* Traumatic Brain Injury (TBI) has long-term devastating effects for which there is no accurate and effective treatment for inflammation and chronic oxidative stress. As a disease that affects multiple signalling pathways, the search for a drug with a broader spectrum of pharmacological action is of clinical interest. The fact that endocrine disruption (e.g hypogonadism) has been observed in TBI patients suggests that endogenous therapy with testosterone, or its more androgenic derivative, dihydrotestosterone (DHT), may attenuate, at least in part, the TBI-induced inflammation, but the underlying molecular mechanisms by which this occurs are still not completely clear.

*Aims and methods:* In this study, the main aim was to investigate proteins that may be related to the pathophysiological mechanism of TBI and also be pharmacological targets of DHT in order to explore a possible therapy with this androgen using network pharmacology.

*Results and conclusions:* We identified 2.700 proteins related to TBI and 1.567 that are potentially molecular targets of DHT. Functional enrichment analysis showed that steroid (p-value: 2.1–22), lipid metabolism (p-value: 2.8–21) and apoptotic processes (p-value: 5.2–21) are mainly altered in TBI. Furthermore, being mitochondrion an organelle involved on these molecular processes we next identified that out of 32 mitochondrial-related proteins 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), peroxisome proliferator activated receptor gamma (PPGARG) and prohibitin are those found highly regulated in the network and potential targets of DHT in TBI. In conclusion, the identification of these cellular nodes may prove to be essential as targets of DHT for therapy against post-TBI inflammation.

## 1. Introduction

Traumatic brain injury (TBI) is a debilitating, complex, and heterogeneous pathology, affecting mostly young people at full productive age. The causes of brain trauma are diverse, but most are related to drug- and alcohol-driving, cell phone use whilst driving, and accidental falls. There is still no precise treatment that can effectively protect the brain following initial trauma nor from the secondary damage caused by persistent and chronic inflammation. This demonstrates the urgent need to investigate new treatments to improve patient outcomes, specifically to reduce motor and cognitive damage in the chronic period of the pathology.

One of the major features of TBI pathology is endocrine disruption. This affects the levels of hormones in the blood and brain and has positively been correlated with worse outcome and prognosis in TBI patients [1–4]. One of the clinical findings that can explain this hormonal dysregulation is the hypopituitarism observed in post-TBI patients. For instance, TBI patients report having altered blood levels of testosterone (T). Testosterone, when metabolized by the enzyme 5α-reductase, is converted to dihydrotestosterone (DHT) and, similarly to T, DHT is also reduced in patients suffering from TBI [4,5]. This significant reduction of DHT in both blood and brain may be related to the worsening of clinical symptoms, with severe consequences for the development of brain processes such as cognition and learning, in addition to generating important motor sequelae. To reduce most of these detrimental effects due to androgenic dysfunction, treatment with DHT has

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been shown to exert neuroprotection [6–9], thus improving brain functions and limiting motor and cognitive damage by ameliorating inflammation and preserving mitochondrial function [10–12].

Mitochondria are both responsive to androgens and severely impacted by TBI. An increase in cellular calcium concentration due to TBI (*in vitro* and *in vivo*) contributes to mitochondrial dysfunction [13–17] which may be related to a significant increase in the generation of reactive oxygen species (ROS), which if not neutralized, may initiate cell death mechanisms [18]. This oxidative stress can be generated by an imbalance in endogenous concentrations of cellular antioxidants and oxidants after a TBI, leading to damage to the mitochondrial membrane, thus increasing the permeability of the mitochondrial transition pore increasing the generation of ROS, which is one of the major contributors to DNA damage and cell apoptosis [12,19]. During this phase, cytochrome *C* oxidizes and migrates to the cytosol following an increased permeability of voltage-dependent anion channel (VDAC) located in the outer mitochondrial membrane, promptly activating the apoptotic protease activating factor 1 (APAF-1)-mediated intrinsic apoptotic pathway, thus triggering the initiation of other apoptotic factors such as caspases 3, 7 and 9. Mitochondria-dependent ROS also lead to an imbalance of intracellular cholesterol and lipids metabolism which may provoke lipotoxicity by aberrant accumulation of oxidized fatty acids and lipid droplets dysregulation [20]. One of the proteins significantly altered in TBI and that may participate in disease progression is neuroglobin, a hormonal regulated protein considered to be utterly critical for mitochondria integrity and dynamics due to its ability to interact with mitochondrial respiratory complexes, boost oxidative phosphorylation, reduce oxygen and nitrogen reactive species, and inhibit apoptosis by retaining cytochrome *C* within this organelle [15,21–24]. Considering the androgenic alterations in TBI and how supplemental treatment with DHT may be of clinical interest, in this paper we aimed to investigating how DHT regulates proteins involved in the pathology of TBI at the mitochondrial level using system pharmacology.

## 2. Materials and methods

### 2.1. Identification of DHT-regulated proteins involved in TBI pathology

The first step was to collect the list of proteins that are regulated by DHT using the databases DrugBank (https://go.drugbank.com/), Similarity Emseable Approach (SEA, https://sea.bkslab.org), Comparative Toxigenomic database (CTD, http://ctdbase.org) and SwissPrediction (http://swisstargetprediction.ch), whose algorithms use the SMILES code of each drug and, according to the degree of similarity, predict which proteins can be considered as its targets. As inclusion criteria, we limited our search to words like "DHT, "dihydrotestorone" and *"Homo sapiens*". A total of 1,600 DHT-regulated proteins were predicted across the four databases.

