Original Article

Genetic variants of ALOXs genes in polyunsaturated fatty acid/arachidonic acid metabolism associated with type-2 diabetes development

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Abstract: Poly-unsaturated fatty acids (PUFAs)/arachidonic acids (AAs) and their derived eicosanoids play potent roles in triggering inflammation during obesity and diabetes development. Recent studies have indicated functional roles of ALOX5, ALOX12, ALOX12B, and ALOX15 in the development of insulin resistance and islet β-cell dysfunction. However, the impact of their genetic variants on type 2 diabetes (T2D) development in Asian patients remains unclear. In this study, 1,682 healthy controls and 788 patients with T2D were enrolled for genotyping those four ALOX genes by the TaqMan method. A total of eight Han Chinese-specific SNPs (two SNPs for each gene) were selected for this study. Of the SNPs tested, genetic variations in ALOX12 (rs312462) and ALOX12B (rs.4792199 and rs.4792216) were found to be significantly associated with T2D development. Multivariable regression analysis further revealed the genetic variation at rs.4792199 as a potential independent marker as age (older than 52) or BMI (higher than 25) for T2D prognosis. When T2D patients were stratified according to age and BMI, SNPs in both ALOX12 (rs312462) and ALOX12B (rs.4792199 and rs.4792216) showed significant impact on T2D development. The present study confirms the involvement of ALOX12 and ALOX12B genetic variants in conferring susceptibility to T2D development, possibly through alterations in PUFA/AA metabolism.

Keywords: Type 2 diabetes (T2D), lipoxygenases (ALOXs), polyunsaturated fatty acid (PUFA), arachidonic acid (AA), eicosanoids

Introduction

Chronic inflammation is a clinical feature associated with type 2 diabetes (T2D) and its related complications, especially in obese patients. Emerging evidence has supported the point of view that dysregulated lipid metabolism in adipose tissue is the key step in altering cellular cross-talk between adipocytes and surrounding/infiltrative immune cells, like macrophages, resulting in pro-inflammatory cytokine secretion [1, 2]. Accumulation of bioactive lipid species, including diacylglycerol, ceramides, and fatty acyl-coenzyme A, can also impair insulin signaling by inactivation of insulin receptors and their substrates, leading to insulin resistance [3, 4]. Since the inter-relationship between accumulation of lipid toxicity and chronic inflammation is bi-directional [5], a greater rate of fatty acid breakdown and uptake in diabetes patients has been suggested as an unfavorable marker for detrimental outcomes. Enzyme regulation involved in lipid metabolic programming, therefore, plays important roles in T2D development.

Metabolism of polyunsaturated fatty acid (PUFA) and primarily arachidonic acid (AA) has grabbed attention in T2D studies in recent years due to the signaling involvement in immune/inflammatory response [6, 7]. AAs are components of phospholipids in cell mem-
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branes, especially abundant in the brain, muscles, and liver, the three major energy-sinks for glucose and fatty acids in human body. Their well-known metabolites are eicosanoids, functioning as a complex family of lipid mediators or intracellular second messengers to regulate a wide variety of physiological and pathological processes [8, 9]. More importantly, these derivatives have been reported to affect glucose-stimulated insulin secretion from pancreatic β-cells [10, 11].

At least three types of enzymes have been found to participate in the metabolism of PUFAs and AAs, lipoxygenases (ALOXs), cyclooxygenases (COXs), and cytochrome p450 epoxygenases (CYP450s) [9, 10]. The initial products during PUFA/AA conversion are hydroperoxyeicosatetraenoic acids (HpETEs) and hydroxyeicosatetraenoic acids (HETEs) through oxygenation reactions predominantly catalyzed by various ALOXs [9]. Currently, the well-studied ALOXs include ALOX5, ALOX12, ALOX12B, and ALOX15. They promote PUFA/AA oxidation at 5-, 12-, 12R-, and 15- positions, respectively. These ALOXs are expressed in many tissues and inflammatory cells, playing roles in inflammation response and associated disorders, including T2D. For example, ALOX5 has been found as a mediator for inflammation-induced vascular complications [12, 13] and critical for the development of T2D-ratinopathy [14]. ALOX12 and ALOX15 are functionally similar in AA metabolism and activate to trigger adipose tissue inflammation [15, 16]. In particular, ALOX12 upregulation triggered by adipokines or hyperglycemia has been identified as a causal factor for islet β-cell dysfunction in T2D [17]. Functioning as stereochemistry to ALOX12, ALOX12B converts AA to 12R-HpETE or 12R-HETE, potent pro-inflammatory mediators in the human body [18].

