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RESEARCH

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Bioinformatic network analysis on the molecular interactions between Parkinson's disease, alpha-synuclein, and infertility condition

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Abstract

Background Parkinson's disease (PD) is a neurodegenerative condition that is characterized by a progressive decline of neural pathways, and its pathology is associated with alpha-synuclein abnormalities. Currently, infertility affects about 10% of individuals of fertile age within the USA. Interestingly, an increased length of fertility is associated with a decreased incidence of PD. Our study utilized QIAGEN's Ingenuity Pathway Analysis (IPA) to identify and analyze molecular pathways that affect the underlying connection between alpha-synuclein (SNCA)-associated Parkinson's disease (PD) and infertility condition (IC). Furthermore, we explored nicotine's potential as a therapeutic in preventing the exacerbation of IC in terms of SNCA.

Results Although the connection between SNCA-related PD and IC is not well explored, the Qiagen Knowledge Base (QKB) showed an overlap of 12 distinct molecules between SNCA and IC. These molecular pathways were established by adding SNCA and IC to "Pathway Explorer" and establishing connections to distinct molecules including transcription regulators, cytokines, and other enzymes/proteins. The Molecule-Activity-Predictor (MAP) tool predicted that SNCA activation would lead to an exacerbation of PD and IC with the potential involvement of dihydrotestosterone (DHT) and caspases. Specifically, it was found that SNCA decreased MAPK8 expression, which led to a downstream upregulation of IC. Activation of nicotine within this overarching molecular network resulted in a downregulation in both PD pathology and IC.

Conclusions Together, these findings reveal a possible connection between infertility condition and genes regularly associated with alpha-synuclein-related Parkinson's disease while identifying nicotine as a potential therapeutic application.

Keywords Ingenuity Pathway Analysis (IPA), Parkinson's disease (PD), Infertility condition (IC), Nicotine, Alpha-synuclein (SNCA)

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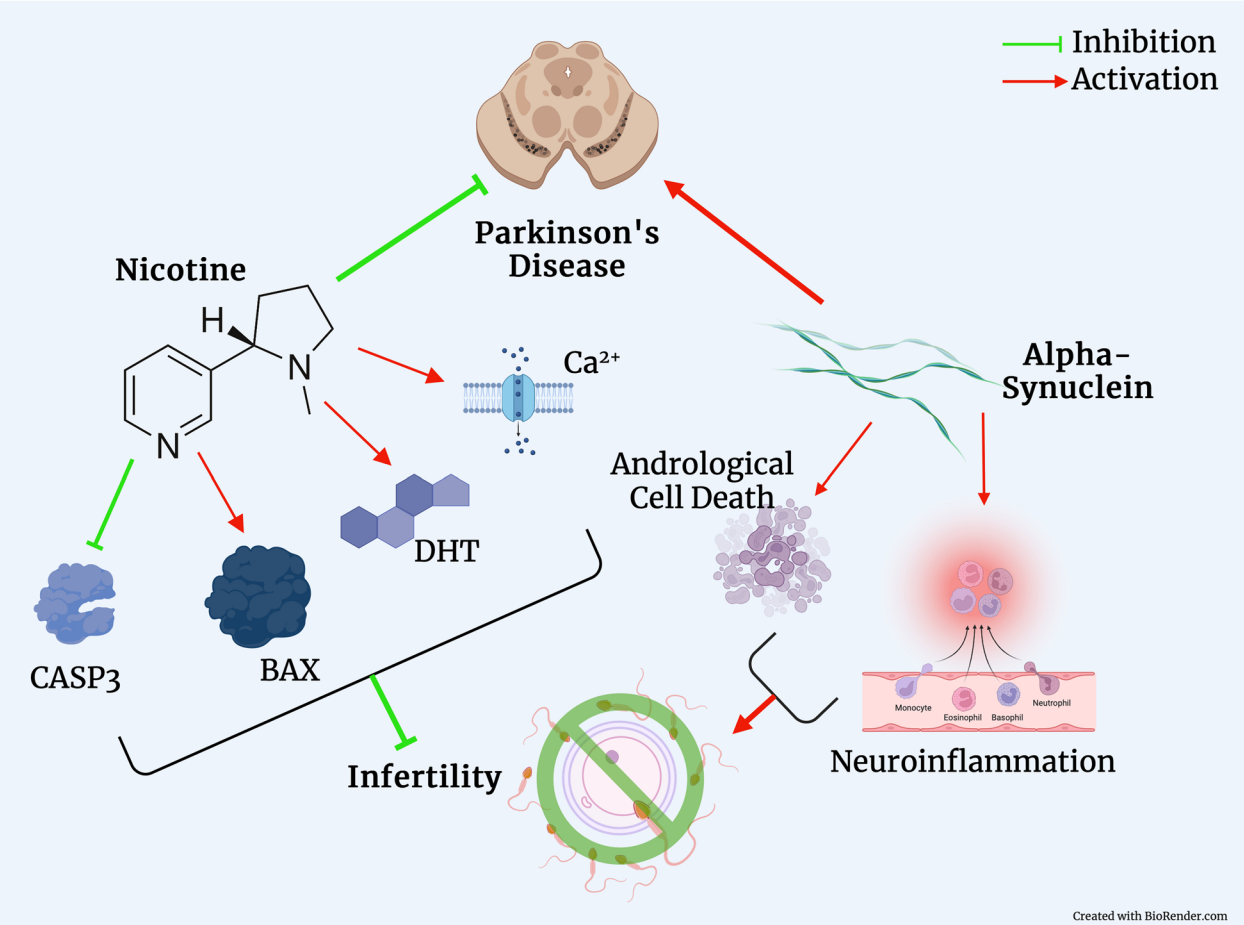
Graphical Abstract



Background

Parkinson's disease (PD) is a chronic neurodegenerative disease that is indicative of motor and nonmotor loss and is characterized by limited mobility as well as decreased muscle control [1]. PD symptoms stem from the loss of striatal dopaminergic neurons as well as neuronal loss in nondopaminergic areas which contribute to the nonmotor symptoms associated with the disease [1]. The typical age of onset for Parkinson's disease is 55–65 years, affecting 1–2% of the population and increases to 3.5% at the ages of 85–89 years [2]. Although it is uncommon to see PD diagnosed in young patients, 5% of PD cases occur before the age of 40 which is an important part of their reproductive years [3]. The pathogenesis of PD suggests that alpha synuclein (SNCA) neuronal transmission in its oligomerized state can be associated with cytotoxicity that leads to the progression of neurodegeneration, contributing directly or indirectly to 90% of PD cases [4]. Additionally, a missense mutation of SNCA is found to be a major component of Lewy bodies (LBs) which form protein aggregates associated with the prognosis of PD [4–6]. The oligomeric conformations, named protofibrils, interfere in cellular homeostasis by causing neuronal death and the mechanisms of action are through intracellular targets that disrupt synaptic functions [7].

Infertility condition (IC) is defined separately in males and females. For females, IC is defined as the inability to conceive after one year of unprotected sexual intercourse and is sometimes associated with diseases such as endometriosis, uterine fibroids, and thyroid disease, although its cause can remain unexplained in other situations [8]. For males, infertility is defined as low sperm count and low testosterone and is found to be present in 9% of males within reproductive age and about 11% of females [8–10]. Increased length of fertility has been associated with a delay in the age

of PD onset. For females, 1 year of prolonged fertility leads to a delay of PD for 0.7 years [11]. The role estrogen has on PD is not yet well understood; however, endogenous estrogen, as well as a person's number of children, age of menopause, and the reproductive life span were all associated with delayed onset of PD [10]. Prior studies on rat models have shown that elevated dihydrotestosterone (DHT) levels exacerbate infertility through reducing gonadotropins and overall concentration of androgens which led to testicular atrophy [12]. However, other studies have shown that DHT has no deleterious effect on the fertility index of male rats and can even improve their fertilizing ability when they are simultaneously treated with tamoxifen [13]. On the other hand, the elevated DHT levels also affect hippocampal synaptic plasticity, delaying the progression of Alzheimer's disease-associated dementia (AD dementia) [14]. To combat the cytotoxic nature of the oligomeric SNCA, nicotine has been found as a possible therapeutic. Nicotine stimulates the nicotinic acetylcholine receptors (nAChRs) of dopaminergic neurons which are usually damaged in PD and lead to the motor symptoms associated with the disease [15]. Many andrological pathologies have shown that caspases are well-characterized in the apoptotic cascade specifically pertaining to spermatocytes [16]. Caspases are implicated in the infertility condition of males specifically through the impairment of spermatogenesis, upregulation of sperm DNA fragmentation, decreased sperm motility, testicular torsion, and immunological fertility [17].