To identify proteins that are altered or likely participate in TBI pathology, we used the search terms "Traumatic brain injury", "*Homo sapiens*", and "human" in the CTD database, Genecard/Malacards (https://www.Malacards.org/) and OMIM (https://omim.org/). A total of 3,522 proteins were collected.

Bearing in mind that this mining process from those databases might lead to repeated proteins and false positives, after collating all lists, these were combined. Then we proceeded to eliminate the duplicated proteins and using the Uniprot ID of each protein we assured to unify the terms to standardize names and avoid redundancy.

### 2.2. Protein-Protein interaction (PPI) network

After eliminating the repeated proteins and unifying the names, the protein list for DHT contained a total of 1,567 targets, while that for TBI was 2,700. First, the proteins in common between the two lists were identified in a Venn diagram, then imported into Cytoscape (version 3.8.2) for a more detailed analysis of the topology of the network and how the interaction among proteins takes place for a more in-depth study of those that are key in the cellular mechanism. Second, we used the Genemania plugin with a confidence ratio of 0.5 followed by "Analyze network" to calculate the degree of confidentiality and significance of each protein within the network taking into account the number of interactions (degree), closeness (the shortest path from a protein to all the others) and betweenness (the number of paths crossing through a protein). In other words, betweenness values are key to identifying proteins with high level of interactions and interconnections where the flux of information across this node (protein) is significative, making this protein a druggable target within the network. By evaluating these parameters our main goal was to explore potential hubs within the network that, in principle, may be pharmacological and therapeutic targets for DHT, and with involvement in TBI.

To identify subclusters containing mitochondrial-related proteins, we used String in cytoscape with a confidence score cut-off of 0.4, those proteins with a score of 4 or above in the parameter compartment: mitochondria were selected and a new subnetwork was created to explore highly connected subclusters using MCODE with a degree cut-off of 2, K-core of 2, and node score cut-off of 2. This plugin ranks the top clusters, assigns a computed score according to the level of interconnections and importance for further analysis.

### 2.3. Functional annotation of proteins and pathways enrichment

For full functional annotation of proteins according to molecular function and biological processes, the DAVID v6.8 (https://david.ncifcrf.gov/), Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg), and Enrich (https://maayanlab.cloud/Enrichr/) databases were used. Both the lists containing the proteins regulated by DHT and TBI as well as those related to mitochondria were submitted to functional analysis, where the top-10 in each category were filtered out and selected for further analysis. As inclusion criteria a p-value of 0.05 and "*Homo Sapiens*" was selected. All results were catalogued, and graphs were created using Graphpad 9.

## 3. Results

### 3.1. Exploration of protein targets of DHT and TBI

One of the first steps in building the protein-protein interaction network is to identify which proteins are both DHT-regulated and affected by TBI. Using the Drugbank, Swissprediction, SEA and CTD databases, for DHT a total of 9 proteins in Drugbank, 101 in SwissPrediction, 37 in SEA and 1,453 in CTD were predicted. On the other hand, for TBI, 1,589 in CTD, 191 in OMIM and 1,742 proteins in Genecards/Malacards. Next we aimed to identify which of these proteins are common on both lists (DHT vs TBI). Overlapping proteins are shown in circus plot (Fig. 1A), where most are related to cell death, apoptosis, ion transport and inflammation (Fig. 1B). A total of 381 proteins (9.8%) are shared between the two groups (Fig. 2A), and finally the network generated with these common proteins using Genemania in cytoscape contained 388 nodes, 18,792 edges with an average number of 79,119 neighbours. By analyzing the functional categories of these proteins, we identified that those with the highest number of proteins are steroid metabolic process (41/243; adjusted p-value: 2.1–22) (Fig. 2B), regulation of lipid metabolic process (41/265; adjusted p-value: 2.8–21) (Fig. 2C), and regulation of apoptotic signalling (40/255; adjusted p-value: 5.2–21) (Fig. 2D). Because all of these cellular processes are severely altered in TBI and are regulated by androgens [12], next we performed a more in-depth analysis to explore which proteins can be considered novel hubs in each category.

