# Bioinformatics and integrated pharmacology network to identify the therapeutic targets and potential molecular mechanism of alpha-lipoic acid on primary ovarian insufficiency

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**Research Article**

**Keywords:** Primary ovarian insufficiency, infertility, alpha lipoic acid

**Posted Date:** June 30th, 2022

**DOI:** https://doi.org/10.21203/rs.3.rs-1789849/v1

## Abstract

Women experiencing primary ovarian insufficiency (POI) are more likely to experience infertility, and its incidence is increasing worldwide annually. Recently, the role of alpha-lipoic acid (ALA) in the treatment of POI has been reported. However, details of the potential pharmacological targets and related molecular pathways of ALA remain unclear and need to be elucidated. Thus, this study aims to elucidate the potential therapeutic target and related molecular mechanism of ALA on POI. First, the potential targets of POI and ALA related targets were downloaded from online public databases. Subsequently, the overlapped target genes between POI and ALA were acquired, and Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, Protein-Protein interaction (PPI) networks were performed and constructed. Finally, molecular docking was performed to verify protein-to-protein effect. A total of 152 potential therapeutic targets were identified. The biological processes of the intersecting targets were mainly involved in the cellular response to peptides, response to xenobiotic stimuli, and response to peptide hormones. The highly enriched pathways were the cAMP, PI3K/AKT, estrogen, progesterone mediated oocyte maturation, and apoptosis signaling pathways. The top ten hub targets for ALA in the treatment of POI were STAT3, STAT1, CASP3, MTOR, PTGS2, CASP8, HSP90AA1, PIK3CA, MAPK1, and ESR1. The binding between ALA and all top hub targets were verified using the molecular docking analysis. In summary, using the systematic integrated pharmacology network and bioinformatics analysis, this study illustrated that ALA participates in the treatment of POI via multiple-targets and multiple-pathways mechanisms.


## Background

Primary ovarian insufficiency (POI), also known as premature ovarian failure (POF), is defined as the loss of ovarian function before the age of 40 years [1]. It is clinically characterized by follicular atresia, oocyte apoptosis,, amenorrhea, elevated plasma follicle-stimulating hormone (FSH) levels, and decreased serum estrogen levels [2]. According to Dr. Coluam, who first described POI in 1986, the prevalence of POI was at 1% [3]. However, according to the data published by Golezar, who analyzed the databases retrieved from related articles published between 1980 and 2017, the prevalence of POI was 3.7% [4]. Considering the increasing prevalence of POI, the development of effective therapeutic strategies is urgently needed.

To date, the entire concrete pathogenesis theory of POI has not been well investigated. Multiple factors contribute to the etiology of POI, including genetic factors, autoimmunity, infection, and idiopathic factors [5].

In recent years, different approaches have been established to regenerate germs cells in the ovary. For example, dormant follicles can be activated by using phosphatidylinositol-3-kinase (PI3K) activators and phosphatase and tensin homolog (PTEN) enzyme inhibitors *in vitro*[6]. In other studies, ovarian fragmentation has been shown to interfere Hippo signaling in the ovary and lead to follicular growth in the ovary [7].In addition, various types of mesenchymal stem cells have been used in rescuing ovarian functions in patients with POI [8, 9]. However, these therapies are experimental and not widely used clinically. Furthermore, POI-induced infertility cannot be improved using assisted reproductive technologies.

The natural compound alpha-lipoic acid (ALA) is derived from octanoic acid. It is an essential mitochondrial cofactor with a strong anti-inflammatory and antioxidant effects [10]. This molecule has a potential therapeutic effect on female fertility as follows: follicle development in vitro is improved by the reduction of reactive oxygen species (ROS) levels and the increase of antioxidant capacity in follicles[11]. Moreover, it is associated with the prevention of miscarriage [12, 13] and the treatment of endometriosis and vestibulodynia [14, 15]. ALA treatment has shown to be effective in many POI animal models, such as in cyclophosphamide-induced and 4-vinyl cyclohexene dioxide induced POI rats [16, 17]. Although the activities of ALA in the prevention of POI have been discovered, in-depth studies of mechanisms are lacking. In addition, a comprehensive elucidation of ALA's biological pathways and biomarkers has not yet been completed.

In this study, an integrated bioinformatics analysis and pharmacological network was performed to further explore the molecular mechanism of ALA on POI. The potential targets of ALA and POI were obtained from online databases. In addition, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were used to analyze the biological processes (BP), cellular components (CC), molecular function (MF), and enriched signaling pathways of the interacting targets. Subsequently, the hub targets in the protein-protein interaction (PPI) network were analyzed. Finally, molecular docking was used to assess the interactions between ALA and POI hub targets. The workflow diagram is illustrated in Fig. 1.

## Methods

# Candidate targets alpha-lipoic acid

The Canonical SMILES of ALA were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The target genes of alpha-lipoic acid were downloaded from the following online databases: DrugBank database (http://www.drugbank.ca/), Swiss Target Prediction database (http://www.swisstargetprediction.ch/), and SuperPred database (http://prediction.charite.de/). “Homo sapiens” was set to predict the target genes. In Swiss Target Prediction, the probability filter was set above 0. To obtain all targets, the target genes in the databases were merged, and duplicate genes were deleted.

# Targets Of Primary Ovarian Insufficiency

The targets of POI were obtained from the following online databases: DrugBank database (http://www.drugbank.ca/), DisGeNET (http://www.disgenet. org/search), OMIM database (http://omim.org/), GeneCards (https://www.genecards.org/), and PharmGKB database (https://www.pharmgkb.org/). “Homo sapiens” was set to predict the targets. To obtain all targets, the target genes in the databases were merged, and duplicate genes were deleted.

# Intersection Targets Of Alpha-lipoic Acid, And Primary Ovarian Insufficiency

# The intersection targets of ALA and POI were determined using online TBtools (https://www.tbtools.com/home).

# Pathway Enrichment Analysis Of The Intersecting Target Genes

To analyze the functions of the intersecting targets, GO enrichment and KEGG pathway analysis were performed using the org.Hs.eg.db package and ClusterProfiler package of R software[18]. GO analysis contained three categories as following: BP, CC, and MF. These provided a functional classification of the intersecting targets. KEGG (http://www.genome.ad.jp/kegg/) is a knowledge database to provides a systematic analysis of gene functions. Benjamini-adjusted $P < 0.05$ were set as the cutoff value for significance. The results were visualized using the Omcishare website (https://www.omicshare.com/) and the ggplot2 package in the R software.

