PTPN22 is a Potentially Diagnostic Biomarker for Abdominal Aortic Aneurysm

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Research

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Abstract

**Background:** Abdominal aortic aneurysm (AAA) is a severely life-threatening disease; it is generally asymptomatic with diagnosis at a very late stage. Moreover, the genetic components and underpinnings in AAA is considerable with an estimated heritability of up to 70% roughly. Therefore, identifying the biomarkers for AAA is valuable toward predicting and eventually inspecting the high-risk populations.

**Methods:** Herein, we used integrative bioinformatics and experimental analyses to reveal that protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) can be a potentially diagnostic biomarker for AAA.

**Results:** *PTPN22*, whose expression is significantly upregulated in aortic tissues of AAA, was primarily identified as a genetic biomarker for AAA, while it has no diagnostic value for patients with thoracic aortic aneurysm. Moreover, CaCl$_2$-based in vitro AAA model was adopted to experimentally show that Garcinia acid could be a therapeutic drug for AAA.

**Conclusions:** Collectively, these results indicate that *PTPN22* may be a potentially diagnostic biomarker for AAA.

Introduction

Abdominal aortic aneurysm (AAA) is a severely life-threatening disease with an overall incidence rate of 6% in men and 1.6% in women [1]. AAA is hallmarked by an enlargement of localized infrarenal aorta with a diameter larger than 3.0 cm [2]. Unless an rapid increase in size or rupture, AAA is generally asymptomatic and diagnosed at a very late stage [1]. It has been established that the occurrence of AAA is highly associated with unhealthy lifestyles, such as smoking history and high [3]. However, the genetic component and underpinnings in AAA is substantial with an estimated heritability of up to 70% approximately [4]. Difficulties remain regarding the implementation of a screening program; current knowledge towards its genetic underpinnings is insufficient to guide early screening of AAA in the clinic. Therefore, identifying the biomarkers for AAA is valuable toward predicting and eventually inspecting the high-risk populations. [5].

High-throughput platforms-based genetic screening has been highlighted as a well-conducted approach for the diagnosis and prevention of AAA. Through adopting integrative bioinformatics and/or experimental analyses, it is valuable in identifying the potential biomarkers to accurately predict AAA occurrence [6, 7]. As a proof-of-concept, previously, we have also applied this research mode in identifying crucial genes with diagnostic and/or prognostic values in several different types of diseases [8–11].

In the current study, through adopting comprehensive bioinformatics-based analysis, protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) is identified as a potential biomarker for the diagnosis of AAA. Additionally, we also uncover that Garcinia acid could be a therapeutic drug for AAA.

Materials And Methods
Data and collection and processing

Gene expression profiles were retrieved from GEO (http://www.ncbi.nlm.nih.gov/geo). Datasets were analyzed by using GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/). P value < 0.05 and |Log₂FC| ≥ 1 were considered significant.

Functional analysis

To explore the potential biological functions of identified differentially expressed genes (DEGs), Database for Annotation, Visualization and Integrated Discovery (https://david-d.ncifcrf.gov/) was employed to conduct Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses [12]. GeneMANIA (http://genemania.org/) was adopted to obtain the potential proteins interacted with designated protein [13].

Receiver operating characteristics analysis

Receiver operating characteristics (ROC) curve was generated using MedCalc software (version 19.0.7). The area under the ROC curve (AUC) value with 95% confidence interval (CI) was calculated.

Transcription factors analysis

FunRich software (version 3.1.3) and the PASTAA program in Transcription factor Affinity Prediction (TRAP) Web Tools (http://trap.molgen.mpg.de/cgi-bin/home.cgi) were adopted to predict the potential transcription factors targeting the promoter region of PTPN22 [14, 15]. The promoter sequence of designated gene was obtained using Ensembl (https://asia.ensembl.org/index.html), an online genome browser for vertebrate genomes. The motif was generated through using JASPAR (http://jaspar.genereg.net/), a database of transcription factor binding profiles [16].

Cell culture

Vascular smooth muscle cells (VSMCs) were kindly provided by Dr. Tao Zhuang (Shanghai East Hospital, Tongji University School of Medicine). Cells were maintained in DMEM (Gibco, USA) supplemented with 10% FBS (Gibco, USA), 100 U/mL penicillin (Gibco, USA), and 100 μg/mL streptomycin (Gibco, USA). Cells were incubated at 37 °C in a humidified atmosphere supplemented with 5% CO₂.

