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PIN1, a perspective on genetic biomarker for nonalcoholic fatty liver disease (NAFLD)

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ABSTRACT

Objective: A novel genetic and molecular basis of nonalcoholic fatty liver disease (NAFLD) was explored.
Study design: A 38-year-old male, who has no bad living and dietary habits, was diagnosed as NAFLD. The potential pathogenic role of Pin1 was evaluated by enzyme-linked immunosorbent (ELISA) assay and single nucleotide polymorphism (SNP) sequencing.
Results: ELISA determined a six-time higher concentration of plasma Pin1 compared to our previous data. Nine *PIN1* SNPs were sequenced and classified according to their NAFLD-pathogenic risks, suggesting that rs2233678 and rs2287839 may be the most important genotypes that result in Pin1 overexpression and NAFLD development.
Conclusion: In summary, this work explores a novel basis for early-onset NAFLD and highlights that elevated plasma Pin1 may predict NAFLD risk at early stage. Hypothetically, inhibiting Pin1 may benefit NAFLD prevention in the future.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a major cause of liver fibrosis, liver cirrhosis and liver cancers, implying that early diagnosis of NAFLD will benefit the prevention of many liver diseases [1–4]. However, the pathogenic mechanisms of NAFLD are still complicated and even ambiguous until now, and recent studies increasingly emphasized the importance of genetic variations in the pathogenesis of NAFLD. For example, the polymorphisms of several genes such as adiponectin C1Q (ADIPOQ), patatin-like phospholipase domain-containing protein-3 (PNPLA3), Tribbles-1 (TRIB1), and vitamin D-related genes, may play crucial roles in development and progression of NAFLD [5–9].

Recently, we demonstrated the medical application of 3D-printing in liver surgeries and drug hepatotoxicity evaluation [10], and we also elucidated the indispensable role Pin1 in several human diseases [11–13]. The *PIN1* gene encodes the peptidyl-prolyl *cis-trans* isomerase Pin1, which is the only known enzyme that modulates the *cis-trans* configuration of pThr/pSer-Pro motifs in many signaling molecules [13]. Herein, we intend to elucidate the potential pathogenic role of Pin1 in NAFLD.

2. Methods

2.1. Patient investigation

A 38-year-old individual was enrolled in the first department of cardiology in the Affiliated Hospital of Hebei University of Engineering on May 21, 2018. The individual finished a standard questionnaire, signed the informed consent form, and the study was approved by the Ethical Committee of the Affiliated Hospital of Hebei University of Engineering.

2.2. Experimental measurements

5 mL peripheral blood was collected into an anticoagulant vacuum tube. The plasma and the blood cells were separated by centrifugation at 3000 rpm for 10 min and were stored at −80 °C. The Pin1 concentration in the blood and the single nucleotide polymorphisms (SNPs) of the *PIN1* gene were analyzed by ELISA

Abbreviations: NAFLD, nonalcoholic fatty liver disease; SNP, single nucleotide polymorphism; TGF-β1, transforming growth factor-β1; PPARα, peroxisome proliferator-activated receptor α; FGF-2, fibroblast growth factor 2; HIF-1α, hypoxia inducible factor-1α; VEGF, vascular endothelial growth factor.

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(Human Pin1 ELISA kit, Product number: ZCI BIO ZC-32747, Shanghai) [11] and DNA sequencing (Sangon Biotech, Shanghai), respectively. The reagents, primers for PCR, and DNA sequencing methods were provided by the Sangon Biotech (Shanghai) and shown in the Supplementary Material.

3. Results

The young individual (170 cm, 75 kg), who lives in the city of Handan and works in an office, was diagnosed with NAFLD in the Affiliated Hospital of Hebei University of Engineering. The triglyceride concentration in the individual's blood was 3.65 mmol/L, which is much higher than the healthy value range (0.48−1.88 mmol/L). Fatty liver was further confirmed by liver imaging via computer tomography.

A questionnaire survey manifested that the individual has no bad living and dietary habits such as drinking, smoking, working overtime, staying up late, excessive intake of egg, meat, milk and fish, *etc*. The individual has no hypertension and diabetes, and his body mass index (25.9) is approximately in the normal range.

Based on the above information, the underlying NAFLD-pathogenic mechanism for the individual is not clear. Consequently, the physicians and researchers thus suggested a molecular and genetic analysis for the individual, focusing on the concentration of Pin1 protein in the blood and the single nucleotide polymorphisms (SNPs) of *PIN1* gene.

The ELISA result indicated that the Pin1 concentration in the individual's blood plasma (303.113 pg/mL) is significantly higher (about six-time more) than our previous determination of Pin1 concentration (about 50 pg/mL) in eleven volunteers [11].

High Pin1 in plasma may be due to enhanced *PIN1* transcription due to genetic polymorphisms. Among the nine SNPs (Fig. 1, the PCR primers were not shown here because of word count limitation), the Pin1 high-expression genotypes as well as the high NAFLD-pathogenic risks were mainly attributed to rs2233678 and rs2287839 in the *PIN1* gene promoter. For example: ***i***, The SNP rs2233678 (NO. 2 in Fig. 1) was the −842GG homogeneous genotype, and −842G was proven to promote *PIN1* transcription and enhance Pin1 expression (in some cancer patients) when compared with the −842C allele [14]; ***ii***, The SNP rs2287839 (NO. 1) was the −5185GC heterogeneous genotype, and the variant −5185C has a lower affinity with AP4 (a *PIN1* promoter suppressor) and thus may enhance *PIN1* transcription (in some Alzheimer patients) when compared with the −5185G allele [15]; ***iii***, The impacts of other SNPs (located in the promoter or exon 2 or introns), on *PIN1* transcriptional efficiency, mRNA splicing and protein expression still need to be further elucidated. For example, the SNP rs2233679 (NO. 3) was identified as the −667CC homogeneous genotype in the promoter of *PIN1* gene, but the influences of −667C and −667T on Pin1 expression and relevant diseases are still not very identical, necessitating further clinical investigations especially in different ethnic groups [16−19].