# Ppi Network Construction And Hub Targets Analysis

The PPI data was acquired using the Search Tool for the Retrieval of Interacting Genes (STRING; http://string.embl. de/). The intersecting targets were mapped to STRING, and “homo sapiens” was selected as a background. The interaction score on the PPI network was set more than 0.4 as the threshold value. Subsequently, Cytoscape software (version 3.9.0) was used as a biological graph visualization tool to build up PPI networks. Hub genes were selected based on maximum neighborhood component (MNC), degree, edge percolated component (EPC), and closeness using the cytoHubba plugin.

# Molecular Docking

We used molecular docking to evaluate the interaction between hub target genes and ALA. Protein crystal structures were acquired from the protein data bank (http://www.rcsb.org). The two-dimensional structure of ALA was acquired from the PubChem compound database website (https://pubchem.ncbi.nlm.nih.gov/) and processed by energy minimization using Chemoffice software. Subsequently, the original ligands and water molecules of the proteins were removed by PyMOL (http://www. pymol. org/). Proteins were then added with hydrogens using AutoDockTools (version 1.5.7, http://autodock.scripps. Edu). Finally, proteins

and ligands were converted into “PDBQT” format. AutoDock Vina was used to dock ALA with the proteins. Binding affinity values less than 0 kcal/mol was set as the ligand can stably bind to the receptor, and binding affinity values of no more than − 5.0 kcal/mol showed an excellent binding ability.

## Results

## Predictive Targets of alpha-lipoic acid, and primary ovarian insufficiency

We collected 5485 genes associated with POI and 228 genes associated with ALA from the online databases as mentioned in the Methods. The intersecting targets are shown in **Supplementary Table 1**. As shown in Fig. 2, 152 target genes were accquired by intersecting targets for ALA and those for POI.

## Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis of intersecting targets of alpha-lipoic acid and primary ovarian insufficiency

## Go Enrichment Analysis

## To investigate the variable functions of potential targets in POI using ALA treatment, GO enrichment analysis were proceeded. The 152 potential targets were analyzed using the ClusterProfiler package of R 4.0.2. The top ten terms of three part of the GO enrichment analysis were selected based on the involved gene counts and p-values. Then the results were visualized by the R package ggplot2 (Fig. 3A). The top 10 BPs, top 10 CCs, and top 10 MFs are presented in Table 1 and Fig. 3B. The findings showed that the GO functions of the target protein molecules are mainly the cellular response to peptides, cellular response to peptide and steroid hormones, and regulation of calcium hemostasis.

## The top three enrichments in the BP subclassification were the cellular response to peptides, response to xenobiotic stimuli, and response to peptide hormone. In the MF subclassification, the top three enrichments were endopeptidase activity, amide binding, and peptide binding. In the CC subclassification, the top three enrichments were the membrane raft, membrane microdomain, and ficolin − 1−rich granule.

Table 1
Gene Ontology enrichment analysis.

| Category | term | Description | P value | Count |
|---|---|---|---|---|
| BP | GO:1901653 | cellular response to peptide | 1.55E-17 | 29 |
| BP | GO:0009410 | response to xenobiotic stimulus | 5.09E-14 | 28 |
| BP | GO:0043434 | response to peptide hormone | 4.64E-14 | 27 |
| BP | GO:0070997 | neuron death | 6.67E-12 | 23 |
| BP | GO:0006874 | cellular calcium ion homeostasis | 2.83E-10 | 23 |
| BP | GO:0055074 | calcium ion homeostasis | 4.52E-10 | 23 |
| BP | GO:0072503 | cellular divalent inorganic cation homeostasis | 1.14E-09 | 23 |
| BP | GO:0071375 | cellular response to peptide hormone stimulus | 1.06E-12 | 22 |
| BP | GO:0007204 | positive regulation of cytosolic calcium ion concentration | 6.13E-12 | 22 |
| BP | GO:0051480 | regulation of cytosolic calcium ion concentration | 3.04E-11 | 22 |

| Category | term | Description | P value | Count |
|---|---|---|---|---|
| CC | GO:0045121 | membrane raft | 4.12E-07 | 16 |
| CC | GO:0098857 | membrane microdomain | 4.12E-07 | 16 |
| CC | GO:0101002 | ficolin-1-rich granule | 2.42E-08 | 14 |
| CC | GO:0034774 | secretory granule lumen | 3.95E-05 | 13 |
| CC | GO:0060205 | cytoplasmic vesicle lumen | 3.95E-05 | 13 |
| CC | GO:0031983 | vesicle lumen | 3.95E-05 | 13 |
| CC | GO:0098978 | glutamatergic synapse | 4.22E-05 | 13 |
| CC | GO:0045177 | apical part of cell | 0.00049655 | 13 |
| CC | GO:1904813 | ficolin-1-rich granule lumen | 2.42E-08 | 12 |
| CC | GO:0009925 | basal plasma membrane | 3.36E-05 | 12 |
| MF | GO:0004175 | endopeptidase activity | 7.60E-09 | 21 |
| MF | GO:0033218 | amide binding | 5.30E-07 | 18 |
| MF | GO:0042277 | peptide binding | 3.70E-06 | 15 |
| MF | GO:0106310 | protein serine kinase activity | 0.00012877 | 13 |

| Category | term | Description | P value | Count |
|---|---|---|---|---|
| MF | GO:0016705 | oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen | 1.27E-06 | 12 |
| MF | GO:0008237 | metallopeptidase activity | 3.64E-06 | 12 |
| MF | GO:0061629 | RNA polymerase II-specific DNA-binding transcription factor binding | 0.00011322 | 12 |
| MF | GO:0004674 | protein serine/threonine kinase activity | 0.00065227 | 12 |
| MF | GO:0140297 | DNA-binding transcription factor binding | 0.00071405 | 12 |
| MF | GO:0004879 | nuclear receptor activity | 2.35E-09 | 10 |

## Kyoto Encyclopedia Of Genes And Genomes Enrichment Analysis Of The Potential Therapeutic Targets

To illustrate the critical signaling pathways of ALA in the treatment of POI, KEGG enrichment analysis were proceeded. The 152 potential targets were analyzed using the ClusterProfiler package of R 4.0.2. The top twenty pathways were selected based on the involved gene counts and P values, including pathways in cancer, neuroactive ligand-receptor interaction, and Alzheimer's disease (Fig. 4A). Subsequently, the enriched pathway was sorted and classified as shown in Fig. 4B and Table 2. We identified that the top three pathways were signal transduction, endocrine system, and cancers. Based on the above results, the cAMP, PI3K/AKT, estrogen, progesterone-mediated oocyte maturation, and apoptosis-signaling pathways were found to be crucial in the treatment of POI with ALA. These five signaling pathways were retrieved from the KEGG database and shown in **Fig. 5** and Fig. 6.

