Original Paper

Modulation of the Association between the *PEPD* Variant and the Risk of Type 2 Diabetes by n-3 Fatty Acids in Chinese Hans

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Key Words

PEPD · n-3 fatty acids · Type 2 diabetes · Interaction with genetic variants · Chinese Han

Abstract

Background/Aims: Type 2 diabetes (T2D) is modulated by the interactions between genetic and dietary factors. This study sought to examine whether the associations of genome-wide association study (GWAS)-identified genetic variants with T2D risk were modulated by n-3 fatty acids in Chinese Hans. **Methods:** Six hundred and twenty-two T2D patients and 293 healthy controls were recruited. Erythrocyte phospholipid fatty acids were measured by standard methods. Nine GWAS-identified T2D-related single-nucleotide polymorphisms (SNPs) were genotyped. These SNPs were all identified in GWAS of Asian populations with a high minor allele frequency (>0.2). **Results:** Among the 9 SNPs, only rs3786897 at *PEPD* (peptidase D) showed a significant interaction with n-3 fatty acids ($p_{\text{interaction}}$ after Bonferroni correction = 0.027). The rs3786897 A allele was associated with a higher risk of T2D [GA+AA vs. GG: odds ratio (OR) = 2.16, 95% confidence interval (CI) 1.32–3.55] when n-3 fatty acids were lower than the population median, but no significant association (GA+AA vs. GG: OR = 0.63, 95% CI 0.35–1.12) was observed when n-3 fatty acids were higher than the median. **Conclusions:** The association between the *PEPD* genetic variant and the risk of T2D was modulated by n-3 fatty acids. Higher n-3 fatty acids may abolish the adverse effect of the risk allele at *PEPD* for T2D.

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Introduction

Type 2 diabetes (T2D) accounts for 90–95% of diabetes cases and is becoming a common chronic disease globally, especially in developing countries, such as China and India [1]. It was estimated that in Chinese adults, the overall prevalence of diabetes and prediabetes was 11.6 and 50.1% in 2010, respectively [2]. Since 2007, genome-wide association studies (GWAS) on T2D have been conducted in a variety of populations of different ancestries, and >200 T2D-related genetic variants have been identified by GWAS so far [3]. In addition to the genetic architecture of T2D susceptibility, environmental factors are suggested to play a key role in the etiology of T2D. Accumulating evidence suggests that environmental factors, especially dietary factors, may interact with genetic variants to modulate the risk of T2D and related traits [4]. Our genome-wide complex trait analysis has also demonstrated the importance of the genome-wide interaction of genetic variants with dietary factors in explaining the variance of diabetes-related traits in a European American population [5].

The health effect of dietary n-3 polyunsaturated fatty acids (PUFA) has been extensively explored during the past decades. Recent progress indicates that n-3 PUFA have a great potential to interact with genetic variants from various candidate genes to modulate the risk of chronic diseases, such as cardiovascular disease, metabolic syndrome and T2D [6]. Erythrocyte n-3 PUFA, biomarkers of dietary n-3 PUFA intake, have shown interaction with genetic variants at a genome-wide level for the variance contribution of diabetes-related traits in populations of different origins [7]. However, no study has been published in Chinese populations on the modification of n-3 PUFA for the genetic risk of T2D.

Several genetic variants at the *PEPD* (peptidase D) gene have been identified in recent GWAS to be associated with the risk of T2D in Asians [8, 9]. The enzyme encoded by *PEPD* plays an important role in the recycling of proline and collagen metabolisms, while collagen is shown to have a profound impact on β -cell function [10] and is involved in the pathogenesis of T2D [11, 12]. The GWAS-identified genetic variant rs3786897 at *PEPD* is shown to be highly associated with *PEPD* mRNA expression [8], which implicates a potential functionality of the variation. In addition to genetic variants of the *PEPD* gene, there are dozens of genetic variants identified by GWAS associated with the risk of T2D in Asians [3]. The aim of the present study was to examine the modulation of the associations between GWAS-identified genetic variants and the risk of T2D by n-3 PUFA in a Chinese population.

Material and Methods

Study Design and Participants

All diabetic patients meeting the requirements of the World Health Organization criteria for the diagnosis of T2D (World Health Organization, 1999) were recruited from outpatient clinics across 30 hospitals in 20 provinces in China. Fasting blood glucose for all the recruited patients was between 7.0 and 10.0 mmol/l. Exclusion criteria for the study were abnormal vitamin and mineral absorption (abnormal serum vitamin or mineral level, or clinical history); the use of vitamin and mineral supplementation within the last 3 months; severe renal, hepatic, heart or psychiatric diseases (with the exception of diabetic complications); a history of cancer, thyroid disease or alcohol abuse, and pregnancy or lactation. Finally, 622 T2D patients were enrolled in the study.