### 3.2. Steroid metabolic process

One of the neuropathological aspects observed in TBI patients is a

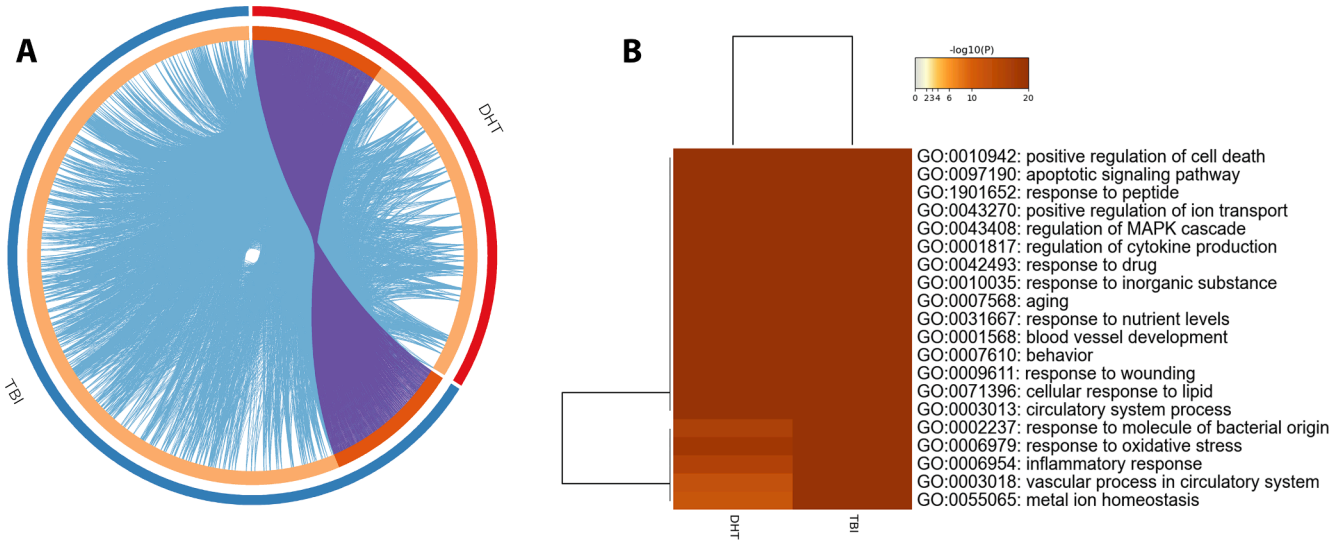


**Fig. 1.** Circus plot representing how proteins integrate and correlate between TBI and DHT, showing mainly those overlapping between these two lists (A). In TBI a total of 2700 proteins were included while in DHT, 1567. Dark orange are those proteins appearing in both lists and those marked with light orange are those present only in one list. The purple lines identify those proteins that are shared by both lists. On the other hand, the blue lines represent those proteins that fall within the same ontological term, which is found significantly enriched and whose size does not exceed 100. The greater the number of purple lines and the wider the arcs are, the greater the overlap between the two lists. (B) Protein enrichment analysis showing how the proteins are differently expressed across ontology terms.

significant decrease in hormone levels in both the blood and brain (hypogonadism) with time of injury. Not only are the levels of gonadal hormones such as estradiol and testosterone reduced, but cortisol has also seen altered in patients suffering from a TBI [25], suggesting serious implications in circadian rhythm affecting not only the brain but also other organs [26]. Interestingly, exogenous administration of glucocorticoids may improve the hypothalamic-pituitaryadrenal axis [27], which may prove to be an alternative therapeutic approach. To better understand this signalling in our model, next we assessed the proteins involved in steroids metabolism that are targets of DHT and involved in TBI. Network analyzer results showed that, out of 41 proteins, SREBF1 (41), HMGCR (37), SREBF2 (32), IDI1 (31), DHCR7 (30), FDPS (29), HMGCS1 (29), FDFT1 (28), INSIG1 (25), and MSMO1 (25) are the top-10 presenting a higher degree (Table 1), an indication of the number of possible interactors within the network. Interestingly, sterol regulatory element-binding transcription factor 1 (SREBF1) although presenting high degree and closeness, it was not the top protein in betweenness. In contrast, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR) ranked second for degree but is the top protein in betweenness and closeness, that is the information flow between proteins considered to be central and the number of times the information passes through them (betweenness) within a close distance (closeness) make this protein an important hub within this network.

### 3.3. Regulation of lipid metabolic process

Out of 41 proteins according to the number of degrees SREBF1 (39), PPARG (33), SREBF2 (33), HMGCR (32), HMGCS1 (30), ACACA (26), FDFT1 (26), DHCR7 (26), FASN (25), and LDLR (24) make up the top-10 (Table 2). Despite being the second protein on this list in relation to the number of degrees, peroxisome proliferator-activated receptor gamma (PPARG) presented the highest betweenness and closeness, making it an important node in this network.

### 3.4. Regulation of apoptotic signalling pathway

From a list of 30 proteins with functional roles in this category, sorting them according to the number of degrees showed that TP53 is by far the top protein with 53, followed by HIF1A (37), MCL1 (35), AKT1 (34), CAV1 (31), TNFAIP3 (28), BCL2L1 (28), AR (27), ICAM1 (26), and CSNK2A1 (25) (Table 3). For those proteins presenting higher betweenness and closeness, tumor protein p53 (TP53), hypoxia inducible factor 1 subunit alpha (HIF1A), and RAC-alpha serine/threonine-protein kinase (AKT1) make most the tops on this list and are key targets. Caveolin-1 (CAV1) although showing high closeness it does present high betweenness in comparison to the ones topping this list.

### 3.5. Subcluster of mitochondrial-related proteins regulated by both DHT and TBI

Androgen receptor is found within mitochondria as it possesses a mitochondria internalization sequence [28], making this organelle a target of androgens [7,28,29], and likely severely affected upon TBI [12,30–32]. Considering the mitochondria as a converging point between lipid metabolism, steroids and apoptotic mechanisms, using String in cytoscape we shortlisted those proteins that either may be localized within this organelle or play a biological role involving mitochondria to explore which of them have an essential participation in the network and be druggable targets of DHT in TBI.

Of the list of 381 proteins in common between DHT and TBI (Fig. 1), 32 have some relationship to mitochondria (Suppl Table 1). Overall, this mitochondrial cluster has 52 nodes, 459 edges and 11,692 neighbours. Using the network analyze tool we calculated which proteins can be important nodes in this network according to the number of interactions (degree), betweenness and closeness. As expected, amyloid beta precursor (APP), mitogen-activated protein kinase 3 (MAPK3), and TP53 form the top 3 with the largest number of interactions (Table 4). However, considering only the ones with shortest path in the network (closeness), prohibitin (PHB) is third on this parameter and fourth on betweenness, and despite not having an expressive number of interactions (22) suggests this protein as a central node in the intersection of the information flow within the network.

