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***Case Control Study***

**Keratin 8/18 variants involved in non-alcoholic fatty liver disease and their association with insulin resistance: a case-control study in Chinese cohort**

Li R *et al*. Keratin 8/18 variants in NAFLD

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**Abstract**

***AIM***

To test the hypothesis that K8/K18 variants predispose humans to Non-alcoholic fatty liver disease (NAFLD) progression and its metabolic phenotypes.

***METHODS***

We selected a total of 373 unrelated adult subjects from our Physical Examination Department, including 200 unrelated NAFLD patients and 173 controls of both genders and different ages. Diagnose of NAFLD were established according to ultrasonic sign of fatty liver. All subjects were tested for population characteristics, lipid profile, liver tests, as well as glucose tests. Genomic DNA was obtained from EDTA-anticoagulated peripheral blood with a DNeasy Tissue Kit. K8/K18 coding regions were analyzed, including 15 exons and exon-intron boundaries.

***RESULTS***

Among 200 NAFLD patients, 10 heterozygous carriers of keratin variants were identified (5%), including 5 amino-acid-altering heterozygous variants and 6 non-coding heterozygous variants. One novel amino-acid-altering heterozygous variant (K18 N193S) and three novel non-coding variants were observed (K8 IVS5-9A→G, K8 IVS6+19G→A, K18 T195T). A total of 9 patients had a single variant and 1 patient had compound variants (K18 N193S+K8 IVS3-15C→G). Only one R341H variants was found in the control group (1 of 173, 0.58%). The frequency of keratin variants in NAFLD patients was significantly higher than in the control group (5% *vs* 0.58%，*P* = 0.015). Notably, the keratin variant was significantly associated with insulin resistance (IR) in NAFLD patients (8.86% variants in NAFLD with IR *vs* 2.5% in NAFLD without IR, *P* = 0.043).

***CONCLUSION***

K8/K18 variants are overrepresented in Chinese NAFLD patients and might accelerate liver fat storage through IR.

**Key words:** Keratin; Variant; Non-alcoholic fatty liver disease; Insulin resistance; Chinese population

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**C****ore tip:** This study presents the first investigation of the association between keratin 8 and 18 (K8/K18) variants and Non-alcoholic fatty liver disease (NAFLD) in a Chinese population. We found an increased frequency of variants in NAFLD patients versus controls. We also identified a new amino acid-altering variant of K18. The results demonstrate that keratin variants are overrepresented in Chinese NAFLD patients and might accelerate liver fat storage through insulin resistance.

Li R, Liao XH, Ye JZ, Li MR, Wu YQ, Hu X, Zhong BH. Keratin 8/18 variants involved in non-alcoholic fatty liver disease and their association with insulin resistance: a case-control study in Chinese cohort. *World J Gastroenterol* 2017; In press

**INTRODUCTION**

Keratin (K) proteins form intermediate filaments (IFs) in epithelial cells, which constitute the major cytoskeletal proteins along with actin filaments and microtubules[1,2]. They are primarily expressed in epithelial tissues, hair and skin appendages, and they are classed as relatively acidic type I IFs (K9–K28, K31–K40) and relatively basic type II IFs (K1–K8, K71–K86)[3,4]. Type I and type II keratins exist as paired polymeric filaments, that display a tripartite structure containing a conserved α-helical central rod domain that is flanked by less conserved N-terminal head and C-terminal tail domains[3,5]. For example, K5/K14 and K1/K10 are found in basal and suprabasal keratinocytes, whereas K8/K18 are found in adult hepatocytes and other simple-type epithelia cells[6]. Moreover, human association studies have identified mutations in keratins that can cause or predispose carriers to a broad range of human diseases, including multiple liver diseases[1,7]. Finally, the importance of K8/K18 in protecting hepatocytes from apoptosis was clearly demonstrated in keratin-related genetically engineered animal models[6, 8].

Non-alcoholic fatty liver disease (NAFLD), regarded as a hepatic manifestation of metabolic syndrome, has become the most common chronic liver disease worldwide, as it affects 20%-50% of the general population in affluent countries[9,10], and 15% of individuals in China[11]. NAFLD is strongly concomitant with obesity, hypertriglyceridemia, type 2 diabetes mellitus, and insulin resistance (IR)[12]. It is generally accepted that the initiating events in NAFLD depend on the development of obesity and IR at the level of adipose tissue and the liver.