The above studies provide strong evidence to support the involvement of these common ALOXs in inflammatory response and T2D development. Inhibitors targeting ALOXs have been under development or clinical trials that aim to reduce insulin resistance and obesity-related comorbid conditions [15-17, 19]. However, their genetic impact on Asian patients with T2D remains largely unknown. This study aimed to explore the genetic association of Han Chinese-specific SNPs in those common ALOX genes. The clinical impact of their genetic variants was studied and discussed.

### Materials and methods

#### Study population

The study cohort consisted of 788 patients diagnosed with T2D at the China Medical University Hospital in Taiwan, between 2009-2016. The control group consisted of 1,682 individuals that received regular physiological checks. They were proven to be healthy based on examinations conducted. T2D-related clinical characteristics of each study subject were verified by clinical reports. General characteristics of T2D patients and non-T2D controls are shown in Table 1. This study was approved by the Institutional Review Board of the China Medical University Hospital.

### Table 1. General characteristics of T2D patients and non-T2D controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>T2D N = 788</th>
<th>Non-T2D N = 1682</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>&lt; 52 years old</td>
<td>187 (23.73%)</td>
<td>1037 (61.65%)</td>
<td></td>
</tr>
<tr>
<td>≥ 52 years old</td>
<td>601 (76.27%)</td>
<td>645 (38.35%)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.0029**</td>
</tr>
<tr>
<td>Male</td>
<td>445 (56.47%)</td>
<td>842 (50.06%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>343 (43.53%)</td>
<td>840 (49.94%)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>&lt; 0.0001***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>284 (36.04%)</td>
<td>933 (55.47%)</td>
<td></td>
</tr>
<tr>
<td>≥ 25</td>
<td>504 (63.96%)</td>
<td>749 (44.53%)</td>
<td></td>
</tr>
<tr>
<td>WHR ratio</td>
<td>&lt; 0.0001***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 0.89</td>
<td>206 (26.14)</td>
<td>1053 (62.60)</td>
<td></td>
</tr>
<tr>
<td>≥ 0.89</td>
<td>582 (73.86)</td>
<td>629 (37.40)</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: T2D, type 2 diabetes; BMI, body mass index; WHR ratio, waist-to-hip ratio. *p-values were calculated by Chi-squared test. Statistical significance expressed as bold italic with a p-value < 0.05. Statistical significance (**: P < 0.01; ***: P < 0.001). BMI was calculated as ((height (cm)/[weight (kg])²). WHR was calculated as (waist (cm)/hip (cm)).
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Table 2. Allele distributions of SNPs in PUFA/AA lipoxygenase genes in Taiwanese patients with T2D and controls

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>MAF Patients (n = 788)</th>
<th>MAF Controls (n = 1682)</th>
<th>OR</th>
<th>95% CI</th>
<th>p-valueᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALOX5</td>
<td>rs1864414</td>
<td>37.37%</td>
<td>35.26%</td>
<td>1.10</td>
<td>0.97-1.24</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>rs10751383</td>
<td>29.51%</td>
<td>31.15%</td>
<td>0.92</td>
<td>0.81-1.05</td>
<td>0.24</td>
</tr>
<tr>
<td>ALOX12</td>
<td>rs311743</td>
<td>48.60%</td>
<td>49.91%</td>
<td>0.92</td>
<td>0.81-1.05</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>rs312462</td>
<td>16.50%</td>
<td>15.01%</td>
<td>1.13</td>
<td>0.96-1.33</td>
<td>0.14</td>
</tr>
<tr>
<td>ALOX12B</td>
<td>rs4792199</td>
<td>30.14%</td>
<td>34.99%</td>
<td>0.80</td>
<td>0.70-0.91</td>
<td>0.0008***</td>
</tr>
<tr>
<td></td>
<td>rs4792216</td>
<td>42.77%</td>
<td>38.88%</td>
<td>1.17</td>
<td>1.04-1.33</td>
<td>0.0094**</td>
</tr>
<tr>
<td>ALOX15</td>
<td>rs1038121</td>
<td>7.74%</td>
<td>8.29%</td>
<td>0.93</td>
<td>0.74-1.16</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>rs4790689</td>
<td>20.62%</td>
<td>20.42%</td>
<td>1.01</td>
<td>0.87-1.17</td>
<td>0.86</td>
</tr>
</tbody>
</table>

