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Genetic Analysis of Advanced Glycation End Products in the DHS MIND Study

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Abstract

Advanced glycation end-products (AGEs) are a diverse group of molecules produced by the non-enzymatic addition of glucose to proteins, lipids, and nucleic acids. AGE levels have been associated with hyperglycemia and diabetic complications, especially in animal models, but less clearly in human studies. We measured total serum AGEs using an enzyme linked immunosorbant assay (ELISA) in 506 subjects from 246 families in the Diabetes Heart Study (DHS)/DHS MIND Study (n= 399 type 2 diabetes(T2D)-affected). Single nucleotide polymorphisms (SNPs) in several candidate genes, including known AGE receptors, were tested for their influence on circulating AGE levels. The genetic analysis was expanded to include an exploratory genome-wide association study (GWAS) and exome chip analysis of AGEs ($\approx 440,000$ SNPs). AGEs were found to be highly heritable ($h^2 = 0.628$, $p=8.96 \times 10^{-10}$). While no SNPs from candidate genes were significantly associated after Bonferroni correction, rs1035798 in the gene *AGER* was the most

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significantly associated ($p=0.007$). Additionally, rs7198427, in *MT1A*, showed a nominally significant p -value ($p=0.0099$). No SNPs from the GWAS or exome studies were identified after correction for multiple comparisons; however, rs17054480 in the *PALLD2* gene on chromosome 4 showed the strongest association ($p=7.77\times 10^{-7}$). Five SNPs at two loci (*ISCA2/NPC2* and *FBXO33*) had p -values of less than 2.0×10^{-5} and three additional SNPs (rs716326 in *MACROD2*, and rs6795197 and rs6765857 in *ZBTB38*) showed a nominal association with p -values of less than 1.0×10^{-5} . These findings provide a foundation for further investigation into the genetic component of circulating AGEs.

Keywords

Genome-Wide Association Study; type 2 diabetes; AGE receptor

Introduction

Advanced glycation end products (AGEs) are a diverse, heterogeneous class of molecules, formed through the non-enzymatic glycation and oxidation of proteins, lipids, and nucleic acids. AGEs have been implicated in a wide range of diseases and phenotypes and are a major pathway of interest in complications of type 2 diabetes (T2D) [1]. AGE formation is accelerated in T2D-affected individuals due to increased concentration of circulating glucose [2]. Previous investigations in diabetic cohorts have associated AGEs with cardiovascular outcomes [3], diabetic nephropathy [4], retinopathy [5], and neuropathy [6]. In addition, AGEs have been shown to be associated with a number of clinical measures and behaviors including estimated glomerular filtration rate (eGFR) [8], soluble vascular cell adhesion molecule 1 [van Eupen, et al. 2013], body mass index (BMI), and smoking [7-9].

While there are numerous association studies investigating the relationship between AGE levels and diabetic complications, there are relatively few prior studies investigating the genetics influencing AGE levels. AGEs were previously reported to be heritable [10] and, in addition, single nucleotide polymorphisms (SNPs) in *AGER* (RAGE; Receptor for Advanced Glycation End Products) and *MT1A* (Metallothionein-1A) were observed to be associated with AGE levels [11-13]. However, there are additional AGE receptors that represent potential candidates for genetic analyses, including AGE-R1 (Oligosaccharyl Transferase-48), AGE-R2 (PRKCSH protein kinase C substrate 80K-H), AGE-R3/ Galectin-3, and SR-A (macrophage scavenger receptors type I and type II). We have evaluated heritability of AGEs and then conducted genetic association analyses with AGE levels in the Diabetes Heart Study (DHS)/DHS Mind Study cohort, an ongoing genetic and epidemiological analysis of families enriched for type 2 diabetes with extensive genetic data [14, 15].

Materials and Methods

Study Population

The DHS is a family-based study examining risk for macrovascular and other complications in T2D. Briefly, the DHS includes siblings concordant for T2D but without advanced renal

insufficiency. When possible, unaffected siblings were also recruited. T2D was clinically defined as diabetes developing after the age of 35 years and initially treated with oral agents and/or diet and exercise, in the absence of historical evidence of ketoacidosis. Diagnoses were confirmed by measurement of fasting blood glucose and glycosylated hemoglobin (HbA1c). Extensive measurements of cardiovascular disease (CVD) risk factors were obtained during baseline exams, which occurred from 1998 to 2006. Ascertainment and recruitment have been previously described in detail [14, 15].

The DHS MIND is an ancillary study to the DHS that included a cognitive testing component and brain magnetic resonance imaging (MRI). The purpose was to investigate the relationships between cognitive function, brain imaging, and vascular disease in T2D. Participants from the original DHS investigation were re-examined on average 6.7 ± 1.6 years after their initial visit. Participant examinations were conducted in the General Clinical Research Center of the Wake Forest Baptist Medical Center. Study protocols were approved by the Institutional Review Board at Wake Forest School of Medicine and all study procedures were carried out in accordance with the Declaration of Helsinki. All participants provided written informed consent before participation.