KEGG pathway analysis using DAVID 6.8 showed a significative correlation of -log10(p), the number of mitochondria-related proteins in each category, fold enrichment and false discovery ratio (FDR) (Fig. 3A). Next, we selected the top-10 categories with lowest FDR and -log10(p) versus protein counts (Fig. 3B), and that included therefore those proteins belonging to regulation of apoptotic process (13 proteins; p-value: 10.38), regulation of release of cytochrome *c* from mitochondria (6 proteins; p-value: 10.35), release of cytochrome *c* from mitochondria (5

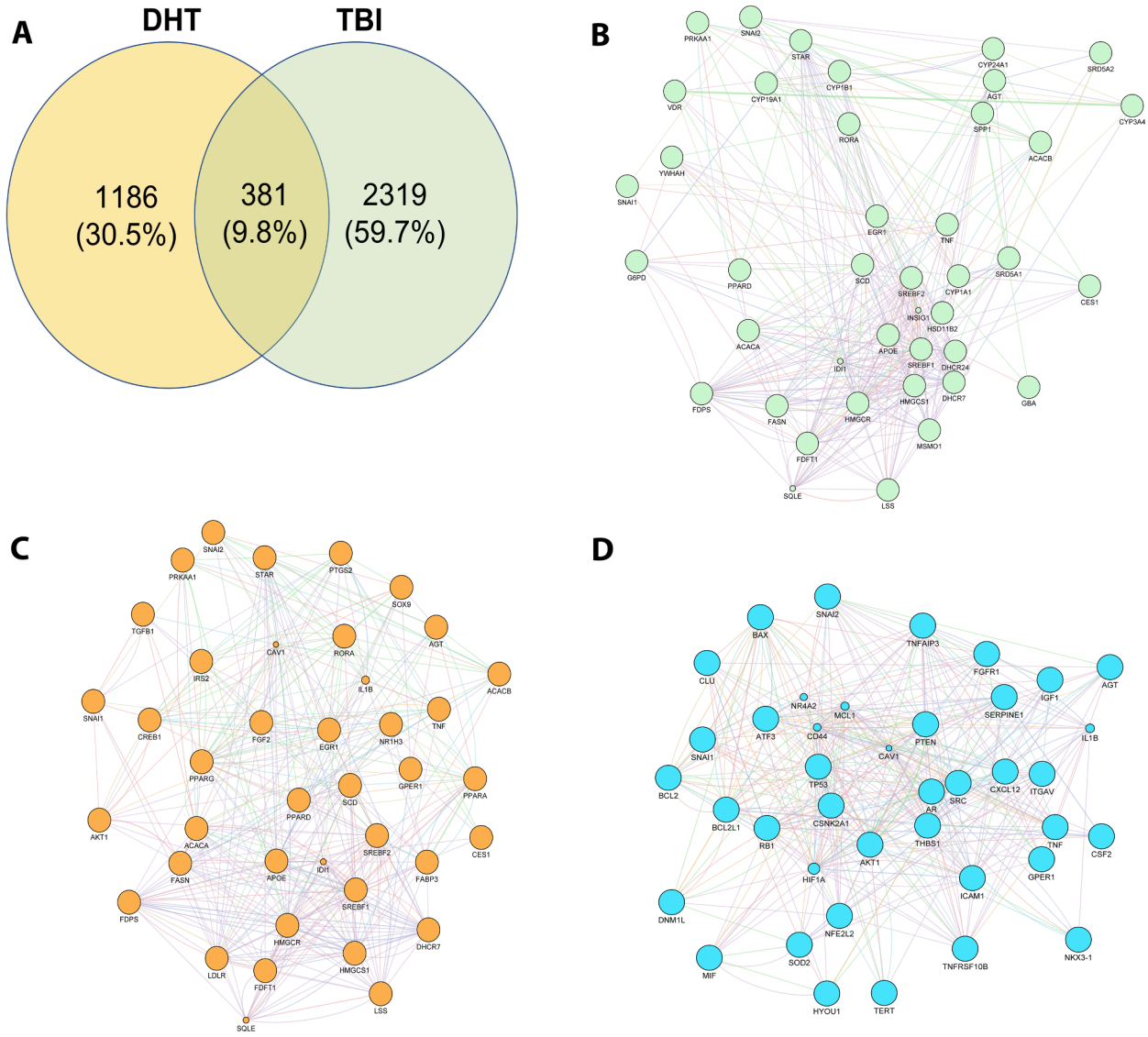


**Fig. 2.** Identification of common proteins between TBI and DHT and assessment of protein–protein network analysis. Venn diagram showing that approximately 8.8% of proteins are shared in DHT and TBI list (A). Enrichment of these shared proteins demonstrate that a high percentage is involved in regulation of steroid metabolic process (B), lipid metabolic process (C) and apoptotic signalling pathways (D).

**Table 1**
Top 10 proteins related to steroid metabolic process according to the number of degrees.

| Name | Degree | Betweenness | Closeness |
|---|---|---|---|
| SREBF1 | 41 | 0.03859 | 0.689655 |
| HMGCR | 37 | 0.052024 | 0.689655 |
| SREBF2 | 32 | 0.015843 | 0.634921 |
| IDI1 | 31 | 0.016716 | 0.677966 |
| DHCR7 | 30 | 0.016741 | 0.666667 |
| FDPS | 29 | 0.014496 | 0.655738 |
| HMGCS1 | 29 | 0.007061 | 0.645161 |
| FDFT1 | 28 | 0.012244 | 0.645161 |
| INSIG1 | 25 | 0.044904 | 0.666667 |
| MSMO1 | 25 | 0.039887 | 0.677966 |