The goal of the present study was the exploration of Parkinson's disease (PD)-related alpha-synucleins (SNCA) and their involvement in infertility condition (IC). A specific focus was given to examining the molecular roles of dihydrotestosterone (DHT) and caspases (CASP) within these intermediary molecular pathways due to previous literature indicating their involvement in IC. Also, nicotine was explored as a potential therapy for SNCA-mediated IC. QIAGEN's bioinformatics tool Ingenuity Pathway Analysis (IPA) was utilized in order to create molecular networks and examine the specific biological roles of these specified molecules. The molecular networks were then compared to findings within the QIAGEN Knowledge Base (QKB) utilizing the canonical pathway analysis and other statistical methods. Unlike a traditional meta-analysis, the present study sought to examine the specific molecular roles of each molecule in the pathway between infertility condition and alpha-synuclein-induced Parkinson's disease based on the curated findings of the QIAGEN Knowledge Base.

Methods

Ingenuity pathway analysis software

IPA is a bioinformatics software tool used for data mining and utilizes the information of canonical pathways which were established from hundreds of thousands of literature studies curated by QIAGEN scientists to aid in interpreting and analyzing various molecular pathways (Fig. 1). A variety of tools were utilized to generate pathways displaying the molecular networks associated with SNCA, IC, and their intermediary molecules to test different functional hypotheses. The bioinformatics tool utilized data from the QIAGEN Knowledge Base (QKB) between February 5, 2022, and April 14, 2022.

IPA "My pathway": identifying overlapping molecules between alpha-synuclein and infertility condition

SNCA and IC were placed in a network by utilizing the "My Pathway" tool. The "Grow" tool was used and found 1179 associated molecules with SNCA and 338 associated molecules with IC. These molecules were found in several cell types/tissues, and all models were considered which included human, rodent, and cell culture datasets. Then, the "Pathway Explorer" tool enabled the exploration of the intermediary molecules between SNCA and IC. There were 12 intermediary molecules found that directly linked SNCA and IC. To ensure the intermediary molecules were downstream of SNCA and upstream of IC, the unidirectional setting within the "Pathway Explorer" tool was utilized. This was done to ensure that only the direct effects of SNCA activation on these intermediary molecules and then on IC were explored. Also, the "Trim" tool was used to remove molecules, such as those not naturally occurring in the biological systems of humans and mammals (i.e., non-endogenous mammalian chemicals, chemical drugs, toxicants). The removal of these identified molecules is based on datasets stored within the QKB. In this instance, molecules not labeled as naturally occurring within humans and mammals were removed from the associated molecule list.

As the activity of a molecule in the pathway is simulated to be inactivated or activated, the "Molecular Activity Predictor" (MAP) tool predicts the activity of cellular functions, diseases, and molecules in response to the simulation of change in expression of a molecular node. The literature findings in the QKB, with the use of the "MAP" tool, demonstrated how the relationship of simulating activation of SNCA affected IC. This analysis provided insight on the possible roles of DHT and caspases have indirectly on promoting IC pathologies. Furthermore, this activity prediction by the "MAP" tool allowed nicotine to be examined as a possible therapeutic agent. For the analyses above and presented further below, the

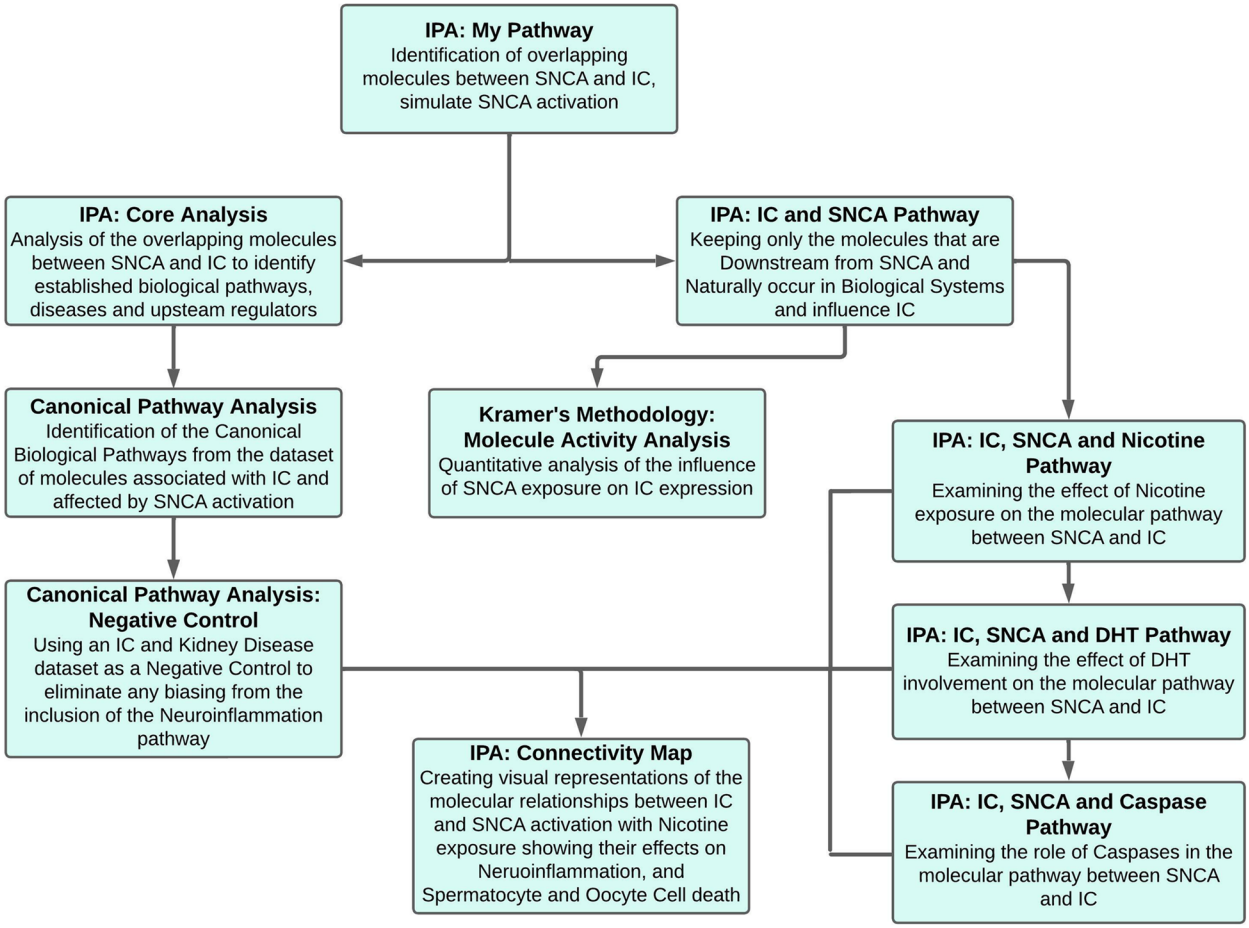


Fig. 1 The workflow utilized from QIAGEN's Ingenuity Pathway Analysis (IPA) bioinformatics software for data mining. The "Grow," "Connect," "Pathway Explorer," and "Molecule Activity Predictor" (MAP) tool within the "My Pathway" feature were utilized to create biological networks that illustrated the connectivity between various nodes. Furthermore, Kramer's methodology for downstream effect analysis was used to quantify the expression changes predicted by the "MAP" tool. Also, the "Core Analysis: Expression Analysis" feature was used to compare the molecules within the generated molecular pathway with canonical pathways stored within QIAGEN's knowledge base (QKB)

sex differences in infertility condition were not differentiated, since the data for female and male infertility was limited within the QKB at the time of the study.