Immunofluorescence analysis
For αSMA immunofluorescence staining, cells were fixed with 4% paraformaldehyde, and blocked by PBS containing 10% normal goat serum as previously introduced [17]. Cells were then incubated with the primary antibody against αSMA (Cat#ab7817, Abcam) followed by the corresponding secondary antibody. After being incubated with DAPI, images were captured using a microscope.

**Quantitative real-time PCR (qRT-PCR) analysis**

Total RNA was purified through using TRizol (Invitrogen, USA); cDNA libraries were then synthesized with Reverse Transcription Reagent kit (Takara, Japan). The cDNA was submitted for Real-Time PCR with SYBR Green mix (Takara, Japan) with Gapdh normalization. The primers (Table S1) were purchased from Sangon Biological Engineering (Shanghai, China). Data were analyzed with the comparative Ct (ΔΔCt) method.

**Statistical analysis**

Data are shown as mean ± standard deviation (SD). Pair groups were compared using two-tailed Student's t-test; multiple groups were compared using One-way ANOVA and Tukey's multiple comparison test. P value < 0.05 was considered as significance. Statistical analyses were performed through using GraphPad Prism 7 (San Diego, USA).

**Results**

**LILRB2 and PTPN22 are two potential diagnostic biomarkers for AAA**

To profile the genetic alterations in aortic tissues collected from AAA patients, two datasets with similar grouping characteristics were retrieved. There were 824 DEGs were identified in AAA patients based on GSE47472 dataset (Fig. 1a); among these DEGs, 206 genes were significantly upregulated, while 618 genes were significantly downregulated (Fig. 1b). For further functional exploration, GO analysis was then conducted. These upregulated DEGs were highly enriched in the biological processes, such as brush border and channel inhibitor activity; these downregulated DEGs were highly enriched in ATPase and hydrolase activities (Fig. 1c). Based on GSE7084 dataset, we identified 303 AAA patients-specific DEGs (Fig. 1d), which are consisted of 194 significantly upregulated and 109 significantly downregulated genes (Fig. 1e). Unlike GSE47472 dataset, these upregulated DEGs were highly enriched in immune response-related biological processes, such as chemotaxis and inflammatory response; these downregulated DEGs were highly enriched in cytoskeleton-related biological processes, such as cytoskeletal protein binding (Fig. 1f). To obtain the potential genes with diagnostic value, we cross-compared upregulated and downregulated genes of both datasets, respectively. Two genes including LILRB2 and PTPN22 were concurrently upregulated (Fig. 1g), while three genes including DIAPH2, MXI1, and TBXA2R were...
concurrently downregulated in both datasets (Fig. S1). To assess the predictive values of \textit{LILRB2} and \textit{PTPN22} for AAA, ROC analysis was conducted based on GSE47472 datasets; the AUC value for \textit{LILRB2} was similar to that for \textit{PTPN22} (Fig. 1h). Collectively, these results reveal \textit{LILRB2} and \textit{PTPN22} are two potential diagnostic biomarkers for AAA.

The sexual and size dimorphisms of identified genes in AAA patients

Given that the prevalence of AAA varies between males and females [2], GSE7084 dataset was then analyzed (Fig. 2a). When compared with control, \textit{LILRB2} was highly expressed in male patients with AAA, whereas the expression of \textit{PTPN22} was significantly upregulated in both male and female patients with AAA (Fig. 2b). The expression of \textit{DIAPH2} and \textit{TBXA2R} were significantly downregulated in male patients with AAA, while the expression of \textit{MXI1} was significantly downregulated in both male and female patients with AAA (Fig. S2a). Considering that the diameter of AAA is reported to be associated with the expression of genetic biomarkers [2], GSE57691 dataset was analyzed (Fig. 2c). There was no significant difference in the expression of \textit{LILRB2} among these groups, whereas the expression of \textit{PTPN22} was significantly upregulated in both small and large AAA samples (Fig. 2d). Consistently, the expression of \textit{DIAPH2}, \textit{MXI1}, and \textit{TBXA2R} were significantly downregulated in both small and large AAA samples (Fig. S2c). However, no significant difference was observed in the expression of these genes between small and large AAA samples.