Table 2
Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis.

| Pathway class | Pathway | count | total genes | P value |
|---|---|---|---|---|
| Endocrine system | Prolactin signaling pathway | 12 | 78 | 5.02E-09 |
| Endocrine system | Renin-angiotensin system | 7 | 24 | 8.58E-08 |
| Endocrine system | Thyroid hormone signaling pathway | 13 | 129 | 2.05E-07 |
| Endocrine system | Estrogen signaling pathway | 12 | 141 | 3.75E-06 |
| Endocrine system | Regulation of lipolysis in adipocyte | 8 | 63 | 8.92E-06 |
| Endocrine system | Insulin signaling pathway | 11 | 145 | 2.88E-05 |
| Endocrine system | Progesterone-mediated oocyte maturation | 9 | 110 | 8.76E-05 |
| Endocrine system | Growth hormone synthesis, secretion and action | 9 | 124 | 2.20E-04 |
| Endocrine system | GnRH secretion | 6 | 67 | 8.48E-04 |
| Endocrine system | Relaxin signaling pathway | 8 | 135 | 1.86E-03 |
| Endocrine system | Renin secretion | 5 | 73 | 7.36E-03 |
| Endocrine system | Adipocytokine signaling pathway | 4 | 70 | 2.94E-02 |
| Signal transduction | TNF signaling pathway | 14 | 114 | 5.20E-09 |
| Signal transduction | Sphingolipid signaling pathway | 12 | 125 | 1.04E-06 |
| Signal transduction | cAMP signaling pathway | 15 | 223 | 4.29E-06 |
| Signal transduction | HIF-1 signaling pathway | 10 | 121 | 3.23E-05 |
| Signal transduction | ErbB signaling pathway | 8 | 88 | 1.04E-04 |
| Signal transduction | VEGF signaling pathway | 6 | 63 | 6.10E-04 |
| Signal transduction | Phospholipase D signaling pathway | 11 | 229 | 1.54E-03 |
| Signal transduction | FoxO signaling pathway | 8 | 139 | 2.24E-03 |
| Signal transduction | NF-kappa B signaling pathway | 9 | 178 | 2.92E-03 |
| Signal transduction | Ras signaling pathway | 11 | 250 | 3.07E-03 |
| Signal transduction | AMPK signaling pathway | 7 | 126 | 5.09E-03 |
| Signal transduction | Calcium signaling pathway | 11 | 273 | 5.94E-03 |
| Signal transduction | PI3K-Akt signaling pathway | 15 | 445 | 7.30E-03 |
| Signal transduction | Jak-STAT signaling pathway | 8 | 171 | 7.83E-03 |
| Signal transduction | mTOR signaling pathway | 7 | 163 | 1.93E-02 |
| Signal transduction | Rap1 signaling pathway | 8 | 226 | 3.56E-02 |
| Cancers | Pathways in cancer | 31 | 552 | 1.35E-09 |
| Cancers | Prostate cancer | 13 | 103 | 1.35E-08 |
| Cancers | Pancreatic cancer | 10 | 81 | 8.45E-07 |
| Cancers | MicroRNAs in cancer | 14 | 176 | 1.27E-06 |
| Cancers | Proteoglycans in cancer | 14 | 212 | 1.13E-05 |
| Cancers | Acute myeloid leukemia | 8 | 72 | 2.43E-05 |

| Pathway class | Pathway | count | total genes | P value |
|---|---|---|---|---|
| Cancers | Central carbon metabolism in cancer | 8 | 74 | 2.97E-05 |
| Cancers | Non-small cell lung cancer | 7 | 70 | 1.57E-04 |
| Cancers | Colorectal cancer | 8 | 94 | 1.66E-04 |
| Cancers | Chronic myeloid leukemia | 7 | 79 | 3.34E-04 |
| Cancers | Choline metabolism in cancer | 8 | 107 | 4.05E-04 |
| Cancers | Chemical carcinogenesis | 7 | 86 | 5.62E-04 |
| Cancers | Hepatocellular carcinoma | 10 | 172 | 5.92E-04 |
| Cancers | Endometrial cancer | 6 | 63 | 6.10E-04 |
| Cancers | Small cell lung cancer | 7 | 94 | 9.59E-04 |
| Cancers | Breast cancer | 9 | 156 | 1.18E-03 |
| Cancers | Renal cell carcinoma | 6 | 75 | 1.53E-03 |
| Cancers | Glioma | 6 | 79 | 2.01E-03 |
| Cancers | Viral carcinogenesis | 10 | 209 | 2.59E-03 |
| Cancers | Melanoma | 5 | 76 | 8.70E-03 |
| Cancers | Gastric cancer | 6 | 153 | 4.30E-02 |

# Protein-protein Interaction Network And Hub Targets

More evidence has shown that besides single genes, diseases are also regulated via interactions among multiple targets. Thus, to further explore the molecular mechanisms of the pharmacological actions of ALA on POI, STRING database were used to construct a PPI network. A PPI network with 150 nodes and 1739 edges was obtained (Fig. 7A); In the network, the nodes and edges represent proteins and protein-protein associations, respectively.

The top 10 hub target genes, including STAT3, STAT1, CASP3, MTOR, PTGS2, CASP8, HSP90AA1, PIK3CA, MAPK1, and ESR1, which were considered potential therapeutic targets of ALA for POI, were obtained and calculated using cytoHubba with maximal clique centrality (MCC). As shown in Fig. 7B, ranks are upgraded and expressed as color changes from red to yellow. The network characteristics of the potential hub targets are presented in Table 3.

Table 3
Characteristics of the top ten hub targets in the protein-protein interaction network.

| target | MCC | Degree | Betweenness | Closeness |
|---|---|---|---|---|
| STAT3 | 3423630 | 90 | 1384.52384 | 94.16667 |
| STAT1 | 3094808 | 52 | 366.59263 | 82.5 |
| CASP3 | 2941666 | 90 | 1967.04854 | 95.33333 |
| MTOR | 2861270 | 80 | 1058.38605 | 92.33333 |
| PTGS2 | 2758124 | 80 | 1850.07319 | 92.83333 |
| CASP8 | 2382960 | 52 | 148.11404 | 81.33333 |
| HSP90AA1 | 2043148 | 98 | 2196.35486 | 96.5 |
| PIK3CA | 1793095 | 70 | 892.21449 | 86.33333 |
| MAPK1 | 1787784 | 68 | 849.24786 | 86.83333 |
| ESR1 | 1606622 | 82 | 1656.15806 | 92.08333 |

# Molecular Docking

Molecular docking was performed to evaluate the interaction between the top ten hub targets and ALA. Table 4 shows the affinity binding values of hub targets and ALA. A smaller affinity value represents a higher affinity between ALA and the protein. As shown in Table 4, the affinity between all targets and ALA was less than − 5 kcal/mol, which indicates a strong binding affinity. Therefore, ALA may improve the POI by regulating the activity of these proteins. Figure 8 and Fig. 9 show the binding mode of ALA with hub proteins.