**Table 2**
Top 10 proteins related to lipid metabolic process according to the number of degrees.

| Name | Degree | Betweenness | Closeness |
|---|---|---|---|
| SREBF1 | 39 | 0.030032 | 0.684210526 |
| PPARG | 33 | 0.068704 | 0.684210526 |
| SREBF2 | 33 | 0.041486 | 0.684210526 |
| HMGCR | 32 | 0.021403 | 0.672413793 |
| HMGCS1 | 30 | 0.008624 | 0.65 |
| ACACA | 26 | 0.024212 | 0.639344262 |
| FDFT1 | 26 | 0.012098 | 0.629032258 |
| DHCR7 | 26 | 0.010928 | 0.629032258 |
| FASN | 25 | 0.034154 | 0.661016949 |
| LDLR | 24 | 0.028858 | 0.672413793 |

proteins; p-value: 10.19), positive regulation of apoptotic process (9 proteins; p-value: 9.02), apoptotic mitochondrial changes (5 proteins; p-value: 8.76), negative regulation of apoptotic process (10 proteins; p-value: 8.59), positive regulation of programmed cell death (8 proteins; 7.90), cellular response to oxidative stress (6 proteins; p-value: 7.37), positive regulation of gene expression (9 proteins; p-value: 7.36), and positive regulation of macromolecule metabolic process (8 proteins; p-value: 6.91) as top biological processes (Fig. 3C).

Regarding proteins related to molecular function (Fig. 3D), top categories include protein serine/threonine/tyrosine kinase activity (4 proteins; p-value: 6.96), MAP kinase activity (3 proteins; p-value: 5.87), ubiquitin-like protein kinase binding (6 proteins; p-value: 5.3), protein

**Table 3**
Top 10 proteins related to apoptotic signalling according to the number of degrees.

| Name | Degree | Betweenness | Closeness |
|---|---|---|---|
| TP53 | 53 | 0.071211083 | 0.735849057 |
| HIF1A | 37 | 0.055643111 | 0.709090909 |
| MCL1 | 35 | 0.024442865 | 0.661016949 |
| AKT1 | 34 | 0.058131462 | 0.672413793 |
| CAV1 | 31 | 0.021315137 | 0.661016949 |
| TNFAIP3 | 28 | 0.024447502 | 0.661016949 |
| BCL2L1 | 28 | 0.01758909 | 0.609375 |
| AR | 27 | 0.026381118 | 0.609375 |
| ICAM1 | 26 | 0.023608825 | 0.65 |
| CSNK2A1 | 25 | 0.034967115 | 0.65 |

**Table 4**
Top 10 proteins from mitochondrial-related subclusters according to the number of degrees.

| Name | Degree | Betweenness | Closeness |
|---|---|---|---|
| APP | 37 | 0.097480748 | 0.64556962 |
| MAPK3 | 46 | 0.070126255 | 0.671052632 |
| TP53 | 45 | 0.063221589 | 0.614457831 |
| PHB | 22 | 0.05570298 | 0.62195122 |
| MAPK1 | 36 | 0.045991504 | 0.62195122 |
| BCL2L1 | 35 | 0.039251703 | 0.593023256 |
| CASP3 | 27 | 0.037625591 | 0.607142857 |
| AKT1 | 34 | 0.031587165 | 0.62195122 |
| FOXO3 | 27 | 0.030974662 | 0.586206897 |
| HSPA5 | 24 | 0.02920137 | 0.614457831 |

serine/threonine kinase activity (6 proteins; p-value: 4.81), BH domain binding (2 proteins; p-value: 4.43), BH3 domain binding (2 proteins; p-value: 4.43), ubiquitin protein ligase binding (5 proteins; p-value: 4.22), estrogen 16-alpha-hydroxylase activity (2 proteins; p-value: 4.16), kinase binding (6 proteins; p-value: 4.10), and death domain binding (2 proteins; p-value: 3.86) (Fig. 3E).

To identify key mitochondrial subclusters in this network (Fig. 4A), we used the MCODE tool in cytoscape. Three subclusters were generated, the top-one (Fig. 4B) contains 11 nodes with a score of 5.800, followed by cluster 2 (18 nodes and score 5.647) and three (5 nodes and score 3.000). In relation to subcluster 1 (CLU, SGK1, FOXO3, CCNB1, HSPA5, AKT1, APP, MAPK10, MAPK3, PRKCA and MAP2K2), the top biological processes with 20.4% of participation are cellular and metabolic process as well as biological regulation, followed by response to stimulus and signalling with 16.30% participation each, and finally developmental process, multicellular organismal process and localization with only 2% each (Fig. 4C).

In subcluster 2 (TP53, HDAC4, PHB, CDK2, NAE1, GLUD1, SOD2, BRAF, KSR1, ATM, GSTK1, BAX, PRNP, BCL2L1, NR3C1, DNM1L, HNRNPU, and BCL2L11) (Fig. 4D), 27.70% proteins are involved in cellular process, 21.3% in biological regulation, 17% in metabolic process, 12.8% in signalling, 10.60% in response to stimulus, 4.30% in localization, and finally 2.10% proteins in processes such as biological phase, and reproduction (Fig. 4E). Finally, with the participation of only 5 proteins (SETD7, MAPK1, HSPD1, CASP3, and BAD), the top biological processes in subcluster 3 (Fig. 4F) are cellular process, biological regulation, response to stimulus and metabolic process with 15.40% each, followed by localization, signalling, multicellular organismal process and immune system with 7.70% each (Fig. 4G).