\textbf{LILRB2 and PTPN22 have no diagnostic value for TAA patients}

Thoracic aortic aneurysm (TAA) and AAA share several similarities, such as degradation of elastic tissues. However, they also harbor many differences, such as causative genes conferring high risks for patients with TAA or AAA [18]. For instance, circulating fragmented Fibrillin-1 is identified as a biomarker for TAA rather than AAA [19]. Therefore, we then analyzed two TAA-based datasets including GSE140947 and GSE9106 to evaluate the expression and diagnostic value of these hub genes in TAA [20, 21]. Based on GSE140947 dataset (Fig. 3a), the expression of two upregulated genes (\textit{LILRB2} and \textit{PTPN22}) were similar among these groups (Fig. 3b), as well as three downregulated genes including \textit{DIAPH2}, \textit{MXI1}, and \textit{TBXA2R} (Fig. S3a). In addition, GSE9106 dataset, a peripheral blood cells-based RNA profiling of TAA, was also retrieved (Fig. 3c). As shown in Fig. 3d, a total of 205 TAA-specific DEGs was identified, which includes 180 significantly upregulated and 25 significantly downregulated genes, respectively (Fig. 3e). Unlike AAA, these upregulated DEGs were highly enriched in the biological processes, such as extracellular region and vessel development; these downregulated DEGs were highly enriched in the processes, such as phosphatase activity, cell motion, and regulation of S phase (Fig. 3f). Consistent with GSE140947, no significant difference in the expression of \textit{LILRB2} and \textit{PTPN22} was observed between control and TAA samples (Fig. 3g). Only the expression of \textit{DIAPH2} was significantly decreased in TAA
samples; the expression of *MXI1* and *TBXA2R* in TAA were even significantly increased (Fig. S3b). To evaluate the diagnostic value of *LILRB2* and *PTPN22* in TAA, ROC analysis was also conducted based on GSE47472 dataset. As shown in Fig. 3h, the AUC values for *LILRB2* (p value = 0.4576) and *PTPN22* (p value = 0.4448) were 0.547 and 0.550, respectively. These results suggest that *LILRB2* and *PTPN22* are two AAA-specific diagnostic biomarkers.

**Mechanical and therapeutic validations in vitro and mouse AAA experimental models**

Given that no mouse homologous genes were obtained for *LILRB2* and *DIAPH2* of human, therefore, *Ptpn22*, *Tbxa2r*, and *Mxi1* were then selected to analyze. As shown in Fig. 4a, based on GSE51227 dataset [22], when compared with sham group, the expression of *Ptpn22* was significantly increased (Fig. 4a), while the expression of *Tbxa2r* was significantly decreased in aortic tissues collected from AAA mice induced by PPE (Fig. S4a). Inconsistently, no significant difference in the expression of *Mxi1* in aortic tissues was observed between both groups (Fig. S4a). B cell infiltration is commonly detected in both human and mouse AAA tissues; the formation of AAA induced by CaCl$_2$ is suppressed evidently in B cell-deficient mice, highlighting the important function of B cells in the pathogenesis of AAA. GSE109639 dataset illustrating the expression of *Ptpn22* and *Tbxa2r* in aortic tissues collected from WT and muMT mice treated with vehicle and for 7 days, respectively (Fig. 4b) [23]. Consistently, the expression of *Ptpn22* was upregulated apparently in CaCl$_2$-induced aneurysmal tissues in WT mice, which was downregulated in muMT mice (Fig. 4c). Moreover, the expression of *Tbxa2r* was downregulated remarkably in CaCl$_2$-induced aneurysmal tissues in WT mice; no remarkable alteration emerged in muMT mice (Fig. S4b). A similar trend was observed for *Mxi1* between WT and muMT mice in response to CaCl$_2$ treatment (Fig. S4b). These evidences show that the expression of *Ptpn22* is also significantly upregulated in mouse AAA models. Next, we aimed to test whether CaCl$_2$ can also be used as an effective inducing drug for AAA in vitro. When compared with control, VSMCs treated with CaCl$_2$ (100 mmol/L) showed remarkably diminished aSMA that did not extend entirely across cell body (Fig. 4d and S4c), which was consist with a previous report [24]. Additionally, as shown in Fig. 4e the expression of *Ptpn22* was also significantly increased in VSMCs after treatment with CaCl$_2$ (100 mmol/L). These data thus suggest that CaCl$_2$ can be used as an effective drug for in vitro induction of AAA. The immune response play critical roles in the occurrence and progress of AAA [18, 23]; Garcinia acid possesses well immunomodulatory functions [25–27]. Therefore, we next aimed to explore whether GA can be a potential therapeutic candidate for AAA through using CaCl$_2$-induced VSMCs in vitro AAA model. As shown in Fig. 4f, CaCl$_2$-induced diminished aSMA in VSMCs was effectively eliminated by Garcinia acid (Fig. S4d). FunRich and TRAP databases were adopted to identify the potential transcription factors regulating *PTPN22*. HNF4A was identified to directly bind with the promoter region of *PTPN22* with a relatively high score (0.91); the corresponding consensus motifs were also presented (Fig. 4g). Moreover, PTPN22 showed interactions with 20 proteins/genes (Fig. S4e), among which 3 molecules have been reported to be important in AAA.
progress including PRKCD, EGFR, and PDGFRB [28–30]. Collective, these data highlight the important role of immune response and Garcinia acid as a potential therapeutic drugs for AAA.