Table 4
Docking parameters and results.

| Targets | PDB ID | Affinity(kcal/mol) |
|---|---|---|
| CASP3 | 4QUD | -5.4 |
| CASP8 | 2Y1L | -5.2 |
| ESR1 | 1GWR | -5.1 |
| HSP90 | 1QTN | -5.0 |
| MAPK1 | 2Y9Q | -5.1 |
| MTOR | 3FAP | -5.7 |
| PI3K3A | 6PYS | -5.1 |
| STAT1 | 1BF5 | -5.0 |
| STAT3 | 6QHD | -5.2 |
| PGS2 | 5F19 | -5.4 |

# Discussion

POI is a devastating disease that significantly impairs ovarian function [19]. Ovarian failure can lead to reduction in sexual hormones production and regular ovulation, and thus cause infertility issues and menopause symptom in women [20]. However, a cure for POI is currently not possible. As previously stated, several findings have confirmed that ALA effectively improves POI;

however, the therapeutic mechanism of ALA on POI are not fully understood. Therefore, in our present study, systematic pharmacology network was used to reveal the potential mechanism of action of ALA against POI for the first time.

In the present study, 152 potential targets for ALA to prevent POI were collected using network pharmacology and bioinformatics. The top enriched BPs of the intersected targets were principally related to response to peptides, xenobiotic stimuli, and peptide hormones. Furthermore, based on KEGG pathway enrichment analysis, multiple signaling pathways were related to the treatment of POI with ALA. Among those, the cAMP signaling pathway, estrogen signaling pathway, PI3K/AKT signaling pathway, progesterone mediated oocyte maturation signaling pathway, and apoptosis signaling pathway were enriched and closely related to POI. Other studies have shown that the cAMP/PKA signaling pathway is involved in modulating the steroidogenic activity of luteinizing hormone (LH) and FSH, thereby regulating ovarian function and development[21]. By inhibiting the PI3K/AKT signaling pathway embryonic stem cell-derived extracellular vesicles can promote granulosa cell proliferation and inhibit apoptosis to improve ovarian function [22].

According to our PPI network pharmacology results, the hub targets for ALA in preventing POI are STAT3, STAT1, CASP3, MTOR, PTGS2, CASP8, HSP90AA1, PIK3CA, MAPK1, and ESR1.

STAT1 and STAT3, which belongs to the signal transducer and activator of transcription (STAT) family, are critical signaling molecules and transcriptional activator that regulate cell growth, survival, and proliferation [23]. STAT1 and STAT3 are expressed in granulosa cells, specifically in the primordial follicles [24]. Emerging evidence has shown that STAT3 prevents follicular atresia and enhances ovarian function in POI animal models [25, 26]. The mitogen-activated protein kinase (MAPKs), members of the serine/threonine-protein kinase family, are involved in cell proliferation, differentiation, growth, and many other physiological processes. MAPK1, also known as extracellular signal-regulated kinase 2(ERK2), plays an essential role in regulating the gonadotropin LH-β and FSH-β expression, oocyte meiotic maturation, and ovulation [27, 28]. ALA induces dose-dependent activation of MAPK1 in the endothelial cells[29]. Moreover, phosphoinositide 3-kinase (PI3K) pathway is essential for primordial follicle activation [30]. The presence of ALA in the cortical neurons shows a protective effect through activation of the PI3K/PKG/MAPK1/2 pathway [31].

As the primary female sex hormone, by binding to estrogen receptors (ESRs), estrogen play a crucial role in the maturation and maintenance of the female reproductive system. Estrogen receptor-α (ER-α) and estrogen receptor-β (ER-β) are encoded by ESR1 gene and ESR2 gene, respectively. ER-α is the main estrogen receptor and is widely expressed throughout the female reproductive system. Gustafsson showed that anovulation and completed infertility occurred after knocking out ER-α in female mice, indicating the importance of ER-α in female fertility[32]. As the ER-α encoding gene, ESR1 polymorphisms have been seen as important genetic factors for unexplained female infertility, and controlled ovarian hyperstimulation (COH) outcomes in the in IVF cycle [33, 34]. Reports suggested that ESR1 gene polymorphisms can impair the ovarian reserve and cause POI [35–37]. Moreover, ALA treatment significantly reduced the progression of atherosclerosis by restoring the ESR expression in the mouse model [38]. However, the effect of ALA on ESR in POI requires further validation.

The caspase family participates in the process of mediating cell apoptosis [39]. Caspase 3 and caspase 8, encoded by CASP3 and CASP8, respectively, belong to the cell death protein subfamily and are the crucial executor molecules that participate in various apoptosis signal transduction pathways, such as Fas/FasL pathway [40, 41]. The relation of upregulation of caspase3 to ovarian granulosa cell apoptosis and impairment of ovarian follicular development has been reported [42]. Encoded by the HSP90AA1 gene, heat shock protein 90α (HSP90α), participates in the regulation of ovarian and reproductive functions. The presence of HSP90 antibodies causes immune-mediated destruction of ovarian follicles and POI formation [43, 44]. HSP90 can regulate the cytoskeletal structure and meiotic maturation of oocytes by coupling with MAPK [45].

Prostaglandin endo-peroxidase synthase (PTGS), also known as cyclooxygenase (COX), is a critical enzyme responsible for the rate-limiting step in prostaglandins (PGs) biosynthesis and also a major mediator of luteal function [46]. PGs activate epidermal growth factor (EGF)-like factors in cumulus-oocyte complexes (COCs) through autocrine and paracrine mechanisms [47]. PGE2 is generated by the actions of PTGS enzymes during the nuclear maturation of Gn-induced oocytes [48]. In addition, the treatment of ALA in bovine COCs during oocyte maturation accelerates oocyte nuclear maturation and increases the number of MII-stage oocytes through PGE2 synthesis [49]. The mechanistic target of rapamycin (mTOR) is an important molecule involved in multiple physiological processes, including proliferation, differentiation, apoptosis, and autophagy [50]. Activation of the PI3K/AKT/mTOR

signaling pathway can attenuate POI by reducing oxidative stress and inhibiting apoptosis in granulosa cells [51]. Activation of the AMPK/mTOR signaling pathway can induce POI by triggering the autophagy of theca-interstitial cells [52]. Moreover, ALA exerts an anti-lung cancer cell growth effect through mTOR-mediated inhibition of autophagy [53]. However, the effect of ALA on mTOR-related pathways in POI requires further validation.

Based on the above results, ALA may improve primary ovarian failure by regulating oocyte maturation, sex hormone secretion and function, cell proliferation, autophagy, apoptosis, and the inflammatory response. Moreover, the molecular docking between the top ten hub genes and ALA showed high affinities, implying that the these genes may be crucial in the treatment of POI with ALA.