ᵃAbbreviations: SNP, single nucleotide polymorphism; PUFA, poly-unsaturated fatty acid; AA: arachidonic acid; T2D: type-2 diabetes; MAF, minor allele frequency; OR, odds ratio of minor alleles with reference to major alleles; 95% CI, 95% confidence interval; ALOX5, arachidonate 5-lipoxygenase; ALOX12, arachidonate 12-lipoxygenase; ALOX12B, arachidonate 12-lipoxygenase, 12R type; ALOX15, arachidonate 15-lipoxygenase.

ᵇp-values were calculated by Chi-squared test. Statistical significance expressed as bold italic with a p-value < 0.05. Statistical significance (*: P < 0.05; **: P < 0.01; ***: P < 0.001).

Medical University Hospital (IRB-CMUH), with informed consent provided by each participant.

Genotyping of selected SNPs in ALOX genes

Peripheral blood was collected from individual at clinics and genomic DNA was extracted from theuffy coat, according to standard protocol (Genomic DNA kit; Qiagen, Valencia, CA, USA). DNA fragments containing the SNP sites were amplified by PCR using the TaqMan SNP genotyping assay system (Applied Biosystems Inc. Carlsbad, CA, USA) with probe IDs listed in Table S1. A positive signal was generated when a perfect match formed between the probe and the tested DNA fragment. By reading defined fluorescence signals of PCR products, genetic variations in study cohorts were monitored and recorded.

Statistical analyses and clinical impact

Distributions of allelic and genotypic frequency in patients, as well as in controls, were analyzed and compared by Chi-squared test using SPSS software (version 10.0, SPSS Inc. Chicago, IL, USA). Data are expressed as percentages of the total number of alleles and genotypes. Odds ratios (ORs) were calculated for allelic and genotypic frequencies with 95% confidence intervals (95% CIs), using the wild type allele as the reference. Clinical characteristics associated with a specific SNP were compared between groups using two independent t-tests. A p value less than 0.05 is considered statistically significant. Statistical significance, *: p value < 0.05, **: p value < 0.01, ***: p value < 0.001.

Results

T2D risk association analysis of Han Chinese-specific SNPs in ALOX genes

To study whether genetic variations in genes involved in PUFA/AA metabolism predispose to T2D development, Han Chinese-specific SNPs were selected for genotyping in ALOX5, ALOX12, ALOX12B, and ALOX15 genes (Table S1) [20, 21]. Allelic type analyses indicated the involvement of ALOX12B genetic variants in T2D development. The SNP at rs4792199 showed a protective role (P = 7.6 × 10⁻⁴; OR: 0.80; 95% CI: 0.70-0.91) while the SNP at rs4792216 exhibited a promotive role (P = 9.4 × 10⁻³; OR: 1.17; 95% CI: 1.04-1.33) on T2D development (Table 2). Genotypic analyses confirmed significant association of genotype distributions of rs312462 (in ALOX12) (P = 0.030), rs4792199 (ALOX12B) (P = 0.029), and rs4792216 (ALOX12B) (P = 0.029) with T2D development.