## 4. Discussion

Perhaps the greatest challenge nowadays is the discovery of potential cellular targets involved in mediating the metabolic syndrome in TBI in order to establish a more precise treatment that may not only control the further development of this pathology but also promote a mitigation or resolution of clinical symptoms. Considering that the disruption of hormonal signalling has been observed in post-TBI patients [33–35], greatly affecting how cells regulate homeostatic processes at the mitochondrial level, this suggests that an exogenous supplementation with DHT, one of the most potent androgens, may have enormous potential as a therapy in TBI. Prospective therapeutic strategies aimed at reducing the impairment of ATP production, the dysregulation of cell metabolism and the activation of apoptotic mechanisms are of clinical interest and have been the focus of numerous studies in recent years [8,9,15–17,32]. In this present study, we aimed to identify these proteins and evaluate by system pharmacology those regulated by DHT and that can be druggable targets of this hormone to counteract the cell damage observed during the acute and chronic phases of TBI. Overall, our results demonstrated that HMGCR, PPGARG, and prohibitin are proteins highly regulated by this androgen and that they may play a crucial role on this pathology.

### 4.1. Steroid metabolic process and regulation of lipid process by DHT

In the present study we found that steroid and lipid metabolism are two of the top ontology categories that can be modulated by DHT and are seen altered due to TBI (Fig. 2, Tables 1 and 2). It is quite known that TBI affects the metabolism of cholesterol and impairs the lipid homeostasis within the brain [20]. One of the most striking features of the pathophysiological mechanism of TBI at the molecular level is the disruption of cholesterol-rich membranes such as plasmatic and mitochondrial not only from the plasma membrane but also from the mitochondrial membranes. The brain is capable of synthesizing cholesterol for its own use and, due of TBI it loses its ability to control the efflux mechanism of excess cholesterol causing it to accumulate significantly in this organ, instead of being exported to the liver via bloodstream to be excreted or eliminated in the form of bile salts. Oxidized by-products of this altered brain metabolism include increased levels of 24S-hydroxycholesterol [36,37], an indicative of cholesterol efflux and the main cholesterol derivatives in the brain, 25-hydroxycholesterol (25HC), which have been seen involved in local inflammation that can lead to more severe secondary damage [38–40].

We report that both HMGCR (HMG-CoA reductase) and PPARG are cellular hubs involved in TBI and potential targets of DHT (Tables 1 and 2). HMGCR is the limiting enzyme of the mevalonate pathway responsible for *de novo* cholesterol synthesis. In TBI lipid metabolism dysregulation due to membrane disruption augments inflammation [20], possibly via activation of TLR4 (toll-like receptor 4) [41,42], and leads to aberrant levels of 25HC, an oxidized form of cholesterol with multiple immune, metabolic and apoptotic properties [43]. Although there are no studies reporting the benefits of DHT on the regulation of lipid metabolism by HMGCR in the brain after a TBI, other studies suggest its lipid-lowering actions in other diseases. For example, DHT at 3.0 mg/kg/d reduces body weight, total cholesterol, cholesterol ester, triglycerides, and LDL levels and increases HDL levels in animals after a diet rich in fatty acids [44]. Taking into account that neuroinflammation by itself can favour the accumulation of lipids [45] and provoke a dysfunction of cholesterol metabolism in the brain and that DHT has broad anti-inflammatory effects, it is feasible to hypothesize that this potent androgen may mitigate in part the dysregulation of cholesterol and lipid metabolism by acting on HMGCR. Due to the accumulation of cholesterol and other lipids in TBI, which can lead to lipotoxicity, not only HMGCR but also PPARG may play a key role in the regulation of inflammation induced by this intracellular rise of fatty acids. Together with SREBF1 and SREBF2, PPARGs are master regulators of lipid metabolism in cells. For example, transcriptional activation of PPARG leads to a rise on IkBα, a protein that maintains NF-kB inactive in the cytosol, avoiding its nucleus translocation and hence inducing the activation of pro-inflammatory signalling with consequent release of cytokines and chemokines [46,47]. On the other hand, it has been shown that increased 25HC decreases PPARG levels, which may contribute to neuroinflammation in TBI [46]. Furthermore, AR