Quantitative analysis of the effect activation of alpha-synuclein on infertility condition

The changes in expression of the activation of SNCA on IC determined by the "MAP" tool were quantified using the algorithm "Kramer's Methodology for Downstream Effect Analysis," which uses the information from the QKB to generate data points [18]. The algorithm solely considers literature data points downstream of the initial connection; thus, only the unidirectional analysis in Fig. 2a and b was utilized for the analysis. The activation *z*-scores for the intermediary molecules downstream of SNCA and upstream of IC were determined by Kramer's algorithm using 78 references from the QKB to further elucidate the predicted effect of SNCA activation on IC expression determined by the "MAP" tool. The scale for the expression change (*z*-score) for each molecule is between −2 and +2. The value of +2 represents a strong excitatory relationship, whereas −2 represents a strong inhibitory relationship. Thus, Kramer's analysis was utilized to quantify the change in magnitude of the activity of intermediary molecules upon IC when SNCA is activated.

Core analysis: expression analysis of canonical pathways

The molecular pathway exhibiting the relationship between SNCA and IC generated with the "My Pathway" tool was further analyzed by utilizing the IPA tool "Core Expression Data Analysis" and provided multiple analyses including an "Upstream Analysis," analysis of diseases related to the molecular pathway, and "Canonical Pathway Analysis." The "Canonical Pathway Analysis" involves the generated pathway between SNCA and

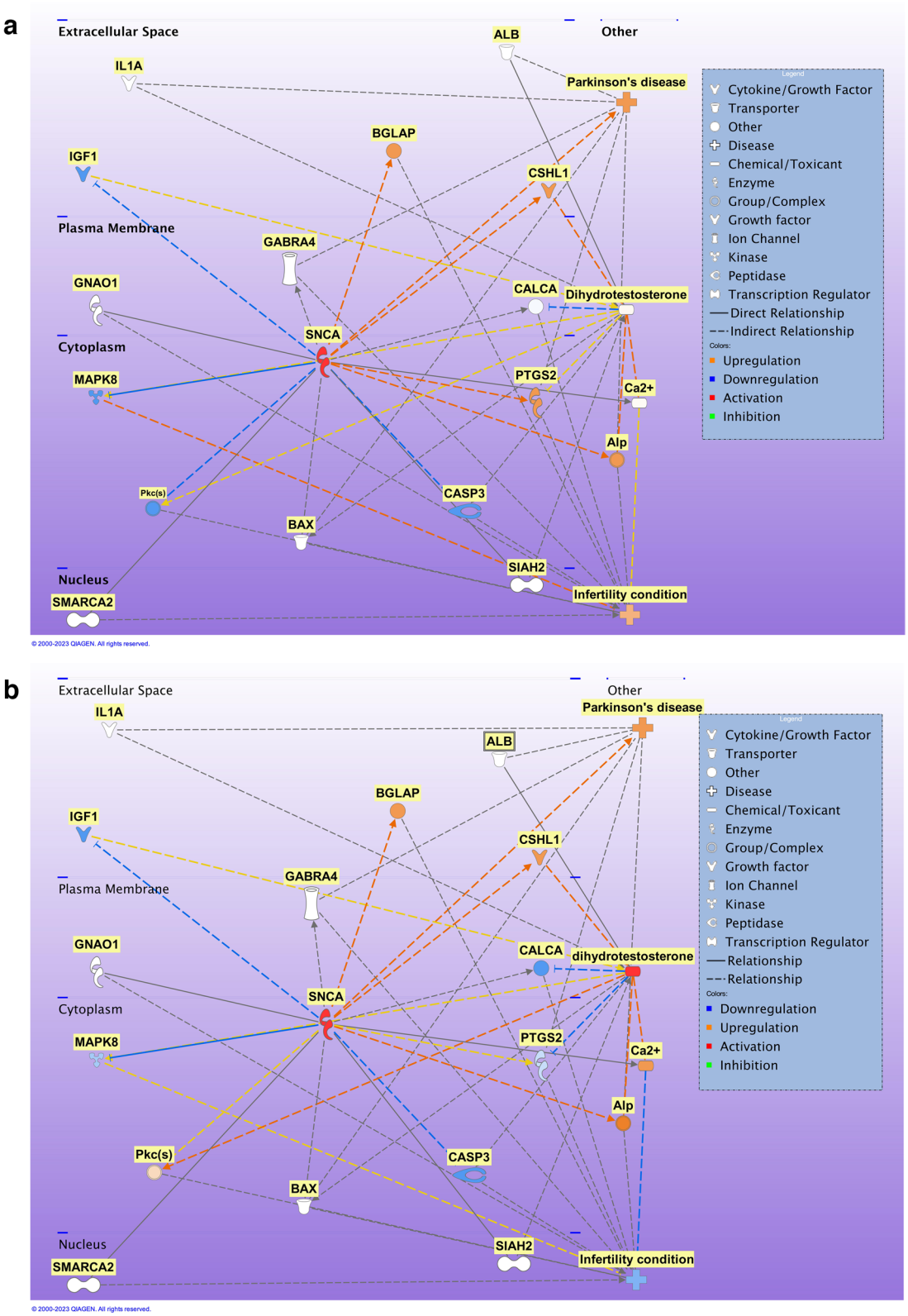


Fig. 2 **a** Molecular network illustrating the connectivity and relationships of the 12 overlapping molecules between activated SNCA and IC with the addition of DHT. **b** Molecular network illustrating the connectivity and relationships of the 12 overlapping molecules between activated SNCA and IC with the addition of activated DHT

IC being compared to the 705 canonical pathways of the QKB resulting in the determination of overlapping molecules between the two datasets. The "Canonical Pathway Analysis" provided an analysis of the biological canonical pathways that likely correlated with the intermediary molecules between SNCA and IC. These canonical pathways are pathways that QIAGEN has established and stored within the QKB based on data from hundreds of thousands of studies which were then verified and established by researchers through manual curation. Additionally, the right tailed *p*-value from the Benjamini-Hochberg-corrected Fisher's exact test was assigned to the molecular dataset to indicate the probability of the number of molecules overlapping between the canonical pathways and the molecular dataset. To mediate any potential biases, a negative control "Canonical Pathway Analysis" was conducted, between kidney disease (not severe enough to be associated with kidney failure) and IC since no priori-associations between the diseases were found during broad literature searches and within the QKB itself. The negative control was run to ensure that the connections found between the molecular data set and PD was not due to biasing of curated literature datapoints within the QKB.

Results

Molecular pathway analysis of molecules mediating the relationship between alpha synuclein and infertility condition

The "MAP" tool generated a connectivity map illustrating the relationship between SNCA and IC. The 12 intermediary molecules in Figs. 2a and b are organized based on the cellular location directly linking SNCA with IC and were the focus of the study as they conveyed the potential pathway between PD-associated SNCA and its effect on IC. The next step involved adding DHT to the pathway as literature studies revealed DHT as a significant contributor to infertility conditions. DHT was connected to the pathway, and its relevant intermediary connections to SNCA, PD, and IC were established using the "Connect" and "Pathway Explorer" tools. A key feature of the "MAP" tool is the ability to visually quantify the level of activation of the molecules based on the opacity of the coloration: a greater degree of coloration represents a greater magnitude of change of activation or inhibition, orange indicates upregulation, and blue indicates downregulation. The "Kramer's Methodology for Downstream Effect Analysis" is able to assign a *z*-score valuation to this opacity by examining the number of associated literature studies that indicate an excitatory relationship (up to +2) or inhibitory relationship (up to −2). The "MAP" tool was utilized to activate SNCA which predicted no activation of DHT, upregulation of IC, and upregulation of PD, which can be visualized by the orange coloration (Fig. 2a). The upregulation of IC by SNCA appeared to be specifically mediated through molecular interactions with mitogen-activated protein kinase 8 (MAPK8) and calcium (Ca^{2+}); however, the connection (upregulation or downregulation) of Ca^{2+} was inverse of its downstream activity which is shown by the yellow coloration of the dotted line. This indicates that the current activity of Ca^{2+} cannot be determined. However, the figure demonstrates that SNCA activation causes a downregulation of MAPK8 which then leads to an upregulation of molecules associated with IC pathology. Then, the MAP tool was utilized to activate SNCA and DHT which predicted the downregulation of IC which can be visualized by the blue coloration, but this maintained the upregulation of PD as observed through the node's orange coloration (Fig. 2b). The connection between MAPK8 and IC switched from indirectly upregulating IC (orange dotted line) to being opposite its downstream activity (yellow dotted line). Meanwhile, the connection between Ca^{2+} and IC changed from opposite its downstream activity (yellow dotted line) to downregulating IC (blue dotted line) when DHT is simultaneously activated with SNCA.