**Discussion**

In the present study, through analyzing multiple aortic aneurysm-derived datasets with multiple methods, we reveal that *PTPN22* is a potential AAA-specific diagnostic biomarker. Furthermore, we also uncover that Garcinia acid could be a potential therapeutic drug for AAA.

AAA is usually asymptomatic before rupture, the present clinical challenges are to diagnose AAA at an early stage and to decipher the biological mechanisms leading to progressive dilatation and finally rupture. To develop novel diagnostic and therapeutic approaches, identification of biomarkers could help to target both objectives [2]. Previous studies have identified AAA biomarkers by studying the levels of different molecules potentially related to AAA pathological mechanisms [31, 32]. Herein, by comparing several AAA versus TAA-derived datasets, we reveal that *PTPN22* may be a valuable AAA-specific diagnostic biomarker. On the one hand, people with high blood pressure, cholesterol, and smoking history are at high risk for AAA, genetic screening among these population is thus valuable. On the other hand, biomarkers-based monitoring the patients diagnosed with AAA at an early stage has long-term benefits [33].

Two independent large cohorts-based genome wide association study for AAA has identified nine risk loci; several genes have been reported to function vitally in the pathogenesis of AAA [7, 34]. Previous studies have also pointed out that targeting these crucial molecules may largely delay and/or prevent the development of AAA [35, 36]. For instance, imatinib can prevent aneurysm progression via inhibiting the expression and activation of MMP9 in experimental AAA models [36]. Here we also identify that *PTPN22* may be a potential therapeutic target for AAA, as supported by the following evidences. First, the expression trend of *Ptpn22* is well replicated in the experimental AAA models induced by both PPE and CaCl₂. Second, HNF4A, the transcription factor targeting *PTPN22*, has also been reported to function importantly in the pathogenesis of AAA [37]. Third, proteins interacting with PTPN22 including PRKCD [28, 38], EGFR [39], and PDGFRB have been reported to function crucially in the development of AAA [40].

**Conclusion**

In summary, our integrative bioinformatics and experimental analyses have highlighted the potential diagnostic and therapeutic values of *PTPN22* for AAA. However, further experimental and/or clinical studies designed to elucidate the mechanism are urgently needed.

**Abbreviations**

AAA: Abdominal aortic aneurysm; PTPN22: Protein tyrosine phosphatase non-receptor type 22; DEGs: Differentially expressed genes; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes;
ROCs: Receiver operating characteristics; AUC: Area under the ROC curve; CI: Confidence interval; TRAP: Transcription factor affinity prediction; VSMCs: Vascular smooth muscle cells.

Declarations

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Authors’ contributions
Xiang Kong and Jianjun Ge supervised the project and conceived the study. Lei Gao and Peng Ruan performed all the experiments and bioinformatics analyses. Kaijing Wang provided technical expertise. Lei Gao and Peng Ruan analyzed the data, and wrote the manuscript with feedback from all authors.

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Availability of data and materials
All analyzed data related to this paper are included.

Ethics approval and consent to participate
Not applicable.

Consent for publication
All the authors have consented for the publication.

Competing interests
The authors declare that they have no competing interests.