However, our present study has some limitations. For instance, the potential target genes selected by the systemic network pharmacology are purely theoretical predictions. It was not clear whether the involved genes and pathways were downregulated or upregulated. Thus, further studied need to be verified by cell and tissue culture invitro, animal models, and clinical experiments.

## Conclusions

In summary, ALA may improve POI by intervening in a series of targets (such as STAT3, STAT1, CASP3, MTOR, PTGS2, CASP8, HSP90AA1, PIK3CA, MAPK1, and ESR1), and signaling pathways (such as the cAMP signaling pathway, PI3K/AKT signaling pathway, estrogen signaling pathway, progesterone mediated oocyte maturation signaling pathway, and apoptosis signaling pathway). Our findings demonstrated the key targets and molecular pathways of ALA in the treatment of POI.

## Declarations

### Competing interests

The authors have no conflicts of interest to declare.

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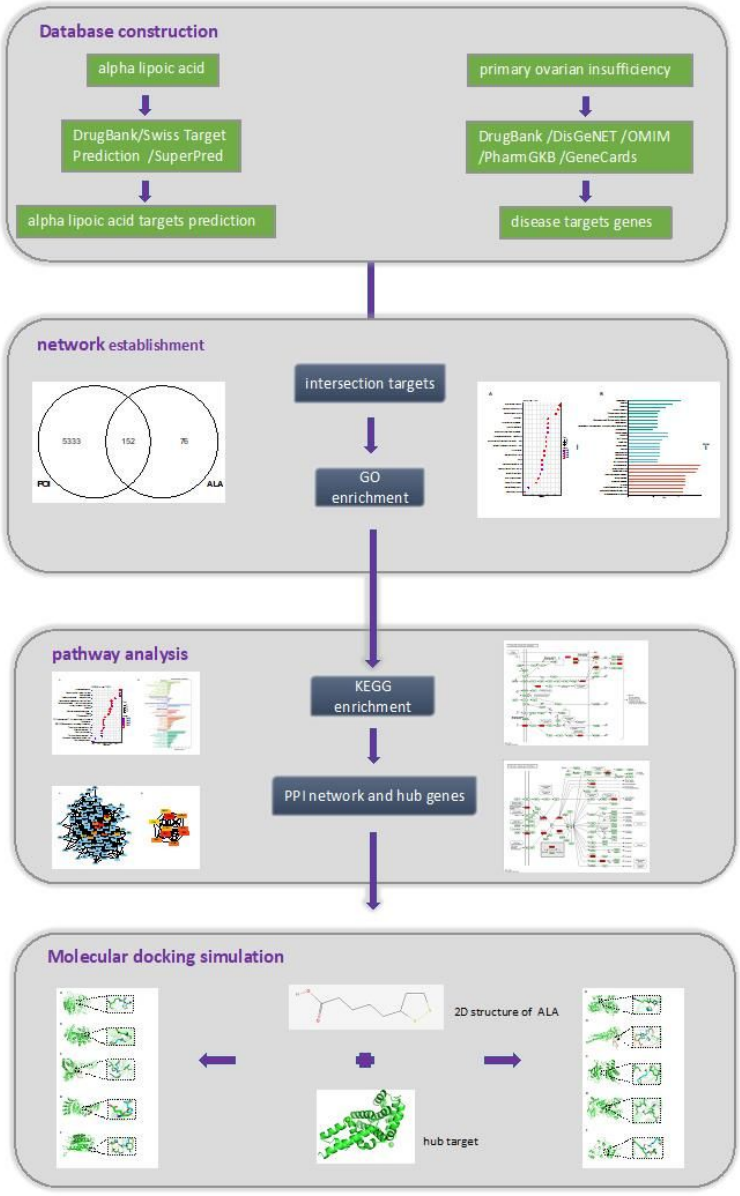
## Figures




## Figure 1

**Workflow chart of researching alpha-lipoic acid in the treatment of primary ovarian insufficiency.**

Research workflow diagram.

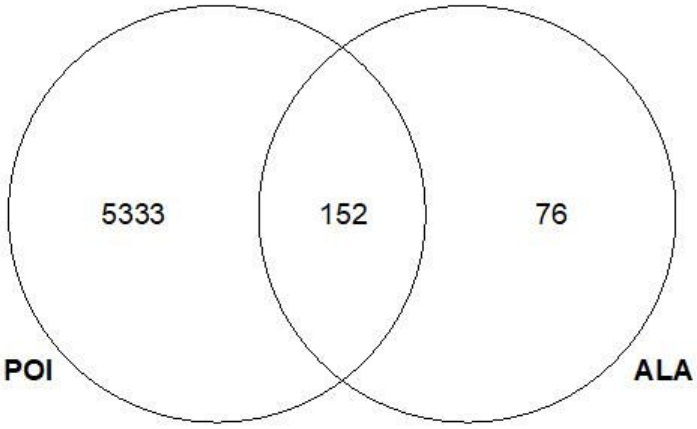



Figure 2

**The intersected targets between alpha-lipoic acid and primary ovarian insufficiency are shown in the Venn diagram**

5485 targets associated with POI and 228 targets associated with alpha lipoic acid (ALA) were acquired using the online database; 152 intersected targets of primary ovarian insufficiency and ALA were obtained.

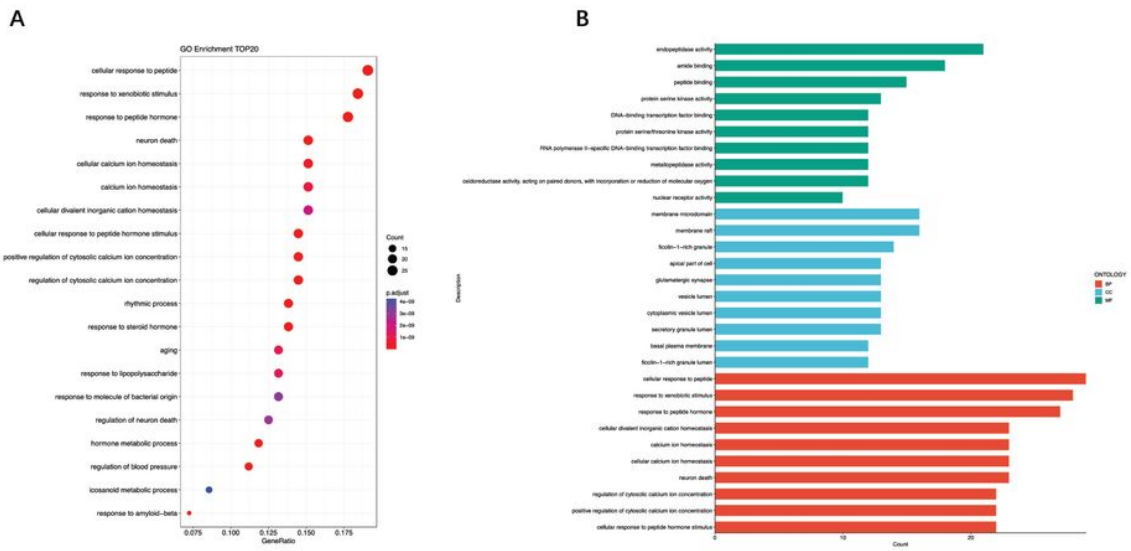





**Figure 3**

**Gene Ontology enrichment analysis of the intersecting targets of alpha lipoic acid and primary ovarian insufficiency.**

A. Bubble chart of top 20 gene ontology (GO) enrichment terms. B. Bar chart of GO biological process (BP) terms, cellular component (CC) terms, and molecular function (MF) terms.