Canonical pathway analysis of the molecular network of molecules affected by activation of alpha-synuclein and infertility condition

The "Core Analysis, Expression Analysis" tool was used to compare the 705 canonical pathways of the QKB with the 12 intermediary molecules associated with SNCA and IC. Figure 3 reveals the 10 canonical pathways with the largest *p*-values [-log(*p*-value)]. The canonical neuroinflammation signaling pathway had the greatest overlap with the molecular data set with a $p\text{-value} = 9.48 \times 10^{-15}$ [-log(*p*-value) = 14.023] and a 3.3% overlap. The neuroinflammation signaling pathway relates to the cell death of andrological cells which is one of the pathologies related to IC, therefore indicating neuroinflammation's potential role in IC [19]. Another significant canonical pathway was the amyloid processing pathway with a $p\text{-value} = 1.8 \times 10^{-9}$ [-log(*p*-value) = 8.745] and 9.6% overlap. The glucocorticoid receptor signaling and adrenomedullin signaling canonical pathways had the greatest overlap with the molecules associated with kidney disease (not severe enough to be associated with kidney failure) and IC. The glucocorticoid receptor signaling pathway with a $p\text{-value} = 5.04 \times 10^{-12}$ [-log(*p*-value) = 11.298] and a 1.9% overlap. The top 10 canonical pathways of the negative control did not include the canonical neuroinflammation signaling and amyloid processing signaling pathway. The results of the negative control strengthen the confidence in the relationship between the SNCA and IC molecular dataset and the

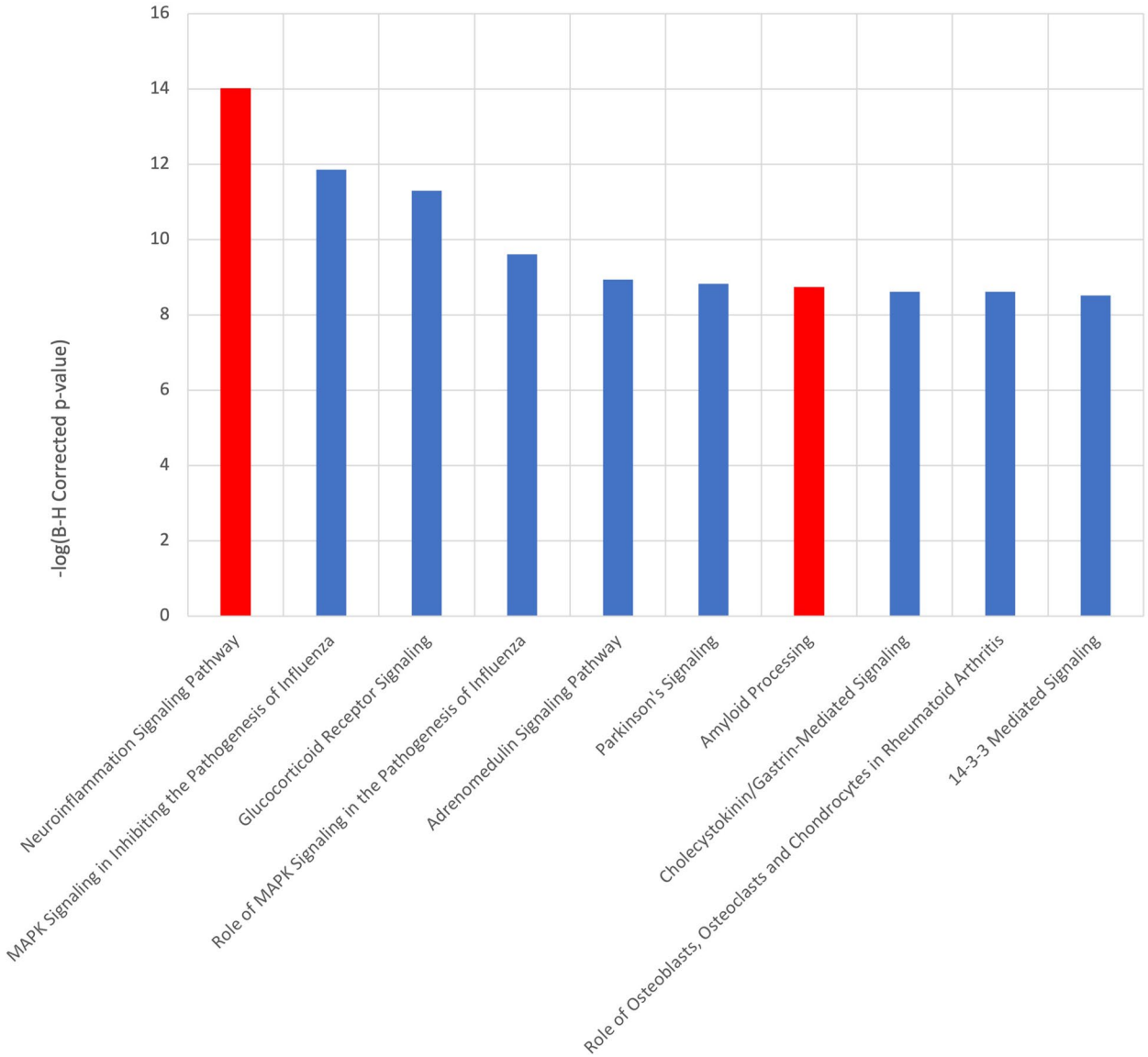


Fig. 3 The top 10 canonical pathways ranked based on their Benjamini–Hochberg corrected *p*-value and evaluated according to the overlap of the molecules within the canonical pathway and the 12 intermediary molecules associated between SNCA and IC

canonical neuroinflammation signaling and amyloid processing signaling pathways because it ensures the exclusion of non-specific and general associations.

Quantitative expression changes for the intermediary molecules between alpha-synuclein and infertility condition utilizing Kramer's methodology for downstream effect analysis

Kramer's methodology for downstream effect analysis was used to calculate the quantitative expression changes predicted by the "MAP" tool in IPA [18]. The *z*-score expression changes for each of the 12 intermediary molecules when SNCA is activated were then plotted in Fig. 4. Of the 12 intermediary molecules, 7 expressed downregulation, while 5 expressed upregulation according to Kramer's methodology for *z*-score quantitation. While a *z*-score of + 2 indicates a strong excitatory effect, a *z*-score of − 2 indicates a strong inhibitory relationship. Thus, when SNCA was activated, there was observed excitation of Ca^{2+}, Alp, BGLAP, PTGS2, and MAPK8. Meanwhile, for SMARCA2, CALCA, GABRA4, GNAO1, CASP3, BAX, and Pkc(s), there was observed inhibition. GABRA4 expressed the greatest inhibitory change with a *z*-score of − 1.732, while Ca^{2+} and Alp expressed the

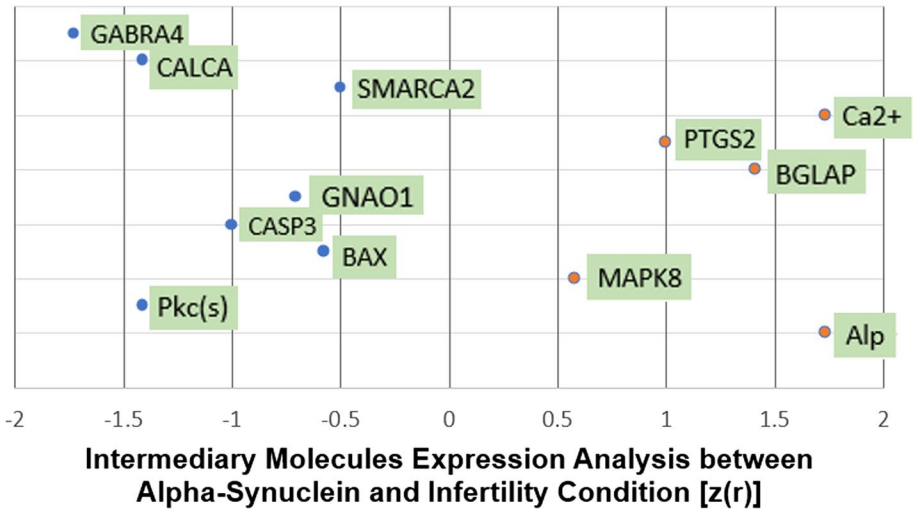


Fig. 4 Visual illustration of the quantitative expression changes calculated by Kramer's methodology for downstream effect analysis of the 12 intermediary molecules between SNCA and IC

greatest excitatory change with *z*-scores of 1.732. The predicted effects from Kramer's methodology closely match the predicted effects from IPA's "MAP" tool. This increases the confidence of the predictions that the "MAP" tool yields based on its algorithm and conveys that any observed differences are likely due to differences in defined points according to the QKB and Kramer's methodology. Therefore, the analysis indicated that the "MAP" tool provides robust and quantifiable, predicted changes in molecular activity that are indicated by the opacity of node coloration.