Healthy controls were recruited from Zhejiang Hospital in Hangzhou through a health check program. All the healthy control participants were free of hypertension, renal disease, hyperlipidemia, hematological disorders, diabetes, a family history of cardiovascular disease or diabetes, alcohol abuse and drug use. After careful screening, 293 healthy controls were included in the study.

The study protocol was approved by the Ethics Committee of the College of Biosystem Engineering and Food Science at Zhejiang University and the Institutional Review Board of Huadong Hospital. All the study participants gave informed written consent to be included in the study.

Measurement of Anthropometric Parameters and Erythrocyte Fatty Acids

Venous blood was collected after an overnight fast. Then, body weight, height, waist circumference, hip circumference and blood pressures were measured by a research assistant trained in standardized procedures. Erythrocyte membrane phospholipid fatty acids were measured using the standard method, as described previously [13]. The total lipid content of erythrocytes was extracted with chloroform/methanol (1:1), followed by the separation of lipids from the phospholipid fraction by thin-layer chromatography. Fatty acids from phospholipids were then converted to methyl ester and extracted into n-hexane and dried on anhydrous Na₂SO₄. Fatty acid methyl esters were filtered by Sep-Pak Silica column before gas chromatography separation and analysis.

Single-Nucleotide Polymorphism Selection and Genotyping

Nine single-nucleotide polymorphisms (SNPs) from 9 different regions, associated with T2D in Asian populations, were selected for genotyping based on the database of the National Human Genome Research Institute (www.genome.gov/gwastudies) [3]. Minor allele frequencies of all the selected SNPs were >0.20. The rs numbers of the 9 SNPs were rs1436953 (*C2CD4A-C2CD4B*), rs11257655 (*CDC123*), rs4712524 (*CDKAL1*), rs2383208 (*CDKN2B*), rs16955379 (*CMIP*), rs3786897 (*PEPD*), rs831571 (*PSMD6*), rs13266634 (*SLC30A8*) and rs1359790 (*SPRY2*; table 1).

QIAamp DNA Blood Mini kits were used to isolate blood DNA according to the manufacturer's instructions (Qiagen, Valencia, Calif., USA). TaqMan SNP genotyping kits with the ABI PRISMA 7900HT Sequence Detection System (Applied Biosystems, Foster City, Calif., USA) were used to genotype the selected SNPs, with an average genotyping success rate of 98%.

Statistical Analysis

SAS (version 9.2 for Windows) was used to perform the statistical analyses. χ^2 tests were conducted to examine the Hardy-Weinberg equilibrium of the selected 9 SNPs. A multivariable logistic regression model was used to assess the interaction of genetic variants with erythrocyte n-3 PUFA for T2D and the main effects of genetic variants or n-3 PUFA on T2D risk, adjusting for age and sex. Erythrocyte n-3 PUFA were dichotomized based on the median level of the population for the interaction analysis. Bonferroni correction was used to adjust for multiple comparisons. The number of multiple comparisons was 9, as the primary aim of the present study was to assess the interaction of 9 T2D SNPs with n-3 PUFA for T2D risk. Therefore, $p_{\text{interaction}} < 0.006$ (0.05/9) was considered to be statistically significant for the interaction analysis. For the main effect, $p < 0.05$ was considered to be statistically significant.

Results

Characteristics of Selected SNPs and Study Participants

The 9 selected SNPs were consistent with Hardy-Weinberg equilibrium, with the minor allele frequency ranging from 0.26 to 0.44 (table 1). Among healthy controls, but not among diabetic patients, significantly different erythrocyte n-3 PUFA compositions were observed across different genotypes of rs3786897 ($p = 0.044$; table 2).

Erythrocyte n-3 PUFA was categorized into 4 groups based on quartiles to investigate its association with the risk of T2D. Compared with the lowest quartile of n-3 PUFA, the risk of T2D was 0.42 [95% confidence interval (CI) 0.27–0.65], 0.57 (95% CI 0.37–0.89) and 0.47 (95% CI 0.31–0.73) for the second, third and fourth quartile of n-3 PUFA, respectively ($p_{\text{trend}} < 0.001$).