There are accumulating examples of K8/K18 involvement in the glucose-insulin cross-talk, that supported the impact of K8/K18 IFs on insulin-dependent glucose metabolism regulation in the liver and its implication in glucose- or insulin-associated diseases[12,13]. Lower fasting glucose levels, increased glucose tolerance and insulin sensitivity, reduced glucose-stimulated insulin secretion and decreased pancreatic insulin content have been shown to occur in K8 knockout mice[14]. The mislocalization of glucose transporter (GLUT) and hexokinase (HK) status may contribute to the modulation of IFs in hepatocytes and hepatoma cells that lack K8/K18[14,15].

Subsequent studies have established that keratin mutation plays an important role in liver diseases and glucose metabolism; we hypothesized that K8/K18 variants may contribute to susceptibility to NAFLD. No studies have been reported on the association between K8/K18 mutations and NAFLD. Therefore, we tested our hypothesis by sequencing 200 NAFLD subjects, and we report herein our positive findings.

**MATERIALS AND METHODS**

***Subjects***

A total of 373 unrelated adult subjects were selected from our Physical Examination Department from January 2011 to December 2012, including 200 unrelated Chinese patients of both genders and different ages (164 males, 36 females, mean age 40.85 ± 9.9 years) and 173 healthy controls who were matched for sex and age (130 males, 43 females, mean age 39.55 ± 9.9 years). Diagnoses of NAFLD were performed under standard clinical evaluation conditions by ultrasonography according to AASLD criteria[16]. Other causes of liver disease were excluded, including increased alcohol intake (> 210/140 g weekly for males/females), viral and autoimmune hepatitis, hereditary hemochromatosis, Wilson’s disease and alphal-antitrypsin deficiency. Controls were confirmed as healthy based on a medical history, general examinations and laboratory examinations at the same hospital. Each participant underwent an anthropometric assessment, including measurements of weight and stature. Body mass index (BMI) was calculated as weight (kg)/stature (m2). All subjects received blood tests after an overnight 12 h fast, including serum triglycerides (TG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), serum uric acid (UA), fasting blood glucose (FBG), fasting insulin (FIN), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). IR was defined by the homeostasis model assessment-IR (HOMA-IR) index. As reported in an epidemiology survey carried out in China, IR was defined as HOMA-IR > 2.69 (*i.e.*, exceeding the 75% percentile of HOMA-IR in normal glucose tolerance subjects)[17,18].

***Genetic DNA extraction and genotyping***

Genomic DNA was obtained from EDTA-anticoagulated peripheral blood using a DNeasy Tissue Kit (Tiangen, Biotech, Beijing, China). The entire K8/K18 coding regions(15 exons and their adjacent exon-intron boundaries) of the DNA fragments was analyzed, which had been amplified with a Touchdown polymerase chain reaction (PCR) protocol using Premix PrimeSTAR ® HS (TaKaRa, Biotechnology, Dalian, China) and previously described primers[19] to obtain high amplification specificity. Messenger RNA sequences of K8 (NM002273) and K18 (NM000224) were used to localize coding variants, while genomic sequences (hKRT8 [M34482] and hKRT18 [AF179904]) were employed for noncoding variants.

***Statistical analysis***

Statistical analyses were performed using SPSS statistical software, version 20.0 for Windows (SPSS Inc., Chicago, IL, United States). Continuous variables were expressed as means ± SD. For continuous variables, a two-tailed *t*-test was used for two group comparisons, whereas Kruskal-Wallis nonparametric one-way analysis of variance was used to compare qualitative variables. K8/K18 variant frequencies in the NAFLD patient and control groups were compared with the two-tailed Fisher exact probability test. All *P*-values less than 0.05 were considered to indicate a statistically significant difference.

**RESULTS**

***Demographics and characteristics of NAFLD patients***

Demographics and clinical information for the NAFLD patients and control groups are summarized in Table 1. A total of 200 patients with NAFLD were included. As expected, most patients were male (82%). Compared with the control group, NAFLD patients showed higher ALT, AST, FBG, TC, TG, LDL and UA levels and lower levels of HDL.

***Keratin variants in NAFLD patients and controls***

We identified keratin heterozygous variants in 10 of 200 NAFLD patients (5%), including 5 carriers of amino-acid-altering heterozygous variants and 6 carriers of non-coding heterozygous variants (Table 2). Among 200 NAFLD patients, 2 (1%) carried R341H heterozygous exonic variants (Figure 1A), which is lower than the previously reported frequency. Additionally, one novel aminoacid-altering heterozygous variant (K18 N193S) and three novel non-coding variants (K8 IVS5-9A→G, K8 IVS6+19G→A, K18 T195T) were observed (Figure 1B). A total of 9 patients had a single variant and 1 patient had compound variants (K18 N193S+K8 IVS3-15C→G). A previously described silent and common heterozygous K8 L227L variant, which we detected in 43% of patients (not shown), is not included in Table 2 or any subsequent analysis.