Genetic impact of SNPs in ALOX genes on T2D development

With protective potential (Table 2), the SNP at rs4792216 (ALOX12B) showed a dominant effect on genotype distribution, indicating that people with TT or CT genotypes were at a lower risk for T2D, compared to people with CC genotype (P = 11.3 × 10⁻⁴; OR: 0.80; 95% CI: 0.68-0.95). Such protective effects can be stronger in a recessive model with higher statistical significance (P = 2.2 × 10⁻⁴; OR: 0.64; 95% CI: 0.49-0.86). Although genetic variations at both rs312462 (ALOX12) and rs4792216(ALOX12B) revealed T2D-promotive effects by genotype analyses, their genetic impact on T2D develop-
ment was recessive. A allele of the SNP at rs312462 (ALOX12) \( (P = 7.9 \times 10^{-3}; \text{OR} = 1.97; 95\% \text{CI: } 1.18-3.28) \) and T allele of the SNP at rs4792216 (ALOX12B) \( (P = 0.016; \text{OR} = 1.31; 95\% \text{CI: } 1.05-1.64) \) recessively increased T2D susceptibility (Table 3). Present data revealed the association of genetic variations in ALOX12 and ALOX12B with T2D development. Genotype analyses for SNPs in ALOX5 (rs1864414 and rs10751383) and ALOX15 (rs1038121 and rs4790689) showed no association with T2D development. However, genetic variations at rs1864414 (ALOX5) did show weakly promotive effects on T2D development in a recessive model \( (P = 0.049; \text{OR} = 1.20; 95\% \text{CI: } 1.00-1.45) \) (Table 3).

### Table 3. Genotype distributions of genetic variants in the ALOX gene family between T2D and control groups

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP genotype</th>
<th>T2D N = 788</th>
<th>Non-T2D N = 1682</th>
<th>p-value</th>
<th>Dominant model</th>
<th>Recessive model</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALOX5</td>
<td>rs1864414</td>
<td></td>
<td></td>
<td>0.144</td>
<td>(GG+TG) v.s. TT</td>
<td>GG v.s. (TG+TT)</td>
<td>1.20 (1.00-1.45)</td>
<td>0.049*</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>232 (29.4)</td>
<td>562 (33.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>523 (66.4)</td>
<td>1054 (62.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>33 (4.2)</td>
<td>66 (3.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs10751383</td>
<td></td>
<td></td>
<td>0.449</td>
<td>(AA+CA) v.s. CC</td>
<td>AA v.s. (CA+CC)</td>
<td>0.90 (0.76-1.06)</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>393 (49.9)</td>
<td>793 (47.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>325 (41.2)</td>
<td>730 (43.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>70 (8.9)</td>
<td>159 (9.5)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ALOX12</td>
<td>rs311743</td>
<td></td>
<td></td>
<td>0.217</td>
<td>(GG+TG) v.s. TT</td>
<td>GG v.s. (TG+TT)</td>
<td>1.02 (0.84-1.23)</td>
<td>0.86</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>204 (25.9)</td>
<td>441 (26.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>402 (51)</td>
<td>803 (47.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>182 (23.1)</td>
<td>438 (26)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>rs312462</td>
<td></td>
<td></td>
<td>0.030*</td>
<td>(AA+GA) v.s. GG</td>
<td>AA v.s. (GA+GG)</td>
<td>1.06 (0.88-1.28)</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>557 (70.7)</td>
<td>1209 (71.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>202 (25.6)</td>
<td>441 (26.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>29 (3.7)</td>
<td>32 (1.9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALOX12B</td>
<td>rs4792199</td>
<td></td>
<td></td>
<td>0.029*</td>
<td>(TT+CT) v.s. CC</td>
<td>TT v.s. (CT+CC)</td>
<td>0.80 (0.68-0.95)</td>
<td>0.0113*</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>383 (48.6)</td>
<td>726 (43.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>335 (42.5)</td>
<td>735 (43.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>70 (8.9)</td>
<td>221 (13.1)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs4792216</td>
<td></td>
<td></td>
<td>0.029*</td>
<td>(TT+CT) v.s. CC</td>
<td>TT v.s. (CT+CC)</td>
<td>1.18 (0.99-1.41)</td>
<td>0.0621</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>266 (33.8)</td>
<td>633 (37.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>370 (47)</td>
<td>790 (47)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>152 (19.3)</td>
<td>259 (15.4)</td>
<td></td>
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</tr>
<tr>
<td>ALOX15</td>
<td>rs1038121</td>
<td></td>
<td></td>
<td>0.263</td>
<td>(CC+TC) v.s. TT</td>
<td>CC v.s. (TC+TT)</td>
<td>0.90 (0.76-1.06)</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>667 (84.6)</td>
<td>1413 (84)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>120 (15.2)</td>
<td>259 (15.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>1 (0.1)</td>
<td>10 (0.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>rs4790689</td>
<td></td>
<td></td>
<td>0.859</td>
<td>(AA+CA) v.s. CC</td>
<td>AA v.s. (CA+CC)</td>
<td>1.00 (0.84-1.19)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>495 (62.8)</td>
<td>1056 (62.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>261 (33.1)</td>
<td>565 (33.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>32 (4.1)</td>
<td>61 (3.6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: T2D, type 2 diabetes; OR, odds ratio; CI, confidence interval; ALOX5, arachidonate 5-lipoxygenase; ALOX12, arachidonate 12-lipoxygenase; ALOX12B, arachidonate 12-lipoxygenase, 12R type; ALOX15, arachidonate 15-lipoxygenase.