Associations of the 9 SNPs with the Risk of T2D

The associations of the 9 SNPs with the risk of T2D were examined under an additive model. Among the 9 SNPs, only rs2383208 at *CDKN2B* was significantly associated with a risk of T2D ($p = 0.042$ for the additive model). Compared with rs2383208 GG homozygotes, A allele carriers showed a 61% higher risk of T2D [odds ratio (OR) = 1.61, 95% CI 1.09–2.36; table 3].

Table 1. Characteristics of the 9 selected SNPs

SNP	Gene	p _{HWE}	MAF	Alleles (minor allele)
rs1436953	<i>C2CD4A, C2CD4B</i>	0.587	0.343	A/G (A)
rs11257655	<i>CDC123</i>	0.249	0.419	C/T (C)
rs4712524	<i>CDKAL1</i>	0.951	0.438	A/G (G)
rs2383208	<i>CDKN2B</i>	0.903	0.401	A/G (G)
rs16955379	<i>CMIP</i>	0.774	0.255	C/T (T)
rs3786897	<i>PEPD</i>	0.729	0.440	A/G (G)
rs831571	<i>PSMD6</i>	0.057	0.347	C/T (T)
rs13266634	<i>SLC30A8</i>	0.828	0.391	C/T (T)
rs1359790	<i>SPRY2</i>	0.563	0.290	C/T (T)

MAF = Minor allele frequency; HWE = Hardy-Weinberg equilibrium.

Table 2. Fatty acid profiles of T2D patients and healthy controls by rs3786897 genotypes

	T2D patients (n = 622)			Healthy controls (n = 293)		
	GG (n = 114)	GA (n = 318)	AA (n = 189)	GG (n = 59)	GA (n = 136)	AA (n = 94)
C18:3n-3	0.49±0.05	0.67±0.06	0.71±0.08	0.52±0.08	0.57±0.05	0.61±0.10
C20:5n-3	1.62±0.16	1.78±0.11	1.66±0.17	1.49±0.18	1.91±0.16	1.82±0.18
C22:5n-3	0.96±0.05	0.97±0.04	0.96±0.05	1.19±0.07	1.23±0.04	1.23±0.06
C22:6n-3	2.74±0.13	2.75±0.12	2.55±0.11	2.66±0.22	3.35±0.18	3.28±0.23
n-3 PUFA	5.81±0.23	6.15±0.22	5.87±0.25	5.86±0.34	7.05±0.26	6.94±0.37*
n-6 PUFA	30.9±1.06	31.7±0.58	30.7±0.75	33.7±0.82	34.9±0.59	33.6±0.66
PUFA	36.7±1.11	37.8±0.66	36.6±0.84	39.6±1.05	41.9±0.76	40.5±0.90
MUFA	26.3±1.02	24.5±0.58	26.0±0.78	22.5±0.78	20.4±0.70	21.1±0.75
SFA	37.2±0.59	37.8±0.32	37.6±0.49	37.7±0.56	37.6±0.53	38.3±0.44

C18:3n-3 = α -Linolenic acid; C20:5n-3 = eicosapentaenoic acid; C22:5n-3 = docosapentaenoic acid; C22:6n-3 = docosahexaenoic acid; MUFA = monounsaturated fatty acid; SFA = saturated fatty acid. * p < 0.05 for the difference of fatty acids across three rs3786897 genotype carriers under an additive model.

Table 3. Associations of rs3786897 and rs2383208 with the risk of T2D

rs3786897	OR (95% CI)	p value ¹	rs2383208	OR (95% CI)	p value ¹
AA vs. GG	1.09 (0.72–1.68)	0.663	AA vs. GG	1.72 (1.12–2.65)	0.013
GA vs. GG	1.29 (0.87–1.91)	0.207	GA vs. GG	1.53 (1.02–2.30)	0.041
GA+AA vs. GG	1.21 (0.84–1.75)	0.311	GA+AA vs. GG	1.61 (1.09–2.36)	0.016

¹ p values were adjusted for age and sex.

Interactions of the 9 SNPs with Erythrocyte n-3 Fatty Acids for the Risk of T2D

The interactions of the 9 SNPs with total n-3 PUFA were examined, and only rs3786897 at *PEPD* showed a significant interaction with n-3 PUFA in modulating the risk of T2D (p_{interaction} = 0.003). Significant interaction remained after correcting for multiple testing (corrected p_{interaction} = 0.027).