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References


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**Figures**

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Figure 1

Identification of LILRB2 and PTPN22 as two potential diagnostic biomarkers for AAA. a Volcano plot illustrating DEGs between normal controls and AAA patients based on GSE47472 dataset. Red, significantly upregulated genes; blue, significantly downregulated genes; gray, no significant difference. Fold change $\geq 2$ and $p < 0.05$ were considered significant. b Number of significantly expressed DEGs in AAA patients. c Top enriched GO terms of significantly upregulated and downregulated DEGs in AAA patients, respectively. d Volcano plot illustrating differentially expressed genes (DEGs) between normal control and AAA samples based on GSE7084 dataset. Red, significantly upregulated genes; blue, significantly downregulated genes; gray, no significant difference. Fold change $\geq 2$ and $p < 0.05$ were considered significant. e Number of significantly expressed DEGs in AAA patients. f Top enriched GO terms of significantly upregulated and downregulated DEGs in AAA patients, respectively. g Venn diagram of significantly upregulated DEGs between GSE47472 and GSE7084. (H) ROC curve of LILRB2 and PTPN22 Based on GSE47472 dataset.

Figure 2
The sexual and size dimorphisms of PTPN22 in AAA patients. 

A. Schematic diagram of GSE7084 dataset. 
Aortic tissues collected from male and female patients with AAA are profiled using RNA sequencing. 

b. Relative expression of LILRB2 and PTPN22 based on GSE7084 dataset. Data are shown as mean ± SD. *, significantly different from the corresponding control; *, p < 0.05. 

c. Schematic diagram of GSE57691 dataset. 
Aortic tissues collected from patients with small and large AAA are profiled using RNA sequencing. Small AAA, mean maximum aortic diameter = 54.3 ± 2.3 mm; large AAA mean maximum aortic diameter = 68.4 ± 14.3 mm. 

d. Relative expression of LILRB2 and PTPN22 based on GSE57691 dataset. Data are shown as mean ± SD. *, significantly different from control; ***, p < 0.001.

Figure 3
LILRB2 and PTPN22 have no diagnostic value for TAA. a Schematic diagram of GSE140947 dataset. Distal and mid aortic smooth muscle cells (ASC) are derived from normal controls; SC of aneurysmal neck and belly are derived from TAA. These cells are subsequently profiled using RNA sequencing. b Relative expression of LILRB2 and PTPN22 based on GSE140947 dataset. Data are shown as mean ± SD. c Schematic diagram of GSE9106 dataset. Peripheral blood cells collected from normal controls and TAA patients were profiled using RNA sequencing. d Volcano plot illustrating DEGs between normal controls and TAA patients based on GSE9106 dataset. Red, significantly upregulated genes; blue, significantly downregulated genes; gray, no significant difference. Fold change ≥ 2 and p < 0.05 were considered significant. e Number of significantly expressed DEGs in TAA patients. f Top enriched GO terms of significantly upregulated and downregulated DEGs in TAA patients, respectively. g Relative expression of LILRB2 and PTPN22 based on GSE9106 dataset. Data are shown as mean ± SD. (H) ROC curve of LILRB2 and PTPN22 based on GSE9106 dataset.

Figure 4

Mechanical and therapeutic validations in vitro and mouse AAA experimental models. a Relative (Rel.) expression of Ptpn22 in aortic tissues collected from sham and AAA mice induced by PPE based on GSE51227 dataset. Data are shown as mean ± SD. *, significantly different from sham; ***, p < 0.001. Schematic diagram (b) and heatmap (c) based on GSE109639 dataset illustrating the expression of Ptpn22 in aortic tissues collected from WT and muMT mice treated with vehicle and CaCl2 for 7 days, respectively. WT, wild type; muMT, B cell-deficient. d Representative immunofluorescence images of
VSMCs treated with different concentrations of CaCl2. Cells were stained with αSMA (green); nuclei were labelled with DAPI (blue). Representative images of two independent experiments are shown. Scale bar, 50 μm. e VSMCs was treated by CaCl2 (100 mmol/L), and the expression of cellular Ptpn22 was assessed by qRT-PCR. Data are shown as mean ± SD from four biological replicates. *, significantly different from control; **, p < 0.01. f Representative immunofluorescence images of VSMCs treated with Garcinia acid in the absence/presence of CaCl2. Cells were stained with αSMA (green); nuclei were labelled with DAPI (blue). Representative images of two independent experiments are shown. Scale bar, 50 μm. g Venn diagram of the potential transcription factor targeting PTPN22 obtained by FunRich and TRAP. Predicted binding site in the promoter region of PTPN22 and the corresponding consensus motif of HNF4A is presented.

**Supplementary Files**

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