***A keratin variant is associated with*** ***biochemical parameters in patients with NAFLD***

We found an increased frequency of variants in NAFLD patients versus controls (5% *vs* 0.58%，*P* = 0.015). To explore whether K8/K18 variants could affect the biochemical parameters, we compared non-carriers with variant carriers in all NAFLD patients. We found that there was no significant difference in clinical features, including BMI, liver biochemistry, glucose, lipids and HOMA-IR (Table 3). However, after dividing patients into those with or without IR, keratin variants showed a significant association with IR in NAFLD patients (Table 2; 8.86% variants in NAFLD with IR *vs* 2.5% in NAFLD without IR, *P* = 0.043).

**Discussion**

Previous reports suggested that K8/K18 variants predispose carriers to various types of liver injury, but it is not known whether K8 or K18 variants show an association with NAFLD. For the first time, our present study investigates the association between K8/K18 variants and NAFLD in a Chinese population. We analyzed K8/K18 variants in NAFLD patients and observed the potential association of keratin variants with NAFLD.

Our results demonstrate that keratin variants are overrepresented in NAFLD patients. Moreover, K8 has the most keratin variants, which is consistent with data from patients with chronic hepatitis C, primary biliary cirrhosis and acute liver failure. This finding highlights the importance of K8/K18 gene variants in NAFLD. The frequency of keratin variants presented here is somewhat lower compared with patients who suffer from other types of liver disease (*e.g.*, 13.1% and 12.4%[20,21]), as previously reported, but it is close to that observed in an Asian cohort in a United States study[22]. Multiple K8/K18 variants have been verified and R341H represents the most common amino acid-altering K8/K18 variant in Chinese populations. As confirmed herein, the K8 variant R341H also exclusively associates with the intronic K8 IVS7+10delC deletion[19]. We also found a new amino acid-altering variant N193S of K18, in which the patient simultaneously carries an intronic variant IVS3-15C→G. It remains to be determined whether this nucleotide error at one site acts in conjunction with the other.

Hepatic steatosis occurs when insulin signaling is impaired, with the development of IR driven by adipose tissue and the liver, along with the sustained excess delivery of fatty acids to the liver. Here, we observed a correlation of IR with keratin variants. This association suggests that K8/K18 mutations result in increased in liver fat, probably through the hepatic insulin resistance, in accordance with other studies. For example, early work using K8-null mice found reduced fasting blood glucose levels, increased glucose tolerance and insulin sensitivity, altered insulin vesicle morphology, and reduced pancreatic insulin levels[14]. Similarly, a study conducted in Finland shows that K8/K18 loss in hepatoma cells leads to distinct alterations in HK status, which are associated with a differential modulation of insulin signaling-dependent regulation of glucose-mediated glycogen formation and proliferative capacity[23]. However, differences in K8/K18 IF loss cause the mis-localization of the GLUT 1-4 transport proteins. Additionally, it is important to consider the cellular context, namely cultured hepatic cells[23] *vs in vivo* embryonic[24] conditions. This reveals distinctive increases in glucose uptake, glucose-6-phosphate formation, lactate release, and glycogen formation in K8/K18 IF- deficient hepatocytes versus respective IF-containing counterparts. Moreover, compelling evidence suggests that the shape and distribution of mitochondria are modulated by cytoskeletal proteins, including keratin[25]. Together, these findings suggest that hepatocyte keratins have a central role in the systemic regulation of glucose. Although the molecular mechanisms that underlie these metabolic perturbations are unclear, K8/K18 IFs likely modify liver fat content via the insulin signaling pathway[26,27].

This study is limited by the absence of liver biopsies to evaluate the severity of hepatic steatosis and fibrosis stage. The absence of histological evidence may weaken the clinical relevance of these genetic effects, although the abdominal ultrasound is generally applied in larger surveys as a noninvasive and convenient tool to diagnose NAFLD[12]. Moreover, cell-based and transgenic mouse studies with genetic K8/K18 mutants or knockouts are needed to verify that keratin variants can cause or predispose carriers to the development of pathologies.

As studies of Asian populations have been limited, we are the first to discuss an association between keratin variants and NAFLD. Our findings provide novel evidence for keratin proteins as modulators in the development of NAFLD and in the pathophysiology of insulin resistance. Further studies of K8/K18 mutations as drivers of insulin resistance and liver fat accumulation are needed. These observations raise the question of how naturally occurring keratin mutations affect liver fatty in humans, and whether such mutations may similarly affect *in vitro* models or animals.