*p-values were calculated by Chi-squared test. Statistical significance expressed as bold italic with a p-value < 0.05. Statistical significance (*: P < 0.05; **: P < 0.01; ***: P < 0.001).
Table 4. Multivariable regression analysis on the risk factors of T2D

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>β coefficients</th>
<th>SE</th>
<th>T</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.016</td>
<td>0.001</td>
<td>23.29</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>Gender (Male/Female)</td>
<td>0.017</td>
<td>0.017</td>
<td>1.04</td>
<td>0.298</td>
</tr>
<tr>
<td>BMI</td>
<td>0.021</td>
<td>0.002</td>
<td>9.63</td>
<td>&lt; 0.001***</td>
</tr>
<tr>
<td>WHR</td>
<td>0.045</td>
<td>0.031</td>
<td>1.44</td>
<td>0.151</td>
</tr>
<tr>
<td>rs4792199 (CC+TC/TT)</td>
<td>0.061</td>
<td>0.026</td>
<td>2.37</td>
<td>0.018*</td>
</tr>
<tr>
<td>rs47922216 (CC+TC/TT)</td>
<td>-0.036</td>
<td>0.022</td>
<td>-1.61</td>
<td>0.107</td>
</tr>
</tbody>
</table>

*Abbreviations: T2D, type 2 diabetes; SE, standard error; T, the t statistic; BMI, body mass index; WHR ratio, waist-to-hip ratio. *p-values were calculated by Chi-squared test. Statistical significance expressed as bold italic with a p-value < 0.05. Statistical significance (*: P < 0.05; **: P < 0.01; ***: P < 0.001).

Discussion

T2D is a metabolic and chronic disorder frequently complicated with cardiovascular disease, strokes, diabetic nephropathy, neuropathy, and retinopathy [22, 23]. Epidemiological studies have also revealed higher incidence rates of certain types of cancers in Asian T2D patients [24, 25]. Molecular mechanisms involved in such pathogenesis have been found to be related to chronic inflammation [1, 2, 26, 27]. Clinical investigation has confirmed that circulating inflammatory markers, like eicosanoids, are increased during T2D development and their levels appear to predict onset and progression of diabetic complications [8, 28]. Therapeutic agents against inflammatory and immune processes are, therefore, considered as potential useful interventions that may benefit T2D patients [26, 27]. ALOXs are dioxygenases frequently expressed in immune, epithelial, and tumor cells, participating in the biosynthesis of pro- and anti-inflammatory lipid mediators, including eicosanoids [19, 29, 30]. Thus, ALOXs have been implicated in a variety of physiological functions, including inflammation, skin disorders, and tumorigenesis. The present study confirmed that SNPs in ALOX12 (rs312462) and ALOX12B (rs4792199 and rs4792216) genes determine genetic susceptibility to T2D development. In addition to their biochemical influence on inflammatory processes, this study provides evidence revealing the genetic impact of dioxygenases on this devastating disorder.