Molecular pathway analysis of nicotine's activation on the molecular relationships between alpha synuclein and infertility condition

The "Grow" and "Pathway Explorer" tools were used within the "My Pathway" function in order to create an intermediary molecular pathway between SNCA and IC. Then, nicotine was individually connected into the pathway through each of the 12 intermediary molecules using the "Pathway Explorer" tool. It was found that specific molecular nodes had findings within the QKB to support the connectivity between nicotine and these specific intermediary molecules. Figures 5a and b indicate that nicotine has significant connections to Pkc(s), PTGS2, GNAO1, BAX, Ca^{2+}, Alp, and CASP3. Furthermore, the "MAP" tool was utilized in order to observe the effect that nicotine exposure has on the pathway between SNCA and IC. As compared between Figs. 5a and b, nicotine activation which indicates nicotine exposure caused a predicted downregulation of both IC and PD. The decreased opacity of the orange coloration demonstrates downregulation as the nodes for both IC and PD are significantly more opaque in Fig. 5a than in Fig. 5b, where nicotine is activated. Nicotine activation also decreased the downregulation of Pkc(s), downregulated GNAO1 and BAX, upregulated Ca^{2+}, and switched CASP3's activity from downregulation upon exclusive SNCA activation to upregulation when there is simultaneous SNCA and nicotine activation. However, there was no change in MAPK8's activity as it was not associated with nicotine either directly or indirectly. These figures suggest that nicotine exposure influences both IC and PD through the established intermediary pathway between SNCA and IC.

Molecular pathway analysis of caspases involvement on the intermediary molecular interactions between alpha synuclein and infertility condition

Like the previously generated pathways, to evaluate the role of caspases in their mediation between SNCA activation and IC, the "Grow" and "Pathway Explorer" functionalities within IPA were utilized. Using the 12 intermediary molecular pathways between SNCA and IC, each of the caspases between CASP1 to CASP14 were connected to the network to examine their effects. It was found that CASP1 is directly connected to SNCA and then connected to CASP3, 4, 5, 7, 8, 10 (note: CASP3 was already directly connected to SNCA). Also, CASP2, 4, 5, 7, 8, 9, 10, and 14 are upregulated when SNCA is activated and modulate the activity of BAX, CASP8, MAPK8, CASP3, and Ca2+. Figures 6a and b shows a top-down pathway of alpha synuclein being activated in the presence of general caspases and IC. As SNCA is activated in Fig. 6a, shown with the molecule highlighted in red, there is a downregulation of caspase as marked by the blue

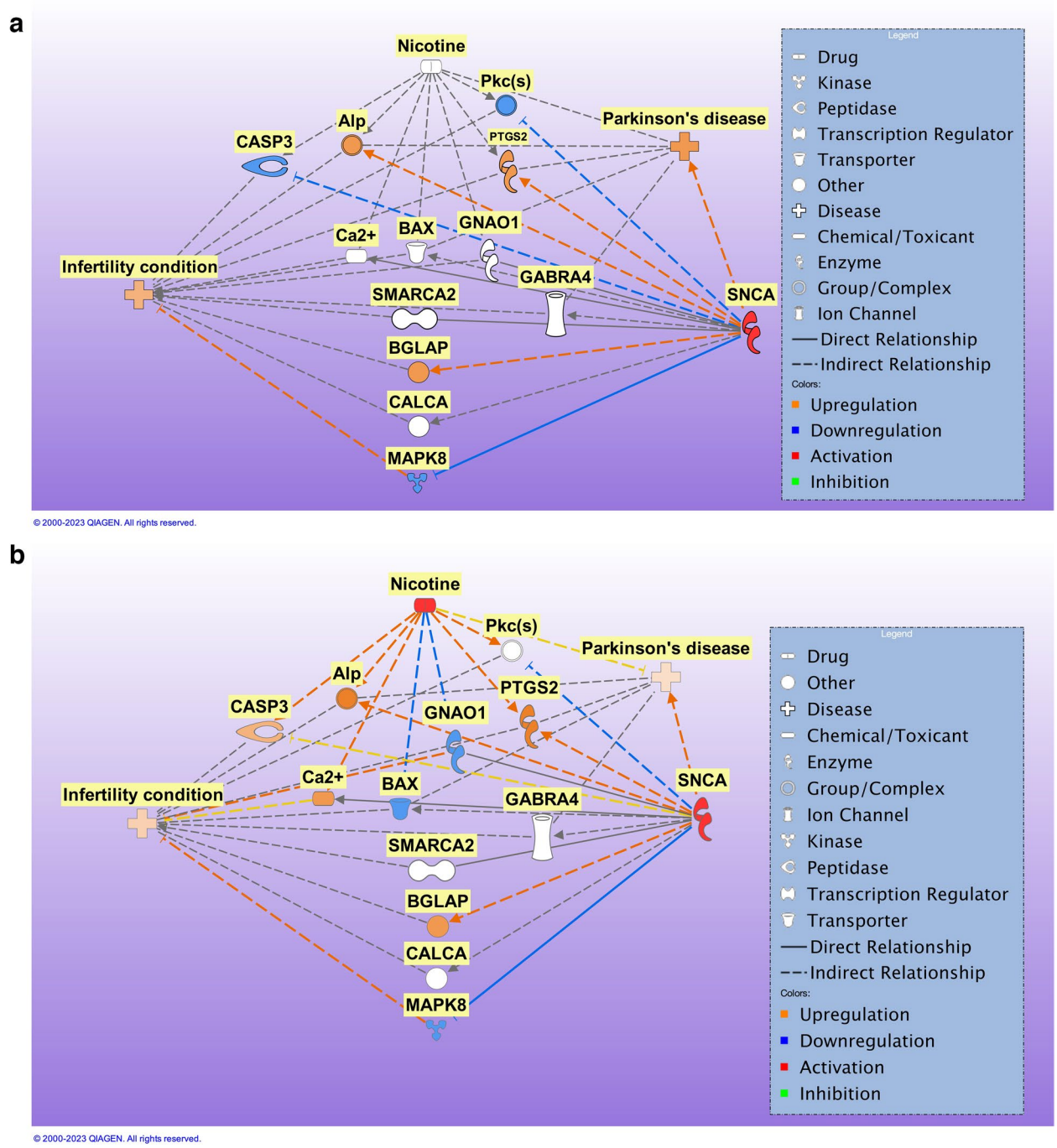


Fig. 5 **a** Molecular network illustrating the connectivity and relationships of nicotine to the 12 overlapping molecules between activated SNCA and IC. **b** Molecular network illustrating the connectivity and relationships of nicotine to the 12 overlapping molecules between activated SNCA and IC with simultaneous activation of nicotine

lines connected from SNCA to the caspase node. The activation of SNCA also leads to the upregulation of Alp, PTGS2, BGLAP, and the downregulation of Pkc(s). The downregulation of general caspases leads to the downregulation of CASP8 and CASP3. The downregulation of the general caspase node and the upregulation of BGLAP lead to decreased MAPK8 activity, which then upregulate IC. In Fig. 6b, SNCA is activated which leads to an upregulation of PD as seen in Fig. 6a as well. However, in Fig. 6b, the general caspases node is activated in