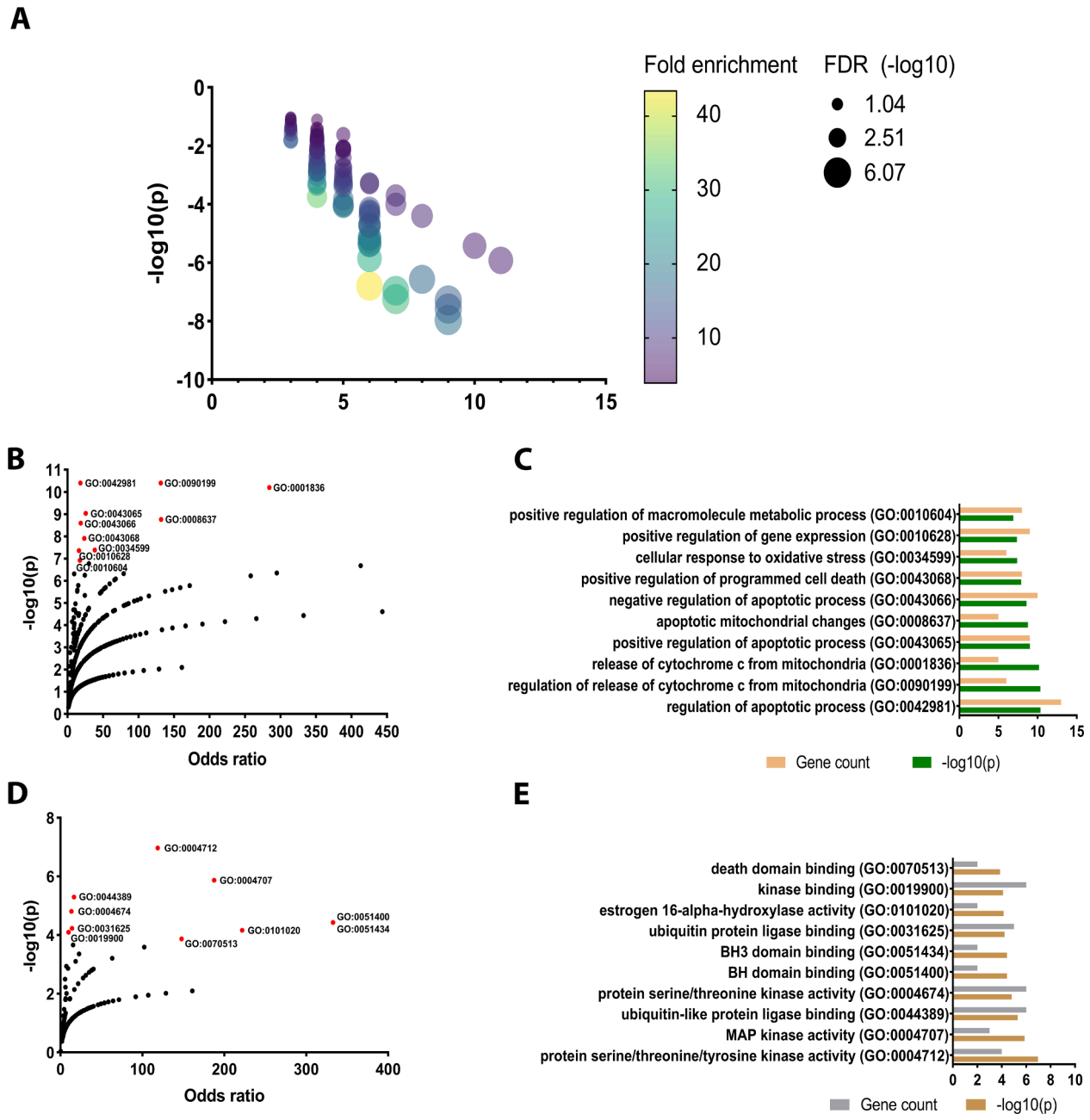


**Fig. 3.** Protein enrichment of mitochondria-related targets between TBI and DHT. Correlation between -log (p-value), false discovery rate, fold enrichment, protein count and top enriched categories. Enrichment analysis according to biological process (B and C) and molecular function (D and E).

activation by DHT downregulates PPARG mRNA and protein levels in mesenchymal cells [48], thus inhibiting adipogenesis on these cells, suggesting a crosstalk between androgenic and PPARG signalling on the regulation of lipid metabolism.

### 4.2. *Participation of mitochondria in lipid metabolism and apoptosis*

Apoptosis is one of the trending ontology categories that has been significantly associated to mitochondria subclusters (Fig. 2D and 3). Interestingly, a mitochondrial protein identified in our study whose key participation in metabolic processes, whilst linked to apoptosis in TBI, is prohibitin (Table 4). Importantly, prohibitin seems like to be significantly oxidized in TBI, suggesting an altered functional capacity of this protein associated with one of the main TBI pathological mechanisms [49]. This protein is part of the prohibitin family, composed of two subunits – PHB1 and PHB2, and both can be found in the nucleus, cytosol and mitochondria [50,51]. Once translocated to mitochondria from the cytosol PHB1 is found to a greater extent localized in the inner mitochondrial membrane which, upon complexing with PHB2, forms a ring-like assembly, playing a crucial role in regulating the dynamics and stability of mitochondria and assembly of mitochondrial respiratory chain complexes. For instance, previous studies showed that PHB controls OPA1 (optic atrophy 1), which is an essential protein for the formation of mitochondrial cristae and for mitochondrial fission/fusion [52,53] and is very much likely found affected in TBI. In this regard, in previous studies by our group we identified OPA1 as significantly decreased after TBI *in vitro*, being this in parallel associated to increases in reactive oxygen species and alterations in mitochondrial membrane potential [15]. In addition to the biological processes mentioned previously, PHB complex when interacting with the anti-apoptotic HAX-1 (HCLS1-associated protein X-1) is able to inhibit apoptosis and favour mitochondrial homeostasis [54]. This is supported by the fact that