The X-axis represents gene ratio, and Y-axis represents BP,CC, MF in the GO enrichment, respectively. The bubble size shows the number of genes involved in BP,CC, MF process. The adjusted p-value is represented by color; the darker the color, the smaller the adjusted p-value.

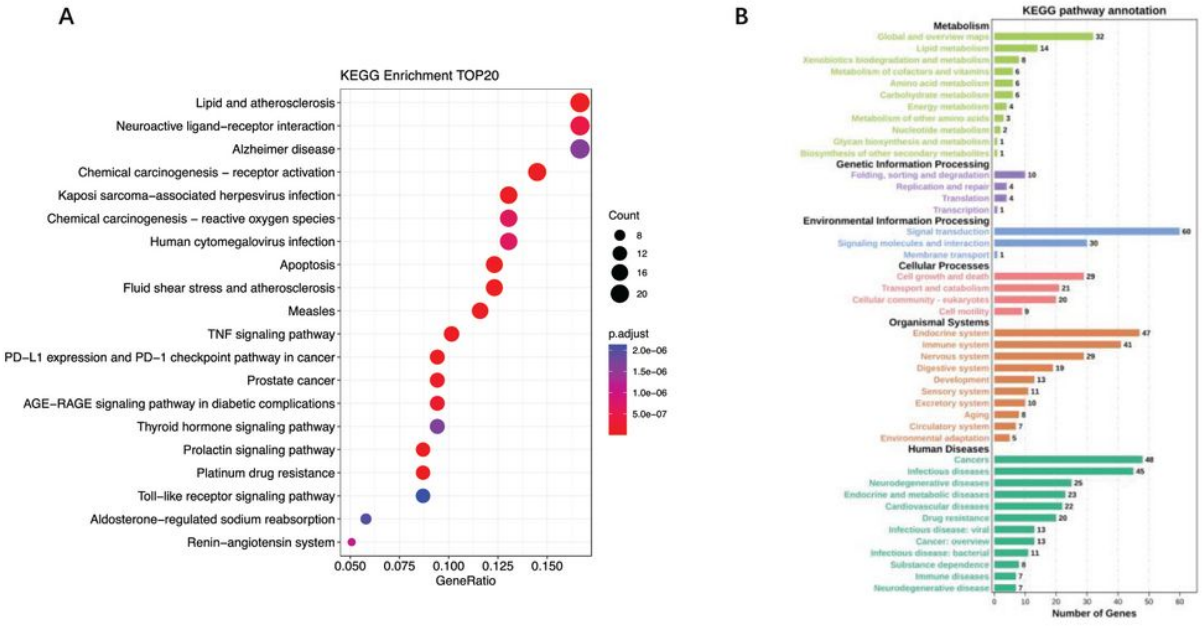


Figure 4

**Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annalysis of the intersected targets of alpha lipoic acid and primary ovarian insufficiency.**

A. Bubble chart of top 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment terms. B. Bar chart of KEGG pathway annotation.

The X-axis represents gene ratio, and the Y-axis represents the KEGG signaling pathway. The bubble size shows the number of genes involved in the KEGG signaling pathway. The adjusted p-value is represented by color; the darker the color, the smaller the adjusted p-valueA. Bubble chart of top 20 Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment terms. B. Bar chart of KEGG pathway annotation.

The X-axis represents gene ratio, and the Y-axis represents the KEGG signaling pathway. The bubble size shows the number of genes involved in the KEGG signaling pathway. The adjusted p-value is represented by color; the darker the color, the smaller the adjusted p-value

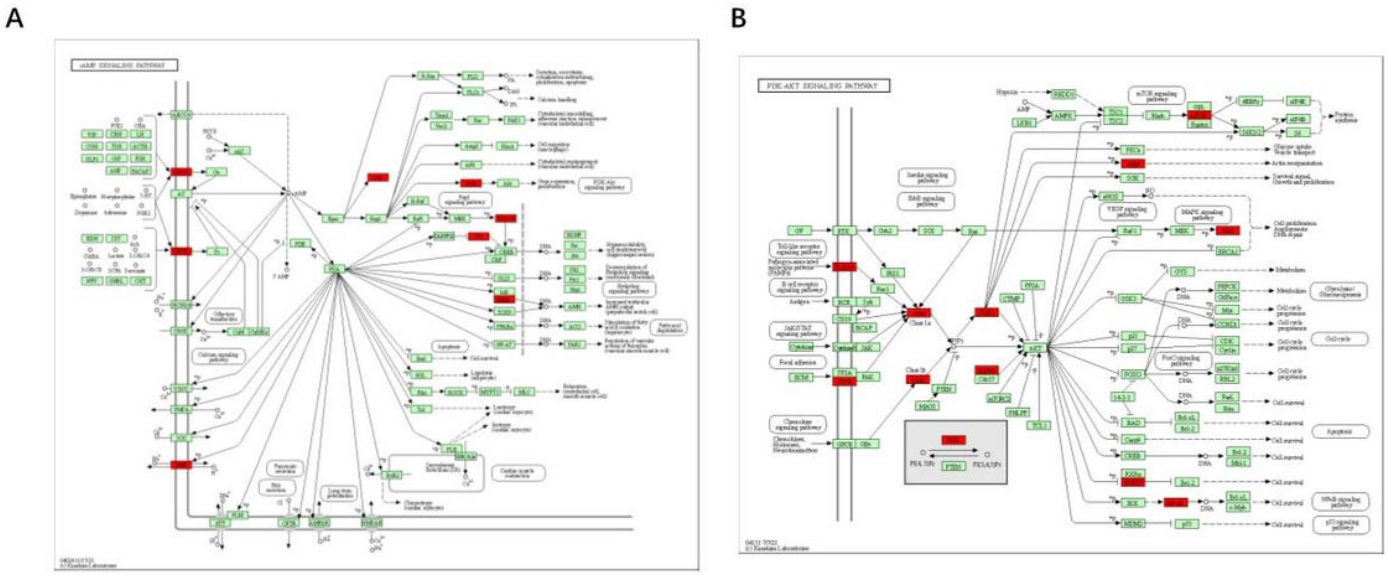



Figure 5

**Distribution of the potential therapeutic targets on significantly enriched pathways.**

A. cAMP signaling pathway. B. PI3K-AKT signaling pathway.

The red nodes represent overlapping targets of alpha-lipoic acid and primary ovarian insufficiency, and the green nodes represent the other genes involved in the pathway.