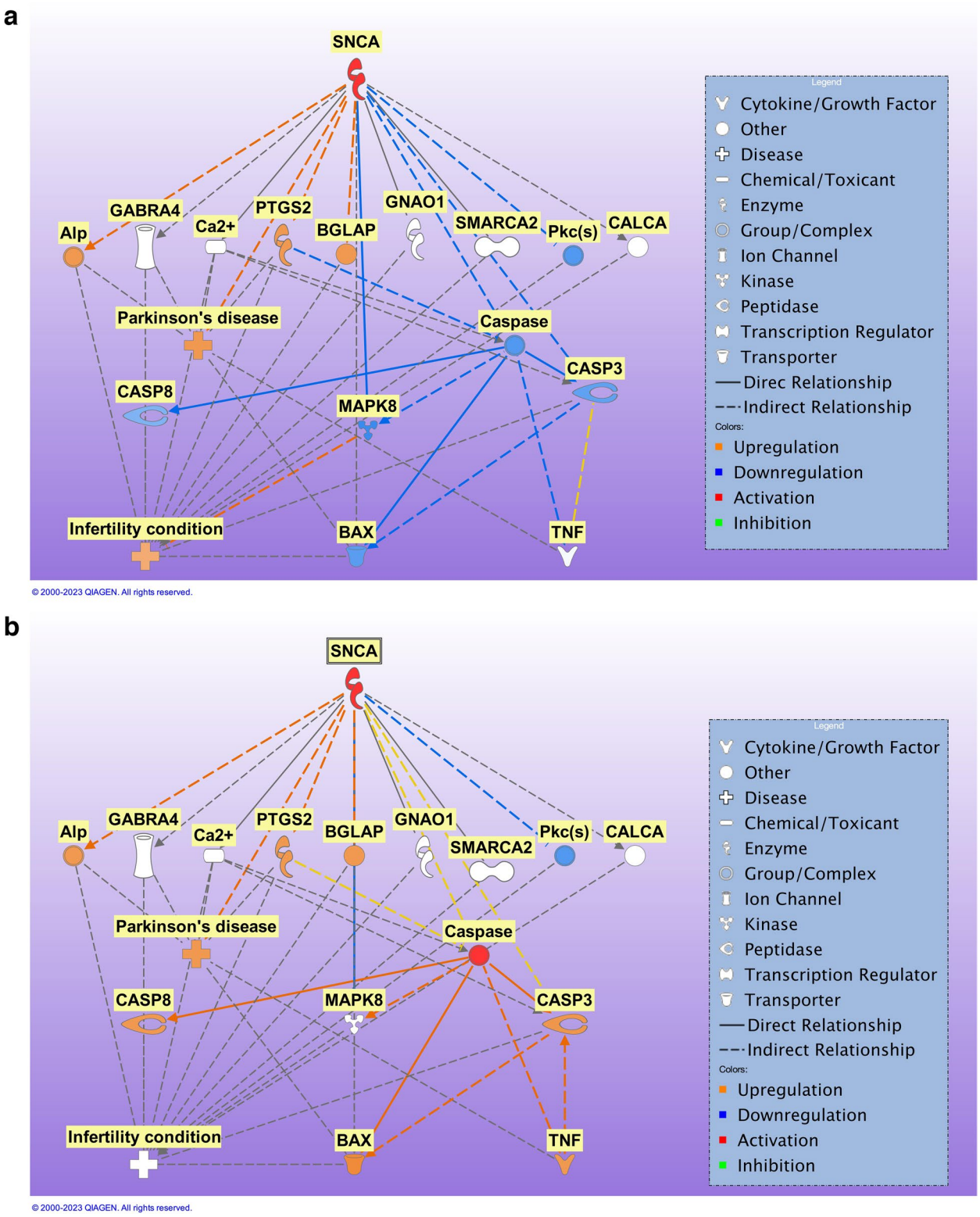


Fig. 6 **a** Molecular network illustrating the connectivity and relationships of CASP3, CASP8, and caspase to the 12 overlapping molecules between activated SNCA and IC. **b** Molecular network illustrating the connectivity and relationships of CASP3, CASP8, and caspase to the 12 overlapping molecules between activated SNCA and IC with simultaneous activation of caspase

conjunction with the activation of alpha synuclein. The activation of general caspases further upregulated CASP8 and CASP3. With the activation of caspase, as well as the continued upregulation of BGLAP, the nodes for MAPK8 and IC exhibit a decrease in opacity. This is indicative that the upregulation that led to IC has been significantly decreased suggesting decreased exacerbation of IC etiology upon increased caspase activity.

Discussion

These data and the accompanying molecular networks indicate that SNCA activation, which is commonly associated with developing PD, exacerbates the etiology of IC. The intermediary molecules illustrating the biological pathway between PD-associated SNCA and IC may elucidate the finding that an increased age of fertility is correlated with a delayed or decreased onset of PD [11]. Additionally, the present findings support the accepted etiology of PD oligomeric neurotoxicity of SNCA [10]. The neurotoxicity of SNCA was focused on within the study due to its well-defined role in PD pathology and the correlation between an increased length of fertility and decreased incidence of PD [4–6]. Our molecular networks and associated intermediary pathways demonstrate that increased SNCA levels, normally associated with PD, exacerbate IC pathology, specifically through molecular pathways involving Ca^{2+} and MAPK8 (Fig. 2), and that nicotine may act as a potential therapeutic agent towards SNCA-associated IC etiology (Fig. 5).

High concentrations of DHT have been shown to exacerbate infertility in rat models by reducing the concentration of gonadotropins and other androgens that are necessary for andrological cell development [12]. Additionally, male rams exposed to high pre-natal concentrations of DHT or testosterone have less motile sperm and severely more occluded seminiferous tubules than the control group [20]. These findings indicate that increased exposure to DHT is seemingly correlated with increased levels of IC. Yet, other studies have shown that DHT implants in tamoxifen-treated male rats actually increased the fertilizing ability of their sperm and had no negative effects fecundity or fertility index [13]. Similarly, Fig. 2a indicates a finding contrary with DHT's role in IC as defined by the previously stated literature. When SNCA is activated within the pathway, IC is upregulated. But, in this intermediary pathway, the node for DHT remains uncolored which indicates that there are currently not enough findings within the QKB to predict its activity change by the "MAP" tool. Thus, it is unable to be assessed what DHT's role is in SNCA-associated IC in humans. One study has shown that decreased levels of DHT due to 5α-reductase inhibitors were associated with a slight decrease in viable sperm, but these changes were found to be readily reversible [21]. Therefore, DHT's role in IC, specifically SNCA-associated IC, is not yet well-characterized in humans and may be a viable subject for further exploration on its role in IC.

Furthermore, DHT has been implicated in the role of neurodegenerative conditions where elevated levels are thought to affect the synaptic plasticity of hippocampal neurons and delay neurodegeneration [14, 22, 23]. But these effects seem to be largely confined to the pathologies of Alzheimer's disease (AD) and multiple sclerosis (MS) since only variable effects have been seen with androgen therapy of PD. Some studies have shown inconsistent results of androgens', such as DHT, effects on worsening/improving PD pathology in castrated mouse models vs. human clinical cases where androgen deprivation therapy seemed to slightly improve PD in humans but had no effect in mouse models [24]. Furthermore, other studies have examined the effect of DHT on glial-derived neurotrophic factor (GDNF) through rat Sertoli cell culture and found that DHT improved GDNF expression [25]. Although not directly shown in the study, this may indicate DHT improving PD by decreasing SNCA through increased GDNF expression. While its role remains controversial, conflicting bodies of research have postulated the neuroprotective effects of GDNF and its role as a preventative against PD [26, 27]. The finding in Fig. 2b supports this theory of GDNF's neuroprotectivity for PD. When SNCA and DHT are activated, IC appears to be downregulated. This exacerbation of PD could possibly be explained by DHT's effects on increasing SNCA and decreasing levels of potentially neuroprotective GDNF. However, the unexpected downregulation of IC due to DHT activation in Fig. 2b remains unclear and may be due to the biological differences between humans and other mammalian models for IC. For example, DHT is known to upregulate Ca^{2+} [28], which is implicated in improving or rescuing fertility in male and female rodent models [29]. In our dataset, specifically shown in Fig. 2b, the activation of DHT led to an increase in Ca^{2+} which is associated with a decrease in IC pathology. Therefore, our data indicates the possibility of DHT as an inhibitor of IC through the Ca^{2+} molecular pathway between SNCA and IC.