Table 4. Association of rs3786897 with the risk of T2D by erythrocyte n-3 fatty acids

	Low n-3 PUFA ($\leq 5.33\%$)		High n-3 PUFA ($> 5.33\%$)	
	OR (95% CI)	p value ¹	OR (95% CI)	p value ¹
AA vs. GG	1.88 (1.07–3.31)	0.028	0.59 (0.30–1.13)	0.112
GA vs. GG	2.40 (1.40–4.12)	0.002	0.65 (0.35–1.19)	0.158
GA+AA vs. GG	2.16 (1.32–3.55)	0.002	0.63 (0.35–1.12)	0.113

¹ p values were adjusted for age and sex.**Table 5.** Association of erythrocyte n-3 fatty acids with the risk of T2D by rs3786897 genotypes

Quartiles of n-3 fatty acids	GG (n = 173)		GA+AA (n = 737)	
	OR (95% CI)	p value ¹	OR (95% CI)	p value ¹
Quartile 1	reference		reference	
Quartile 2	0.33 (0.13–0.84)	0.020	0.46 (0.28–0.76)	0.003
Quartile 3	1.30 (0.48–3.54)	0.614	0.46 (0.28–0.76)	0.002
Quartile 4	0.93 (0.32–2.70)	0.895	0.40 (0.25–0.65)	<0.001
P _{trend}		0.011		0.001

¹ p values were adjusted for age and sex.

When erythrocyte n-3 PUFA were low ($\leq 5.33\%$), SNP rs3786897 A allele carriers, compared with GG homozygotes, had a significantly higher risk of T2D (OR = 2.16, 95% CI 1.32–3.55). However, the genetic effect was not significant when erythrocyte n-3 PUFA were high (table 4). Then, we categorized the n-3 PUFA into 4 groups according to quartiles to examine whether the risk of T2D changed by increasing n-3 PUFA among different rs3786897 genotype carriers (table 5). For GG homozygotes, subjects in the second quartile, compared with the first quartile of n-3 PUFA, showed a significantly decreased risk of T2D (OR = 0.33, 95% CI 0.13–0.84); however, no significant association was observed for the subjects in the third or fourth quartiles, compared with the first quartile of n-3 PUFA. For rs3786897 A allele carriers, a significant dose-response relationship between n-3 PUFA and the risk of T2D was observed ($p_{\text{trend}} = 0.001$), and the most significant association was found in the fourth quartile (OR = 0.40, 95% CI 0.25–0.65).

Discussion

The present study, for the first time, reported that n-3 PUFA may modulate the association of a GWAS-identified genetic variant at the *PEPD* gene with the risk of T2D in a Chinese population. The genetic effect of the *PEPD* rs3786897 A allele on the risk of T2D may be abolished when n-3 PUFA are high. On the other hand, with an increase in n-3 PUFA quartiles, the risk of T2D decreased in the rs3786897 A allele carriers, but the trend was weak in the GG carriers.

With the first publication of GWAS on T2D in 2007, hundreds of genetic variants have been identified to be associated with T2D risk [3]. In Asian populations, accumulating GWAS on T2D have been conducted in the past few years, and dozens of SNPs associated with T2D have been

identified. For example, a recent GWAS in east Asians has identified three novel loci associated with T2D: *MIR129-LEP*, *GPSM1* and *SLC16A13* [14]. Another GWAS in a Chinese population identified SNP rs10229583 near *PAX4* as a new locus of T2D [15]. Two SNPs at *GRK5* and *RASGRP1* were identified as novel T2D-related variants in another GWAS in Chinese populations [16]. A few more GWAS in Asians have discovered additional genetic variants not identified in GWAS in white populations [8, 17–19]. As genetic variations in individuals cannot be changed, identifying beneficial dietary or lifestyle factors which could modify the genetic effects may be critically important for the prevention of T2D [4]. Especially, for individuals already carrying some T2D risk alleles, tailoring ideal dietary or lifestyle strategies based on the genetic background is a key step to prevent the risk. In a meta-analysis of 14 cohort studies, significant interaction of whole-grain intake with the *GCKR* variant (rs780094) for fasting insulin was observed, where among individuals with the insulin-raising allele, a greater whole-grain intake was associated with a smaller reduction in fasting insulin [20]. In another observational study, researchers created a genetic risk score based on 10 GWAS-identified T2D-related SNPs and then found a significant interaction of the genetic risk score with the Western dietary pattern. The Western dietary pattern was not associated with T2D risk among subjects with a lower genetic risk score, while it was positively associated with T2D risk among subjects with a higher score [21].