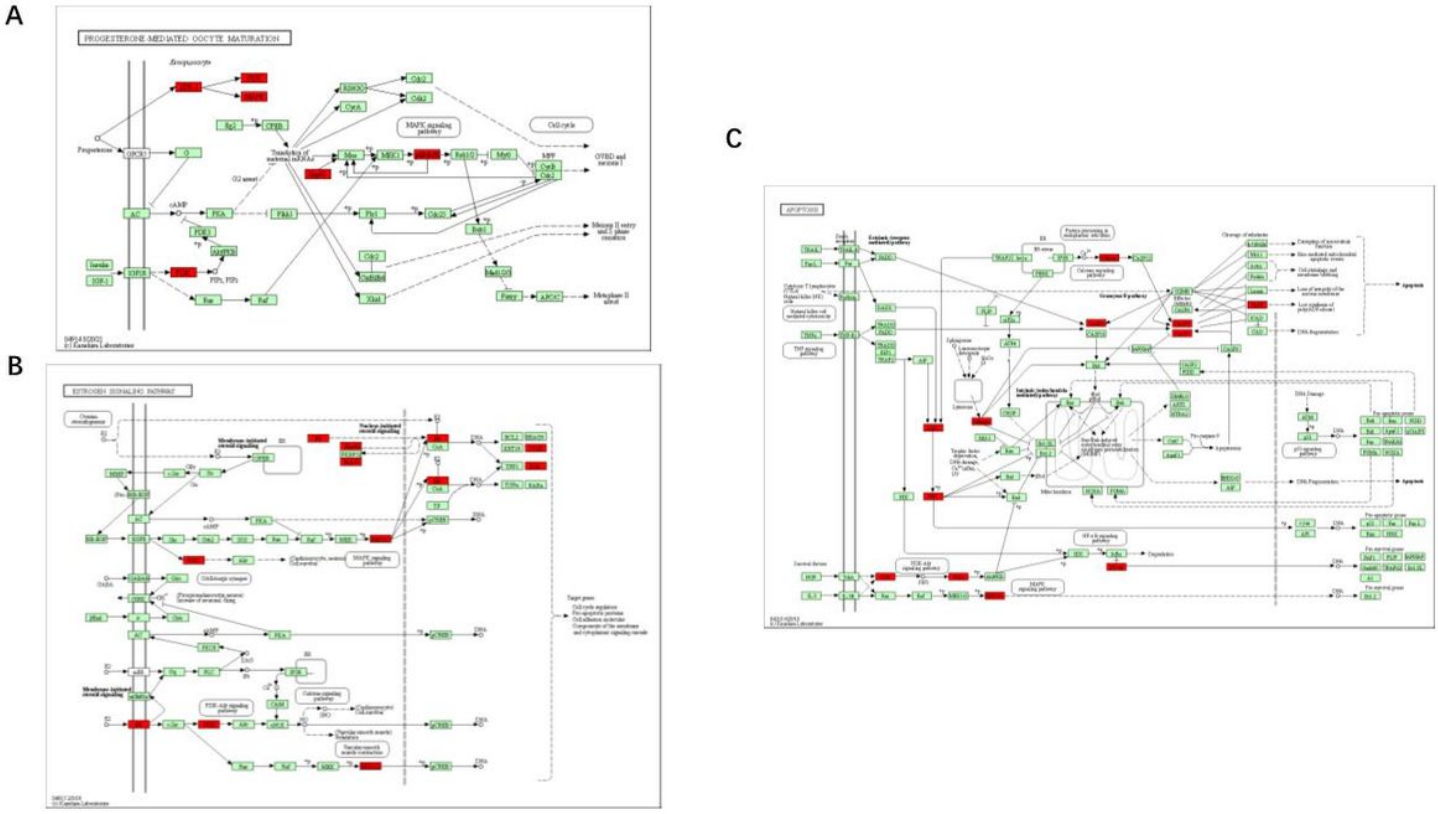



**Figure 6**

**Distribution of the potential therapeutic targets on significantly enriched pathways.**

A. progesterone mediated oocyte maturation. B. estrogen signaling pathway.

C. apoptosis.

The red nodes represent overlapping targets of alpha-lipoic acid and primary ovarian insufficiency, and the green nodes represent the other genes involved in the pathway.