**COMMENTS**

***Background***

Keratins 8 and 18 (K8/K18) protect hepatocytes from various types of injury, and previous reports have suggested that K8/K18 variants predispose carriers to various forms of liver injury. However, it is not known whether K8 or K18 variants have an association with non-alcoholic fatty liver disease (NAFLD). Thus, we tested the hypothesis that K8/K18 variants predispose humans to NAFLD progression and its metabolic phenotypes.

***Research frontiers***

The relationship between keratin variants and liver disease varies in different population. Therefore, studies enrolling histological evidence and more ethnic groups are required. Further studies are also needed to determine the associations and functional consequences of these variants in NAFLD.

***Innovations and breakthrough***

This is the first published study to investigate the relationship between keratin variants and NAFLD. These findings also provide novel evidence for keratin proteins as modulators in the development of NAFLD and in the pathophysiology of insulin resistance.

***Applications***

The association of keratin variants with NAFLD suggests a prominent gene influence on human NAFLD. We should pay more attention to individuals who carries keratin variant, and provide early intervention before disease progression.

***Terminology***

Keratins (K) constitute the largest subgroup of intermediate filaments (IFs) and are expressed primarily in epithelial tissues, hair and skin appendages. Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver dysfunction in the Western world. It’s a spectrum of liver disease that includes simple steatosis, fatty infiltration plus inflammation, and hepatocellular ballooning degeneration, progressing to fibrosis and ultimately cirrhosis.

***Peer-review***

This is a very interesting human study that suggests an association between keratin 8/18 variants and NAFLD. Also the authors successfully showed that IR is a potential mediator of this association. These are completely novel viewpoint in NAFLD research. This study is a good start for future research in this field.

**REFERENCES**

1 **Haines RL**, Lane EB. Keratins and disease at a glance. *J Cell Sci* 2012; **125**: 3923-3928 [PMID: 23104737 DOI: 10.1242/jcs.099655]

2 **Pan X**, Hobbs RP, Coulombe PA. The expanding significance of keratin intermediate filaments in normal and diseased epithelia. *Curr Opin Cell Biol* 2013; **25**: 47-56 [PMID: 23270662 DOI: 10.1016/j.ceb.2012.10.018]

3 **Coulombe PA**, Omary MB. 'Hard' and 'soft' principles defining the structure, function and regulation of keratin intermediate filaments. *Curr Opin Cell Biol* 2002; **14**: 110-122 [PMID: 11792552]

4 **Schweizer J**, Bowden PE, Coulombe PA, Langbein L, Lane EB, Magin TM, Maltais L, Omary MB, Parry DA, Rogers MA, Wright MW. New consensus nomenclature for mammalian keratins. *J Cell Biol* 2006; **174**: 169-174 [PMID: 16831889 DOI: 10.1083/jcb.200603161]

5 **Fuchs E**, Weber K. Intermediate filaments: structure, dynamics, function, and disease. *Annu Rev Biochem* 1994; **63**: 345-382 [PMID: 7979242 DOI: 10.1146/annurev.bi.63.070194.002021]

6 **Omary MB**, Ku NO, Strnad P, Hanada S. Toward unraveling the complexity of simple epithelial keratins in human disease. *J Clin Invest* 2009; **119**: 1794-1805 [PMID: 19587454 DOI: 10.1172/JCI37762]

7 **Strnad P**, Paschke S, Jang KH, Ku NO. Keratins: markers and modulators of liver disease. *Curr Opin Gastroenterol* 2012; **28**: 209-216 [PMID: 22450891 DOI: 10.1097/MOG.0b013e3283525cb8]

8 **Coulombe PA**, Kerns ML, Fuchs E. Epidermolysis bullosa simplex: a paradigm for disorders of tissue fragility. *J Clin Invest* 2009; **119**: 1784-1793 [PMID: 19587453 DOI: 10.1172/JCI38177]

9 **Williams CD**, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, Landt CL, Harrison SA. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology* 2011; **140**: 124-131 [PMID: 20858492 DOI: 10.1053/j.gastro.2010.09.038]

10 **Wong VW**, Chu WC, Wong GL, Chan RS, Chim AM, Ong A, Yeung DK, Yiu KK, Chu SH, Woo J, Chan FK, Chan HL. Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using proton-magnetic resonance spectroscopy and transient elastography. *Gut* 2012; **61**: 409-415 [PMID: 21846782 DOI: 10.1136/gutjnl-2011-300342]

11 **Fan JG**, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. *J Hepatol* 2009; **50**: 204-210 [PMID: 19014878 DOI: 10.1016/j.jhep.2008.10.010]