ALOX12 is expressed in metabolically active tissues, suggested to play a pro-inflammatory role during PUFA/AA metabolism [17, 30]. The major eicosanoid produced by ALOX12 is 12S-HETE, functioning as a chemotaxis of inflammatory cells, like neutrophils monocytes and macrophages, leading to local inflammation [31, 32]. When upregulated in islet cells by high blood glucose or fatty acids, 12S-HETE has been found to generate oxidative stress and ER shock through activation of Nox-1 and ROS, further causing β-cell dysfunction and cell death [17]. Interestingly, functional knockout

Genetic variations in ALOX12 and ALOX12B genes and their correlations with other risk factors

To examine whether T2D-related SNPs could serve as independent markers, multivariate regression analyses were performed to study the impact of genetic variations in ALOX12B (rs4792199 and rs4792216) on T2D susceptibility with other T2D-associated risk factors. As shown in Table 4, significant clinical factors associating with T2D development in the Taiwanese cohort were older age (≥ 52 yr; P < 0.001) and higher BMI (≥ 25; P < 0.001). As with these two factors, a dominant model of the wild type C allele at rs4792199 (ALOX12B) showed genotypic predisposition (P = 0.018) to T2D development after multivariable regression with other related risk factors (Table 4). Furthermore, stratification of T2D patients, according to age and BMI, identified dominant genotypic effects of the SNP at rs312462 (ALOX12) (P = 0.032; OR: 1.55; 95% CI: 1.04-2.01) and wild type C allele at rs4792216 (ALOX12B) (P = 0.045; OR: 0.64; 95% CI: 0.41-0.99) for patients with ages older than 52 and BMIs less than 25 (Table 5). This study also identified dominant genotypic effects of the wild type C alleles at both rs4792199 (ALOX12) (P = 0.036; OR: 1.69; 95% CI: 1.04-2.75) and rs47922216 (ALOX12B) (P = 0.042; OR: 0.66; 95% CI: 0.44-0.98) for patients with ages older than 52 and BMIs higher than 25 (Table 5). Present data confirmed the impact of ALOX12 and ALOX12B genetic risk factors on T2D risk when patients were stratified according to age and BMI.
ALOXs genes in metabolism associate with type-2 diabetes development

Table 5. Effects of ALOX12 and ALOX12B genetic risk factors on T2D risk when stratified according to age and BMIa

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Genotype stratified by age and BMI</th>
<th>OR</th>
<th>95% CI</th>
<th>p-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALOX12</td>
<td>rs312462</td>
<td>AA+AG vs GG, Age &lt; 52, BMI &lt; 25</td>
<td>1.21</td>
<td>0.63-2.32</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA+AG vs GG, Age &lt; 52, BMI ≥ 25</td>
<td>1.12</td>
<td>0.71-1.78</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA+AG vs GG, Age ≥ 52, BMI &lt; 25</td>
<td>1.55</td>
<td>1.04-2.01</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA+AG vs GG, Age ≥ 52, BMI ≥ 25</td>
<td>0.80</td>
<td>0.57-1.13</td>
<td>0.21</td>
</tr>
<tr>
<td>ALOX12B</td>
<td>rs4792199</td>
<td>CC+TC vs TT, Age &lt; 52, BMI &lt; 25</td>
<td>3.49</td>
<td>0.83-14.70</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC+TC vs TT, Age &lt; 52, BMI ≥ 25</td>
<td>1.21</td>
<td>0.66-2.22</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>rs4792216</td>
<td>CC+TC vs TT, Age ≥ 52, BMI &lt; 25</td>
<td>1.65</td>
<td>0.95-2.59</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC+TC vs TT, Age ≥ 52, BMI ≥ 25</td>
<td>1.69</td>
<td>1.04-2.75</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>rs4790689</td>
<td>CC+TC vs TT, Age &lt; 52, BMI &lt; 25</td>
<td>1.12</td>
<td>0.51-2.46</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC+TC vs TT, Age &lt; 52, BMI ≥ 25</td>
<td>1.27</td>
<td>0.73-2.22</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>rs4790689</td>
<td>CC+TC vs TT, Age ≥ 52, BMI &lt; 25</td>
<td>0.64</td>
<td>0.41-0.99</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CC+TC vs TT, Age ≥ 52, BMI ≥ 25</td>
<td>0.66</td>
<td>0.44-0.98</td>
<td>0.042</td>
</tr>
</tbody>
</table>