The "Canonical Pathway Analysis" and associated molecular pathways from IPA suggest that neuroinflammation and amyloid processing may be the main etiologies that exacerbate SNCA-associated IC. Of note, the neuroinflammation signaling and amyloid processing pathways were found to be two of the most significant canonical pathways in the QKB in terms of overlapping molecules to the intermediary pathway between SNCA and IC. Neuroinflammation is well-characterized in PD where PD-associated genes are known to shift the roles

of astrocytes and microglia to become more neurotoxic [30]. Furthermore, amyloid processing leads to the creation of amyloid-β plaques and SNCA oligomeric tangles that spread to the central and peripheral nervous system where they can exacerbate neuroinflammation and cause cell death [31]. Less well-characterized are the roles of these pathways in IC. Neuroinflammation characterized through tumor necrosis factor α-induced protein 3 (TNFAIP3) has been associated with decreased fertility by irregular estrous cycling, insulin resistance, and decreased secondary ovarian follicles in female mice [32]. Furthermore, it has been hypothesized that neuroinflammation causes dysregulation of the hypothalamic-pituitary–gonadal axis neurons which are known to regulate androgens vital to maintaining fertility [32, 33]. Also, amyloid-like substances which are important to sperm maturation in mice and facilitate cellular communication in oocytes have been found to dysregulate fertility and cellular maturation when improperly processed [34]. Together, our results suggest that elevated levels of SNCA, which are associated with PD, exacerbate IC by modulating neuroinflammation and amyloid processing (Fig. 7). This increased level of neuroinflammation exacerbates PD pathology but also acts upon critical fertility pathways and cycles. Furthermore, the dysregulated modulation of amyloid processing by SNCA likely leads to the transmittance of these neurotoxic plaques and tangles to peripheral neurons [9, 31]. Figure 5a demonstrates that increased SNCA increases IC pathology through the molecular pathway of MAPK8 (Fig. 5a).

Nicotine is theorized to have neuroprotective properties in terms of neurodegenerative conditions, including PD which was originally postulated due to the decreased incidence of PD in smokers [35, 36]. It is believed that nicotine's action of neuroprotectivity is due to its activity upon nAChRs. In Fig. 7, we observed decreased incidences of IC and PD. This downregulation is due to the activation of nicotine in the presence of SNCA-associated IC. Nicotine exposure caused an upregulation of Ca^{2+} which is correlated with increased fertility as Ca^{2+} is a necessary ion for the motility of sperm cells and oocyte maturation [28, 29]. Furthermore, it was found that nicotine activation led to the decrease of cell death for both sperm and oocytes which may be a potential biological mechanism for decreasing

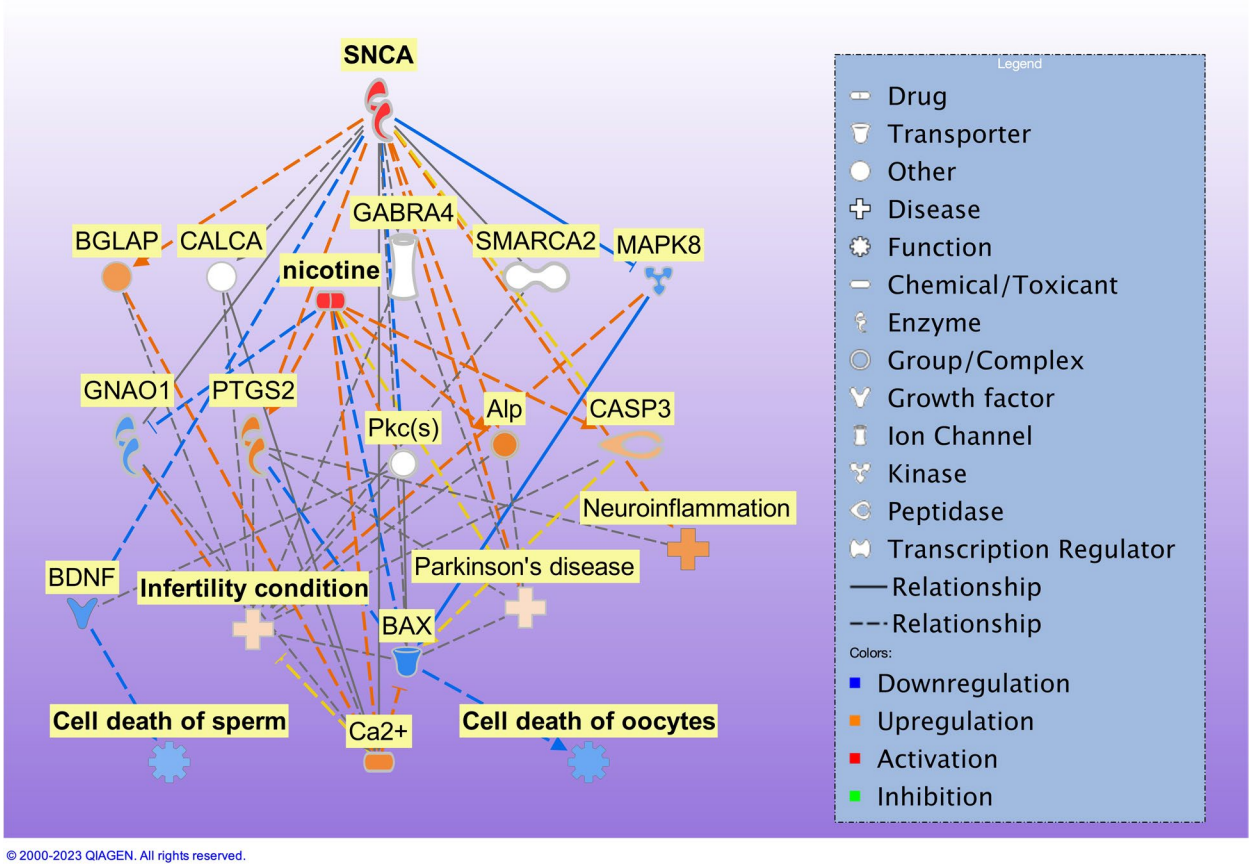


Fig. 7 Molecular network illustrating the connectivity and relationships of nicotine's activation on the molecular pathway of SNCA-associated IC with its effect on neuroinflammation and the cell death of sperm and oocytes

IC (Fig. 7). However, nicotine's effects did not decrease or directly act upon the neuroinflammation pathway, but the PD node still experienced decreased upregulation likely due to its direct connection with nicotine. In corroboration, some research has shown that nicotine exposure in human and rat derived iPSC-derived dopaminergic neurons was able to protect these cells against damage associated with SNCA aggregation [37]. Therefore, nicotine may prevent cellular damage to andrological neurons and cells and minimize IC through a similar mechanism. Additionally, nAChRs are expressed in astrocytes and microglial cells throughout the CNS [38, 39]. Nicotine's binding and activation of these receptors is associated with anti-inflammatory effects through vagus nerve action to the hypothalamic–pituitary–adrenal (HPA) and hypothalamic-pituitary–gonadal (HPG) axes [39]. Furthermore, this cholinergic effect is associated with decreased APP processing and reduced amyloid aggregation that potentially contributes to infertility [40, 41]. As previously explained, preventing the improper processing of amyloid-like substances prevents the transmittance of amyloid plaques and neurofibrillary tangles that would exacerbate cell death. As seen in Fig. 7, nicotine activation downregulates BAX. BAX is a well characterized apoptosis regulator and its inactivation in female rats is associated with extending the fertile period and sustaining the ovarian lifespan [42]. Therefore, nicotine's downregulation of BAX indicates its potential effect on preventing oocyte cell death and sustaining ovarian development. Thus, nicotine has potentially therapeutic effects in terms of SNCA-associated IC, specifically through the BAX molecular pathway. Additionally, this extension of fertility is correlated with a decreased incidence of PD [11, 12].

Another important class of molecules in the network between SNCA and IC is caspases [43]. The regulation of this pathway is integral to the differentiation of spermatocytes and testicular maturity and its dysregulation is implicated in infertility due to decreased sperm motility and DNA fragmentation [44]. Furthermore, recent research has suggested the inflammasome's involvement in inflammatory sterility through CASP1 activation and inflammatory cytokines [45, 46]. The inflammasome is defined as innate immune receptors that regulate CASP1 activation and inflammatory response in response to infection [46]. Additionally, CASP3 activation is involved in the apoptosis of granulosa cells, but its levels are not elevated in apoptotic primordial and primary follicles [44–47]. Of note, CASP1 and CASP8, in addition to CASP3, were found to modulate IC as conveyed in Fig. 6a and b. Figure 8 conveys that CASP1 primarily regulates the caspase response to SNCA-associated IC and its inflammatory involvement. This is corroborated by research findings that have revealed that CASP1 is the apical caspase that leads to CASP3 activation in the inflammasome response [48].