12 **Musso G**, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia* 2012; **55**: 885-904 [PMID: 22278337 DOI: 10.1007/s00125-011-2446-4]

13 **Herrmann H**, Strelkov SV, Burkhard P, Aebi U. Intermediate filaments: primary determinants of cell architecture and plasticity. *J Clin Invest* 2009; **119**: 1772-1783 [PMID: 19587452 DOI: 10.1172/JCI38214]

14 **Alam CM**, Silvander JS, Daniel EN, Tao GZ, Kvarnström SM, Alam P, Omary MB, Hänninen A, Toivola DM. Keratin 8 modulates β-cell stress responses and normoglycaemia. *J Cell Sci* 2013; **126**: 5635-5644 [PMID: 24144696 DOI: 10.1242/jcs.132795]

15 **Roux A**, Gilbert S, Loranger A, Marceau N. Impact of keratin intermediate filaments on insulin-mediated glucose metabolism regulation in the liver and disease association. *FASEB J* 2016; **30**: 491-502 [PMID: 26467793 DOI: 10.1096/fj.15-277905]

16 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology* 2012; **142**: 1592-1609 [PMID: 22656328 DOI: 10.1053/j.gastro.2012.04.001]

17 **European Association for the Study of the Liver (EASL)**; European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016; **64**: 1388-1402 [PMID: 27062661 DOI: 10.1016/j.jhep.2015.11.004]

18 **Zhang L**, Chen S, Deng A, Liu X, Liang Y, Shao X, Sun M, Zou H. Association between lipid ratios and insulin resistance in a Chinese population. *PLoS One* 2015; **10**: e0116110 [PMID: 25635876 DOI: 10.1371/journal.pone.0116110]

19 **Strnad P**, Lienau TC, Tao GZ, Lazzeroni LC, Stickel F, Schuppan D, Omary MB. Keratin variants associate with progression of fibrosis during chronic hepatitis C infection. *Hepatology* 2006; **43**: 1354-1363 [PMID: 16729313 DOI: 10.1002/hep.21211]

20 **Strnad P**, Zhou Q, Hanada S, Lazzeroni LC, Zhong BH, So P, Davern TJ, Lee WM, Omary MB. Keratin variants predispose to acute liver failure and adverse outcome: race and ethnic associations. *Gastroenterology* 2010; **139**: 828-35, 835.e1-3 [PMID: 20538000 DOI: 10.1053/j.gastro.2010.06.007]

21 **Ku NO**, Lim JK, Krams SM, Esquivel CO, Keeffe EB, Wright TL, Parry DA, Omary MB. Keratins as susceptibility genes for end-stage liver disease. *Gastroenterology* 2005; **129**: 885-893 [PMID: 16143128 DOI: 10.1053/j.gastro.2005.06.065]

22 **Usachov V**, Urban TJ, Fontana RJ, Gross A, Iyer S, Omary MB, Strnad P. Prevalence of genetic variants of keratins 8 and 18 in patients with drug-induced liver injury. *BMC Med* 2015; **13**: 196 [PMID: 26286715 DOI: 10.1186/s12916-015-0418-0]

23 **Mathew J**, Loranger A, Gilbert S, Faure R, Marceau N. Keratin 8/18 regulation of glucose metabolism in normal versus cancerous hepatic cells through differential modulation of hexokinase status and insulin signaling. *Exp Cell Res* 2013; **319**: 474-486 [PMID: 23164509 DOI: 10.1016/j.yexcr.2012.11.011]

24 **Vijayaraj P**, Kröger C, Reuter U, Windoffer R, Leube RE, Magin TM. Keratins regulate protein biosynthesis through localization of GLUT1 and -3 upstream of AMP kinase and Raptor. *J Cell Biol* 2009; **187**: 175-184 [PMID: 19841136 DOI: 10.1083/jcb.200906094]

25 **Anesti V**, Scorrano L. The relationship between mitochondrial shape and function and the cytoskeleton. *Biochim Biophys Acta* 2006; **1757**: 692-699 [PMID: 16729962 DOI: 10.1016/j.bbabio.2006.04.013]

26 **Kido Y**, Burks DJ, Withers D, Bruning JC, Kahn CR, White MF, Accili D. Tissue-specific insulin resistance in mice with mutations in the insulin receptor, IRS-1, and IRS-2. *J Clin Invest* 2000; **105**: 199-205 [PMID: 10642598 DOI: 10.1172/JCI7917]