These pathogenic analysis collectively supports a hypothesis that higher *PIN1* transcription and higher Pin1 expression might contribute to an increased risk of NAFLD in young human individuals.

4. Discussions

Considering that Pin1 regulates a series of signaling molecules *in vivo* [13], we speculate that Pin1 may promote the development of NAFLD by the following signaling pathways:

1. Nakatsu et al. firstly demonstrated that over-expressed Pin1 is necessary for methionine choline-deficient diet-induced NAFLD and the subsequent liver fibrosis in mice, whereas Pin1 knock-out mice were extremely resistant to these diseases [1].
2. Yang et al. found that Pin1 is up-regulated in mouse fibrotic liver tissues, that Pin1 plays a key role in dimethylnitrosamine-induced liver fibrosis in mice, and that Pin1 inhibition alleviates dimethylnitrosamine-induced liver fibrosis in mice, and these results may be partially due to the up-regulation of transforming growth factor-β1 (TGF-β1)-mediated fibrogenesis signalings induced by Pin1 [20].
3. The reduced activity of peroxisome proliferator-activated receptor α (PPARα) contributes to NAFLD, whereas enhancing PPARα activity ameliorates NAFLD [1]. Pin1 suppresses the expression levels of PPARα thus possibly resulting in NAFLD, but Pin1 knock-out mice are extremely resistant to NAFLD possibly because of enhanced PPARα expression and its downstream genes [1].
4. Pin1 is necessary for the activation of hepatic NF-κB and is important for the binding of NF-κB with DNA in hepatocytes [1,21]. Furthermore, the activation of NF-κB and its downstream inflammatory cytokines may aggravate NAFLD and liver fibrosis [1,21].
5. Compared to the wild-type mice, Pin1 knock-out mice had fewer fat tissues, which may be attributed to the insulin-signal-transduction mediated by Pin1 [22]. Pin1 enhanced the differentiation of mouse embryo fibroblasts into adipose cells in response to insulin stimulation, signifying that Pin1 may contribute to the obesity-related NAFLD [22].

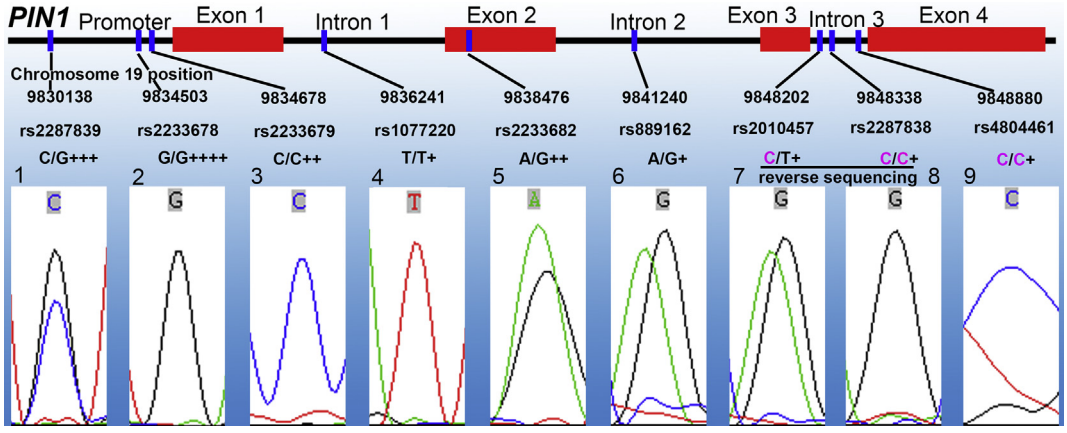

Fig. 1. The gene information of *PIN1* and the genotypes of SNP sequencing data. Annotation: '+' means the predicted NAFLD-pathogenic risk.

6. Fibroblast growth factor 2 (FGF-2) and its receptor FGFR1 promote the proliferation of hepatic stellate cells, and blocking FGF-2 signaling shows a significant anti-fibrosis effect [23]. Recent studies indicated that Pin1-dependent structural regulation of Runx2 is an crucial step in the FGF signaling transduction [23,24].

Besides, Pin1 may also contribute to NAFLD development by up-regulating hypoxia inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), *etc.*, [2,25,26].

5. Conclusion

In summary, the above descriptions primarily elicit the hypothesis that over-expressed Pin1 may play a hazardous role in NAFLD, highlighting that Pin1 may be an important diagnostic biomarker and a potential therapeutic target for NAFLD and other relevant liver diseases. Predictably, PIN1 genotyping could be effective for screening high NAFLD-risk individuals, and Pin1 inhibitors (extracted from natural plants or chemically-synthesized) may have therapeutic efficacy for early NAFLD, thus preventing the development of many fatty liver-related diseases.

Additionally, a recent study found that alcohol stimulates Pin1 over-expression in mouse heart cells [27]. This finding probably supports the speculation that Pin1 may also play a critical pathogenic role in alcoholic fatty liver disease. Nevertheless, we should be clearly aware that large-cohort studies should be necessary to confirm the above predictions, especially when considering that only one patient with NAFLD was included in this short report, and we will try to elucidate them more strictly step by step in the future.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2019.100014.

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