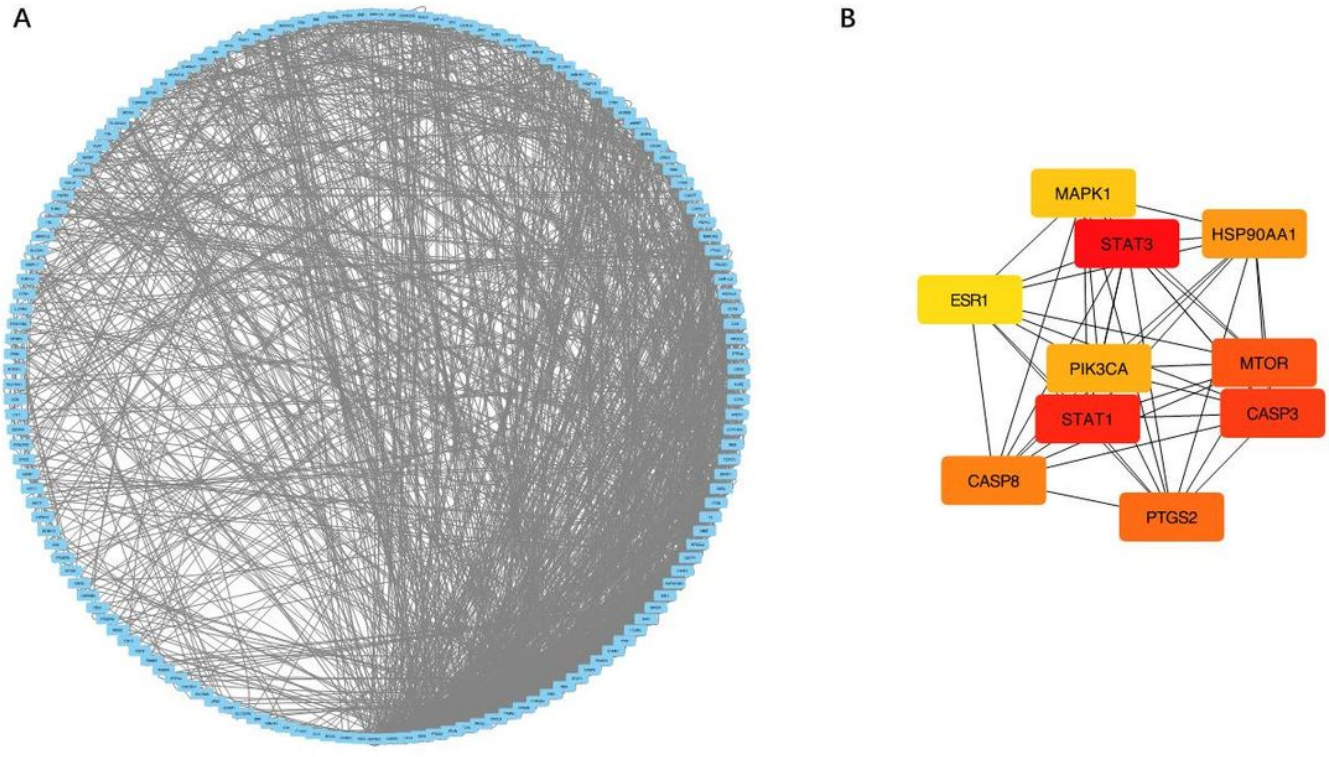
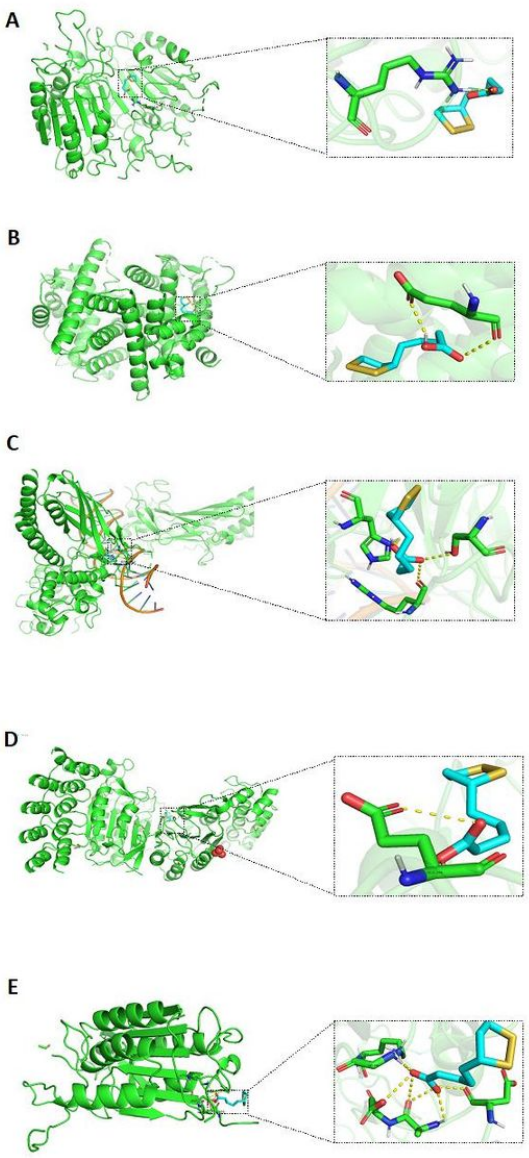



**Figure 7**

**Protein-protein interaction network and hub targets.**

A. Protein-protein interaction network. Network nodes represent the intersected target proteins of primary ovarian insufficiency and alpha-lipoic acid, and edges represent protein to protein associations. B. Top 10 hub targets calculated using cytoHubba. Ranks are upgraded and expressed using color changes from red to yellow.




## Figure 8

**Molecular docking of the hub targets with alpha-lipoic acid.**

A. The binding process between CASP3 and alpha-lipoic acid (ALA). B. The binding process between ESR1 and ALA. C. The binding process between STAT3 and ALA. D. The binding process between CASP8 and ALA. E. The binding process between HSP90 and ALA.

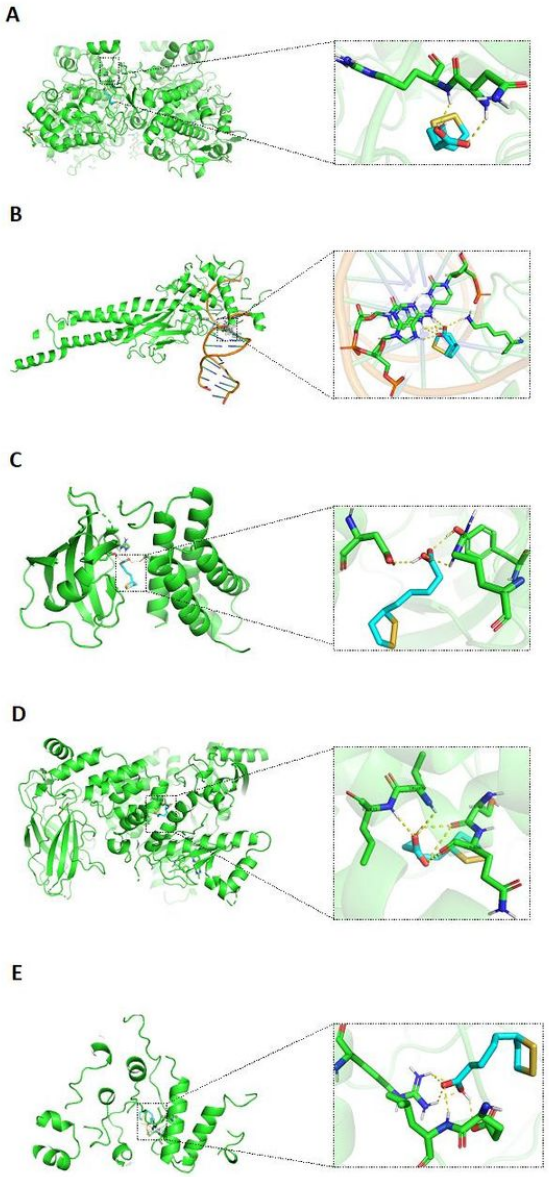



**Figure 9**

**Molecular docking between the hub targets and** alpha-lipoic acid**.**

A. The binding process between PGS2 and alpha-lipoic acid (ALA). B. The binding process between STAT1 and ALA. C. The binding process between MTOR and ALA. D. The binding process between PI3KA and ALA. E. The binding process between MAPK1 and ALA.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable1.xlsx