27 **Hamer I**, Foti M, Emkey R, Cordier-Bussat M, Philippe J, De Meyts P, Maeder C, Kahn CR, Carpentier JL. An arginine to cysteine(252) mutation in insulin receptors from a patient with severe insulin resistance inhibits receptor internalisation but preserves signalling events. *Diabetologia* 2002; **45**: 657-667 [PMID: 12107746 DOI: 10.1007/s00125-002-0798-5]

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**Peer-review report classification**

Grade A (Excellent): 0

Grade B (Very good): B, B

Grade C (Good): 0

Grade D (Fair): 0

Grade E (Poor): 0

**Table 1 Demographics and clinical information of non-alcoholic fatty liver disease patients and control groups**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Controls (*n* = 173)** | **NAFLD patients (*n* = 200)** | ***P* value** |
| Sex (male, %) | 130 (75.14) | 164 (82.00) | 0.107 |
| Age (yr) | 39.55 ± 9.92 | 40.85 ± 9.89 | 0.101 |
| ALT (U/L) | 21.65 ± 12.69 | 43.63 ± 29.16 | < 0.0001 |
| AST (U/L)  FBG (mmol/L)  TC (mmol/L)  TG (mmol/L)  HDL (mmol/L)  LDL (mmol/L) | 24.74 ± 6.52  4.89 ± 0.62  4.71 ± 0.77  1.29 ± 0.99  1.22 ± 0.29  2.80 ± 0.61 | 33.85 ± 12.93  5.40 ± 1.45  5.68 ± 1.09  2.86 ± 2.14  1.07 ± 0.23  3.32 ± 0.95 | < 0.0001  < 0.0001  < 0.0001  < 0.0001  < 0.0001  < 0.0001 |
| UA (µmol/L) | 331.33 ± 88.61 | 412.42 ± 96.60 | < 0.0001 |

Data are presented as mean ± SD. ALT: alanine aminotransferase; AST: aspartate aminotransferase; FBG: fasting blood glucose; TC: total cholesterol; TG: triglyceride; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NAFLD: non-alcoholic fatty liver disease.

**Table 2 Distribution of keratin variants in non-alcoholic fatty liver disease patients and control groups**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Ketain gene** | **Variants** | | **NAFLD** | | | | |
| **Nucleotide** | **Amino acid** | **Control (*n* = 173)** | **Total (*n* = 200)** | **NAFLD with IR (*n* = 79)** | **NAFLDwithout IR (*n* = 121)** | |
| *K8* | 1022G→A  904C→T  1381G→A  IVS5-9A→G(new)  IVS3-26 C→T | R341H  R302C  V461M | 1  0  0  0  0 | 2  1  1  1  1 | 2  1  0  1  1 | 0  0  1  0  0 |
| *K18* | IVS6+19G→A (new) |  | 0 | 1 | 1 | 0 |
| IVS6+17C→T |  | 0 | 1 | 0 | 1 |
| IVS3-15C→G |  | 0 | 11 | 11 | 0 |
| 1590C→G | N193S (new) | 0 | 11 | 11 | 0 |
| 1448A→G | T195T (new) | 0 | 1 | 0 | 1 |
| Total (%) |  |  | 1 (0.58) | 10 (5.00) | 7 (8.86) | 3 (2.48) |