Although the important role of the gene-diet interaction in the prevention of T2D has been increasingly demonstrated, studies in Asian populations are still limited. In the present study, we found that genetic effects of a GWAS-identified T2D SNP rs3786897 could be modified by n-3 fatty acids in a Chinese population. The beneficial effect of n-3 fatty acids on T2D has been well explored in observational studies, especially in studies in Asian populations [22, 23]. In the Shanghai Women's Health Study, a higher intake of long-chain n-3 fatty acids was associated with a lower risk of T2D in a population of 64,193 Chinese women [24]. Plasma n-3 long-chain fatty acids were associated with improved insulin sensitivity in Chinese T2D patients [25]. Our present study supports that a higher level of n-3 fatty acids was associated with a lower risk of T2D, and a higher level of n-3 fatty acids was also able to modify the genetic risk of T2D.

SNP rs3786897 is located in an intron of the *PEPD* gene and was identified as a new locus for T2D in east Asians in a meta-analysis of GWAS [8]. *PEPD* encodes a member of the peptidase family (D), and the enzyme serves an important role in the recycling of proline; in addition, the enzyme plays an important role in collagen metabolism, while diabetic patients showed enhanced collagen degradation via *PEPD* activity [26], and collagen IV had a profound impact on β -cell function [10]. SNP rs3786897 at *PEPD* has been shown to be highly associated with the mRNA expression of *PEPD* in the adipose tissue of 776 individuals, which has shed light on the potential functionality of this variant [8]. In addition, several SNPs at/near the *PEPD* gene (such as rs10425678) have already been suggested to be associated with T2D in a GWAS in the Japanese population [9], but SNP rs3786897 is not in linkage disequilibrium with these previously identified SNPs.

In the present study, we did not observe a significant main genetic effect of SNP rs3786897 on T2D risk, which may be attributed to the interaction with n-3 fatty acids. The precise mechanism behind the interaction of n-3 fatty acids was unclear. However, n-3 fatty acids and related metabolites are natural ligands of PPAR γ , which may form heterodimers with retinoid X receptors and then regulate the transcription of various genes in multiple pathways [27–29]. Of the 9 T2D SNPs tested, only rs2383208 at *CDKN2B* was associated with the risk of T2D. This SNP has been identified as a T2D variant in several Asian populations [9, 16, 30]. There may be other SNPs without a significant main genetic effect due to the limited sample size and influence of gene-diet interaction, caused by other unknown dietary factors. However, we did not find evidence from the literature for the interaction of *PEPD* variants with other dietary factors for disease susceptibility. Nevertheless, the precise mechanism or precise interaction pattern of these T2D-related genetic variants warrants further investigation.

There are several limitations to the present study. Firstly, the sample size is moderate, which may have resulted in insufficient power to demonstrate small interaction effects of some of the SNPs with n-3 fatty acids. Secondly, replication in other populations within China and in other countries is necessary to verify the observed gene-nutrient interaction effects. However, the interaction of n-3 fatty acids with the *PEPD* variant passed the correction for multiple comparisons and the results appeared to be true. Lastly, erythrocyte fatty acids, but not dietary fatty acids, were used for the present study. However, dietary assessment is subject to recall bias and measurement errors, while erythrocyte fatty acids are objective biomarkers of dietary fatty acid intake. Fatty acid composition in adipose tissue was the best indicator of long-term fat intake [31]. However, adipose tissue was seldom available in large epidemiological studies, and, therefore, fatty acid compositions from plasma/serum and erythrocyte lipids were most commonly used in epidemiological studies [31, 32]. Lipids from other sources, such as platelet or buccal cells, were less often used compared with erythrocyte or plasma/serum due to feasibility and availability. Erythrocyte n-3 fatty acids were suggested to be more suitable biomarkers for long-term fatty acid intake compared with plasma/serum [33, 34].

In conclusion, the present study found that the *PEPD* variant rs3786897 interacted with erythrocyte n-3 fatty acids to modulate the risk of T2D in a Chinese population. High n-3 fatty acids may abolish the genetic risk of the rs3786897 A allele. For those carrying the rs3786897 A allele, it is especially important to increase n-3 fatty acid intake to prevent the risk of T2D.

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Disclosure Statement

The authors have no financial or commercial conflicts of interest to report with respect to this work.

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