Additional studies of ejaculated sperm have indicated that there is increased CASP3 activity in low motility sperm, which caused DNA fragmentation [49]. Also, the ratio of pro-apoptotic factors, such as CASP8 and cell survival factors, has been implicated in the maintenance of apoptosis of germ and granulosa cells in the vertebrate ovary, and high levels of this regulated cell death can negatively affect female fertility [50]. Our data reveals the complicated roles of caspases in SNCA-associated IC. While literature findings have indicated that upregulation of caspases leads to increased reproductive cell apoptosis, the data in Fig. 8 reveals that SNCA activation leads to CASP1 upregulation which appears to decrease the upregulation of CASP3 and CASP8. This finding suggests the novel implication that increased CASP1 activity leads to the increased regulation of pro-apoptotic factors such as CASP3 and CASP8 which may prevent disturbances to the apoptotic factor/cell survival factor ratio and thus lead to a prevention of IC pathology through decreased reproductive cell death. Also, some research has revealed that the inhibition of CASP3 prevents the neurotoxicity of androgens, such as testosterone and DHT, on dopaminergic neurons by decreasing the protein kinase-C (Pkc)-induced mitochondrial dysfunction [48]. Although differing from the indication of Fig. 8, our data demonstrates that caspases, specifically CASP1, 3 and 8 are not well understood in the context of neurodegenerative induced fertility and that elevated caspase levels may have a positive synergistic effect on the prevention of IC resulting from increased SNCA, as seen in PD.

The in silica findings from the bioinformatic analyses within IPA have suggested the potential roles of DHT and caspases in the mediation of PD-associated SNCA involvement on IC. Additionally, our findings indicate the possibility of nicotine as a therapeutic agent for SNCA exacerbated IC through its potential to modulate neuroinflammation, reproductive cell death, and amyloid processing. Furthermore, the study conveys the complexity of pathways contributing to infertility and indicates the neurological basis for certain types of fertility. Specifically, it appears that IC resulting from increased SNCA, as found in PD, is modulated by the pathway from SNCA to MAPK8 and Ca^{2+}, as well as other poorly characterized intermediary molecules. Thus, the data revealed that MAPK8 and Ca^{2+} may play a critical role is causing SNCA-associated IC. This study focused on the role that PD-associated SNCA had on IC and found that SNCA upregulated apoptotic factors, neuroinflammation, and amyloid processing which exacerbated cell death and contributed to IC. Furthermore, our study suggested that DHT's upregulation is correlated with decreased IC pathology and that CASP1 activation may modulate the ratio of apoptotic

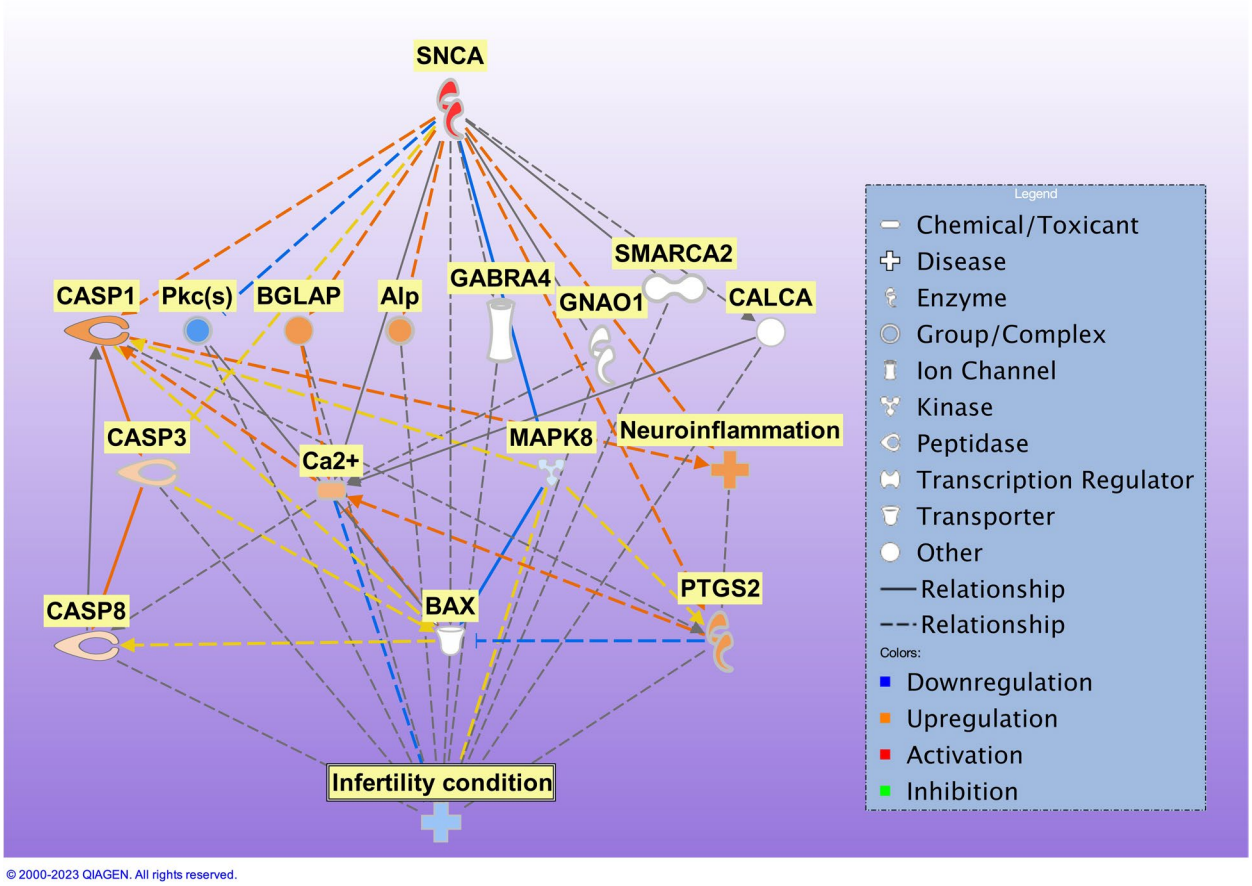


Fig. 8 Molecular network illustrating the connectivity and relationships of CASP1 to CASP3 and CASP8 with their effects on the molecular network of the 12 overlapping molecules between SNCA and IC including neuroinflammation's involvement

and cell survival factors in order to prevent IC pathology. Moreover, our findings corroborated other literature that indicated that an extended period of fertility (decreased infertility) is associated with a decrease in PD pathology. Overall, the findings within the study have demonstrated that the neuronal molecular pathway for IC is complex and not currently well-defined due to potentially conflicting molecular interactions. However, MAPK8 and Ca^{2+} appear to play a critical role in the molecular pathway between increased SNCA causing IC. Nicotine appears to affect the Ca^{2+} pathway between SNCA and IC to decrease IC pathology. The overall complexity of neurologically associated IC warrants further exploration of the different molecular interactions in terms of their neuronal and andrological synergistic effects.

Abbreviations

AD	Alzheimer's disease
Alp	Alkaline phosphatase
APP	Amyloid precursor protein
BAX	Bcl-2-associated X protein
BGLAP	Bone gamma-carboxyglutamic acid-containing protein
Ca^{2+}	Calcium
CALCA	Calcitonin-related polypeptide alpha
CASP	Caspase
DNA	Deoxyribonucleic acid
DHT	Dihydrotestosterone
GABRA4	Gamma-aminobutyric acid receptor subunit alpha-4
GDNF	Glial-derived neurotrophic factor
GNAO1	G protein subunit alpha O1
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary–gonadal
IC	Infertility condition
IPA	Ingenuity Pathway Analysis
LBs	Lewy bodies
MAP	Molecule activity predictor
MAPK8	Mitogen-activated protein kinase 8
MS	Multiple sclerosis
nAChRs	Nicotinic acetylcholine receptors
PD	Parkinson's disease
Pkc	Protein kinase-C
PTGS2	Prostaglandin-endoperoxide synthase 2
QKB	Qiagen Knowledge Base
SMARCA2	SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 2
SNCA	Alpha-synuclein
TNFAIP3	Tumor necrosis factor α–induced protein 3

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