Abbreviations: T2D, type 2 diabetes; BMI, body mass index; SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; ALOX12, arachidonate 12-lipoxygenase; ALOX12B, arachidonate 12-lipoxygenase, 12R type. *p-values were calculated by Chi-squared test. Statistical significance expressed as bold italic with a p-value < 0.05. Statistical significance (**: P < 0.01; ***: P < 0.001).

The current study showed weak association of genetic variations in ALOX5 (rs1864414) and no association in ALOX15 (rs1038121 and rs4790689) with T2D development. To the best of our knowledge, no T2D-associated SNP has been reported in ALOX15 genes to date. However, some studies have revealed the genetic impact of ALOX5 on myocardial infarction [46] and T2D-associated atherosclerosis [47]. Clinical studies have also indicated that components in ALOX5 pathways are highly expressed in arterial walls in patients with atherosclerosis of the carotid and coronary arteries [48]. Notably, ALOX15 was recently found to be either pro- or anti-inflammatory during the development of atherosclerosis, depending on the presence of lipoxins in the arterial walls [49]. Thus, more study is needed to further address its roles in T2D development.

Results from the present study suggest that PUFA/AA lipoxygenase genes ALOX12 and ALOX12B play crucial roles in T2D development, both at protein and genetic levels. Genetic variations in these two genes may also have pleiotropic effects on multiple components associated T2D, such as age and BMI.

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All peripheral blood samples were obtained after written documentation of informed consent from each patient or legal representative. Copies of consent have been collected for archiving by the Human Genetic Center, China Medical University Hospital, Taichung, Taiwan. All the study subjects involved in this study gave their consent to publish the data obtained.

Disclosure of conflict of interest

None.

Abbreviations

T2D, type 2 diabetes; PUFA, poly-unsaturated fatty acid; AA, arachidonic acid; SNP, single nucleotide polymorphism; ALOX5, arachidonate 5-lipoxygenase; ALOX12, arachidonate 12-lipoxygenase; ALOX12B, arachidonate 12-lipoxygenase, 12R type; ALOX15, arachidonate 15-lipoxygenase.

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References

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Table S1. Information of probe TaqMan IDs used for genotyping of ALOX genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Probe ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALOX5</td>
<td>rs1864414</td>
<td>C___2046455_20</td>
</tr>
<tr>
<td></td>
<td>rs10751383</td>
<td>C___2046351_10</td>
</tr>
<tr>
<td>ALOX12</td>
<td>rs311743</td>
<td>C___749093_10</td>
</tr>
<tr>
<td></td>
<td>rs312462</td>
<td>C___749063_20</td>
</tr>
<tr>
<td>ALOX12B</td>
<td>rs4792199</td>
<td>C___9277039_10</td>
</tr>
<tr>
<td></td>
<td>rs4792216</td>
<td>C___9277006_10</td>
</tr>
<tr>
<td>ALOX15</td>
<td>rs1038121</td>
<td>C___8718659_10</td>
</tr>
<tr>
<td></td>
<td>rs4790689</td>
<td>C___29881702_10</td>
</tr>
</tbody>
</table>

aAbbreviations: SNP, single nucleotide polymorphism; ALOX5, arachidonate 5-lipoxygenase; ALOX12, arachidonic 12-lipoxygenase; ALOX12B, arachidonate 12-lipoxygenase, 12R type; ALOX15, arachidonate 15-lipoxygenase.