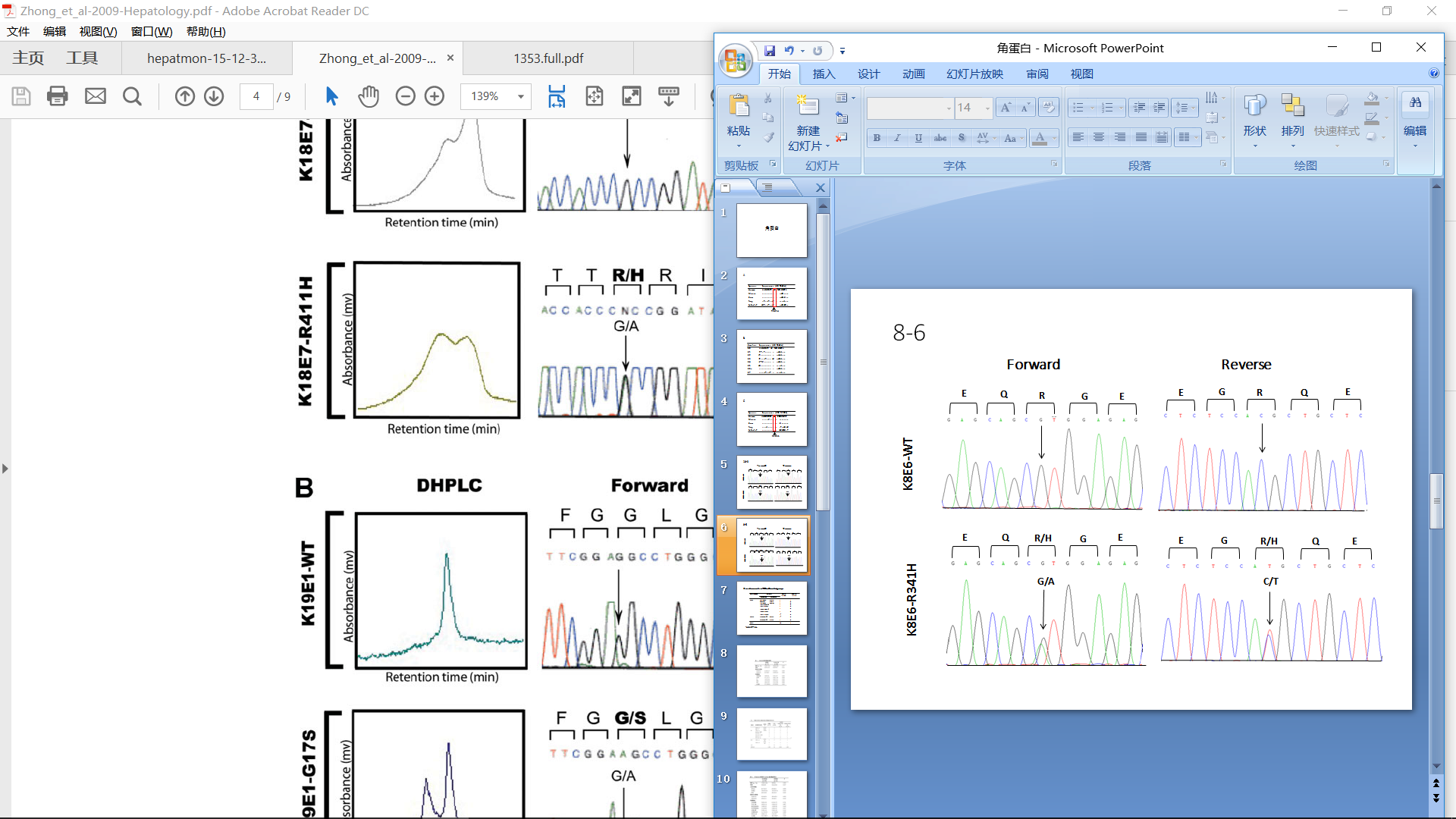
1one patient carries two K8/K18 variants (K18 N193S+K8 IVS3-15C→G). The table displays the number of patients of NAFLD and control groups harboring the listed keratin variants. IR: insulin resistance. IR: insulin resistance; NAFLD: non-alcoholic fatty liver disease.