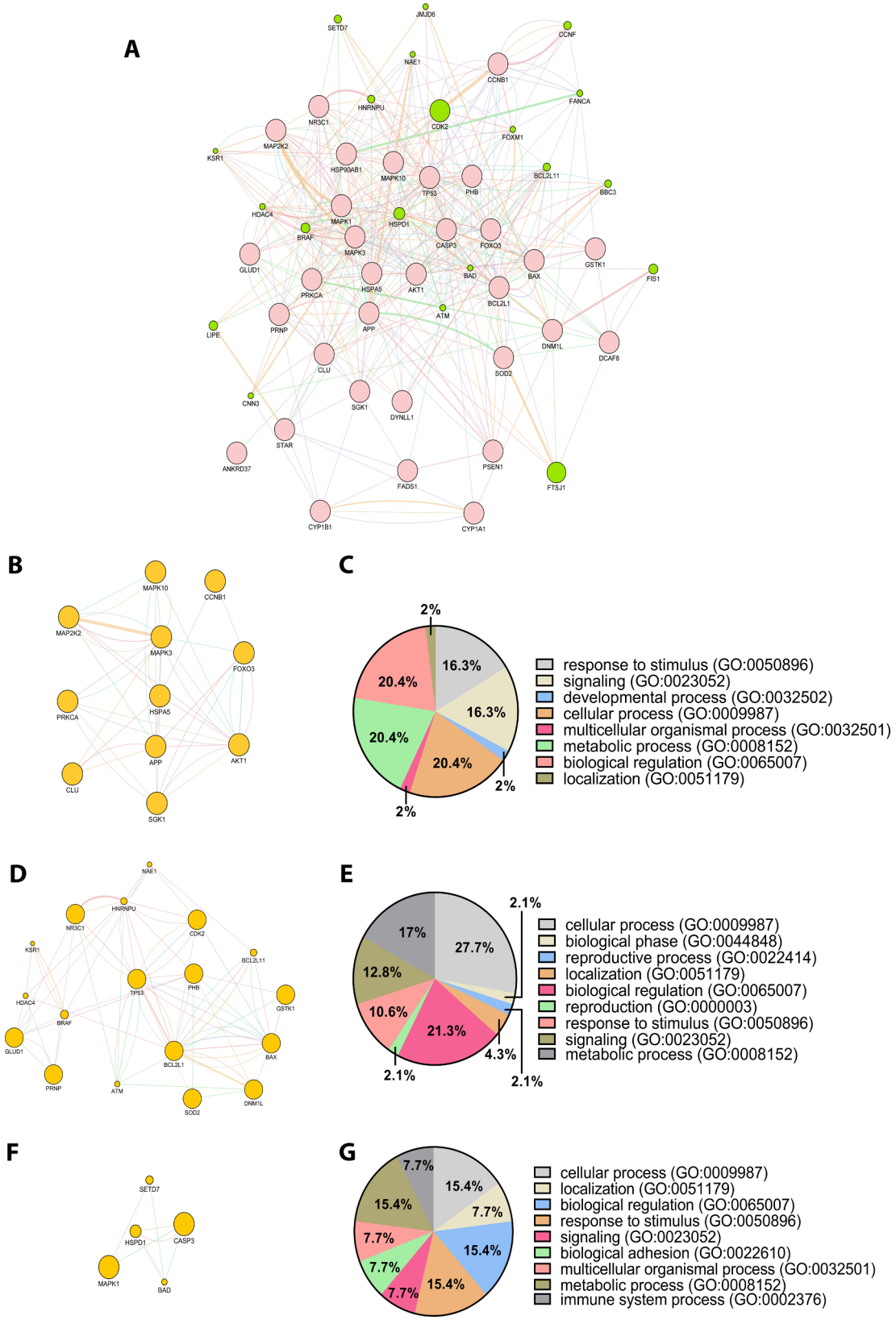


**Fig. 4.** Mitochondrial-related specific subclusters. Cluster 1 containing 11 nodes (B) with a pie chart representing the participation of each ontology term within the network (C). Similarly, cluster 2 has 18 nodes (D) showing that 27.7% of proteins fall within the biological process category (E). Lastly, cluster 3 has 5 proteins (F), of which 15.4% participate in biological regulation, cellular process, response to stimulus and metabolic process (G).

inhibition of PHB by siRNA increases apoptosis, and the fact that PHB complex co-localizes with caspase 3 and PCNA demonstrates its participation in apoptosis [55]. Indeed, in the nucleus, PHB interacts with transcription factors p53 and AR [56–58], suggesting the participation of androgen signalling in the regulation of apoptosis by PHB. After a TBI the levels of PHB complex are significantly elevated in astrocytes and neurons [55], which may be correlated to a destabilization of metabolic processes occurring inside the mitochondria. However, it has been shown that moderate and controlled expression of PHB is neuroprotective by reducing oxidative damage and inflammation in the diseased brain [59]. Considering that by regulating OPA1 prohibitin controls apoptosis [53] and inhibits cell proliferation by repressing the E2 factor (E2F) [60,61] it can be hypothesized that this protein may play a fundamental role in mitigating the pathophysiological mechanism of TBI.

## 5. Conclusions and perspectives

Since there is still no effective and long-lasting treatment against TBI that can significantly reduce inflammation during the chronic period of the disease, studies that aim to explore new therapeutic schemes arise a high interest not only from a clinical perspective but do they also open new opportunities as a hope to improve the quality of life of those patients affected by TBI. Considering that these patients suffer from hypogonadism, which can generate important hormonal alterations not only in the brain but also in the blood, inevitably contributing to the pathological mechanism, a therapy with DHT for repurposing in TBI can mean less burden when compared to the costly and exhausting process of bringing new drugs to market.

By using system pharmacology and enrichment analysis, we identified for the first time that HMGCR, PPGARG, and prohibitin are key novel proteins whose levels may be modulated by DHT and are involved in TBI pathology, hence exerting regulatory functions over mitochondria metabolism. Mitochondria-dependent metabolism of lipids play an essential role, therefore dysregulation of any of these processes may promote mitophagy and/or cytochrome *C* release into the cytosol, leading to the canonical activation of apoptosis. TBI generates profound changes in how lipids are controlled and used as a source of energy or simply as endogenous signalling mechanisms. The mitochondrion is an organelle regulated by hormones, whose levels are altered in TBI, this leads us to hypothesize that mitochondria will no longer be able to carry out lipid metabolism, thus generating a hostile microenvironment due to an intracellular accumulation of lipid intermediates that are highly reactive and can endanger cell survival. By replenishing the pool of androgenic stimulation at mitochondria upon DHT administration with the aim of preserving or maintaining the metabolism, the results of our study indicate that this new pharmacological strategy opens new avenues to better treat TBI patients.

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## Data availability

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

## Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by George E. Barreto, Andrew J. McGovern, David Ramírez and Janneth González. The first draft of the manuscript was written by George E. Barreto and Andrew J. McGovern and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

## Ethics approval

Not applicable.

## Consent to participate

Not applicable.

## Consent for publication

All authors read and approved the final manuscript.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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