**Table 3 Clinical features of non-alcoholic fatty liver disease patients harboring significant keratin variants**

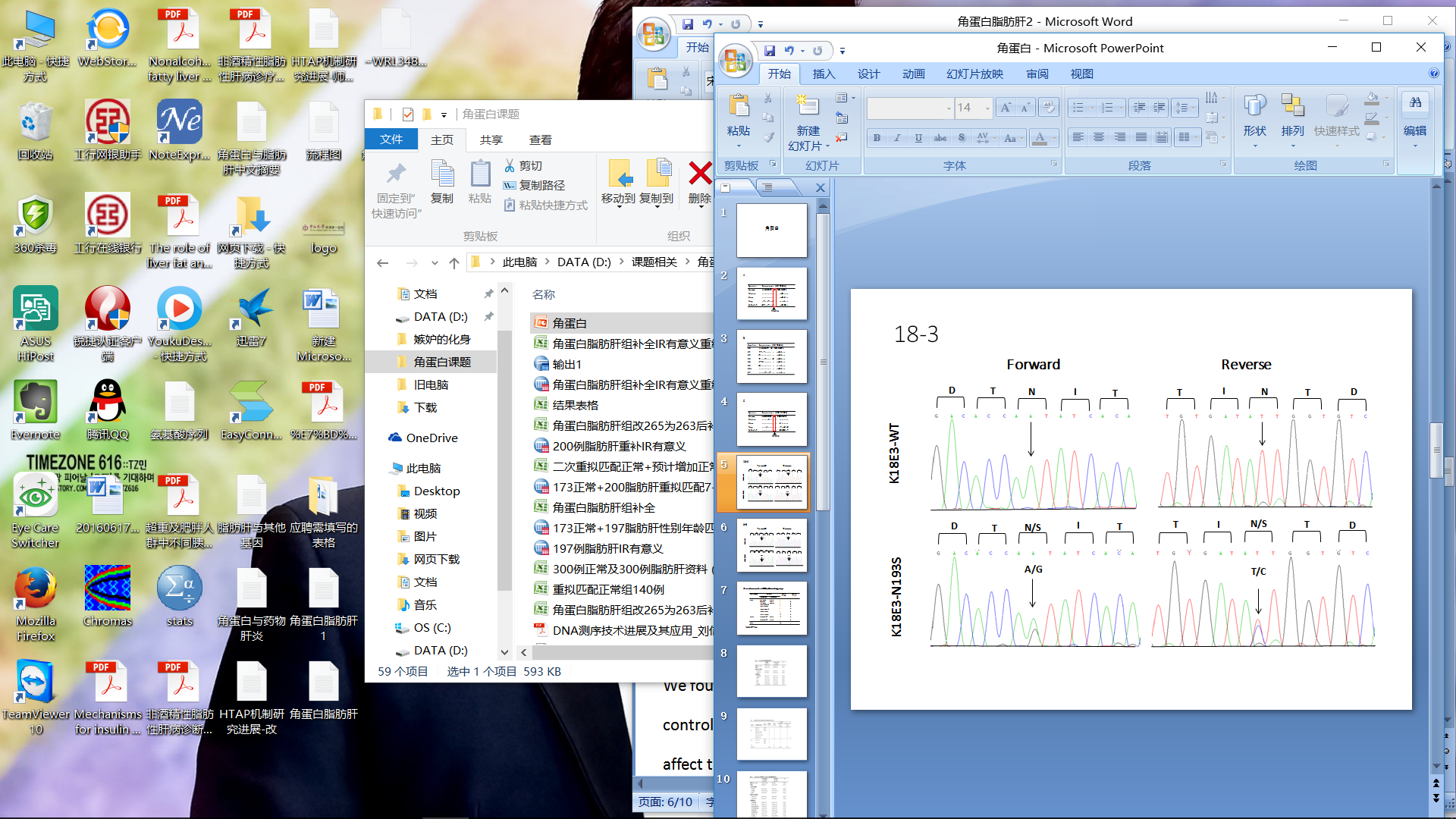
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| --- | --- | --- | --- |
|  | **Keratin variants carriers** | | ***P* value** |
| **No(*n* = 190)** | **Yes (*n* = 10)** |
| Sex (Male, %) | 155 (81.58) | 9 (90.00) | 0.436 |
| Age (yr) | 40.75 ± 9.87 | 42.60 ± 10.63 | 0.603 |
| Diastolic pressure(mmHg) | 82.67 ± 10.47 | 85.80 ± 9.38 | 0.330 |
| Systolic pressure(mmHg) | 130.79 ± 14.82 | 134.20 ± 17.07 | 0.550 |
| BMI (kg/m2) | 26.26 ± 2.91 | 26.10 ± 3.09 | 0.877 |
| ALT (U/L) | 35.93 ± 18.35 | 38.40 ± 25.30 | 0.767 |
| AST (U/L) | 34.03 ± 13.08 | 30.40 ± 9.31 | 0.265 |
| TC (mmol/L) | 5.67 ± 1.02 | 6.10 ± 1.69 | 0.453 |
| TG (mmol/L) | 2.85 ± 2.14 | 3.00 ± 2.35 | 0.850 |
| HDL (mmol/L) | 1.09 ± 0.24 | 1.10 ± 0.18 | 0.828 |
| LDL (mmol/L) | 3.31 ± 0.94 | 3.65 ± 1.12 | 0.374 |
| FBG (mmol/L)  FINS (mmol/L)  HOMA-IR | 5.38 ± 1.43  11.17 ± 4.52  2.64 ± 1.14 | 5.91 ± 1.44  12.73 ± 6.00  3.25 ± 1.50 | 0.281  0.439  0.237 |
| UA (µmol/L) | 414.06 ± 96.89 | 381.50 ± 89.88 | 0.292 |

Data are presented as mean ± SD. BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; TC: total cholesterol; TG: triglyceride HDL: high-density lipoprotein; LDL: low-density lipoprotein; FBG: fasting blood glucose; FINS: fasting insulin; UA: Uric acid.

A



B



**Figure 1 Detection of keratin variants in non-alcoholic fatty liver disease patients and controls.** Genomic DNA was sequenced in the forward and reverse directions. The nucleotide locations of the variants were determined as described in “Patients and Methods”. The heterozygous K8 exon 6-R341H (A) and novel heterozygous variant K18 exon 3-N193S (B) are presented in comparison with their corresponding wide type (WT) samples.