Advanced Glycation End Products

Total serum AGEs were measured using a competitive enzyme linked immunosorbant assay (ELISA) (Lifeome Biolabs; Oceanside, CA). The ELISA was run according to the manufacturer's recommendations using stored serum. The ELISA uses a monoclonal antibody that is specific to AGEs. This ELISA has a minimum detectable dose of AGEs less than 35.2 ng/ml. Intra-assay and inter-assay precision was 2.0% and 22.5%, respectively.

Exome Chip

Additional SNPs, predominately low-frequency and rare coding SNPs, were also captured by the Illumina HumanExome BeadChips v.1.0 (Illumina Inc., San Diego, CA) for which genotype data was available in the DHS [16]. For DHS exome chip data, genotype calling was completed using Genome Studio Software v1.9.4 (Illumina). Samples failing to meet a minimum acceptable call rate of 98% ($n=3$) were excluded from further analyses. An additional 58 samples were included as blind duplicates within the genotyping set to serve as QC samples; the concordance rate for blind duplicates was $99.9 \pm 0.0001\%$ (mean \pm standard deviation (SD)). Exclusion criteria for SNP performance included call rate $< 95\%$ ($n=972$), SNPs with 5 or fewer observances ($n=204,273$) and Hardy-Weinberg Equilibrium (HWE) p -value $< 1 \times 10^{-6}$ ($n=26$); 41,961 SNPs were retained for analysis. Additional QC of exome chip data set was completed to exclude samples with poor quality genotype calls, gender errors, or unclear/unexpected sibling relationships.

Genome-Wide Association Study

Genomic DNA was purified from whole-blood samples obtained from subjects using the PUREGENE DNA isolation kit (Gentra Systems., Minneapolis, MN). DNA was quantitated using standardized fluorometric readings on a Hoefer DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotech, Inc., San Francisco, CA).

A genome-wide association study (GWAS) was completed using the Affymetrix Genome-wide Human SNP Array 5.0 (Affymetrix, CA, USA) as reported [17]. Genotype calling was completed using the BRLLM-P algorithm in Genotyping Console v4.0 (Affymetrix). Samples failing to meet an intensity quality control (QC) threshold and those failing to meet a minimum acceptable call rate of 95% were excluded from further analyses (n=7). An additional 39 samples were included as blind duplicates within the genotyping set to serve as QC samples; the concordance rate for these blind duplicates was $99.0 \pm 0.72\%$ (mean \pm SD). Exploratory analyses of genotype data were performed using PLINK v. 1.07 (<http://pngu.mgh.harvard.edu/purcell/plink/>) and samples with poor quality genotype calls, gender errors, or unclear/unexpected sibling relationships were excluded from further analysis. Exclusion criteria for SNP performance included call rate < 95% (n=11,085), HWE p-value < 1×10^{-6} (n=332), and minor allele frequency (MAF) < 0.01 (n=57,382); 371,951 SNPs were retained for analysis.

Heritability Analysis

Heritability estimates for AGEs were assessed in 506 related individuals from 245 families that had AGE measures. The AGE measurements were natural log transformed to approximate the normality assumptions of the analysis. To determine the contribution of genetic factors to AGE levels, the data in family members were analyzed using Sequential Oligogenic Linkage Analysis Routines (SOLAR) v6.3.4 (Texas Biomedical Research Institute, San Antonio, TX, USA). SOLAR performs a variance components analysis of family data where the total phenotypic variation is partitioned into genetic and nongenetic sources of variation. This approach has been used previously in the DHS [18, 19]. To minimize the bias associated with shared environmental factors, the estimates of heritability (h^2) were based on all available family data and were controlled for covariates related to AGEs including age, gender, BMI, eGFR, T2D affected status, and smoking status (current or former). Five models were developed that incorporated an increasing number of covariates to determine the extent that genetic factors contribute to variation in AGE levels independent of other confounding variables. The first model was unadjusted. The second model was adjusted for age and gender. The third model was adjusted for age, gender, and BMI. The fourth was adjusted for age, gender, BMI, and eGFR. The fifth model was the fully adjusted model and included age, gender, BMI, eGFR, T2D-affected status, and a history of smoking. The significance of the heritability estimates was obtained by likelihood ratio tests.

Genetic Association Analysis

Genetic association was run using SOLAR v6.3.4 (Texas Biomedical Research Institute, San Antonio, TX) which uses variance component methods to account for family structure. AGE measures were natural log transformed prior to analysis to approximate conditional normality. Covariates used in the analysis were age, gender, BMI, T2D affected status, eGFR, and smoking history. All analyses were run using the additive model of inheritance. The Bonferroni corrected p - value for significance for the 121 candidate gene SNPs was set at 0.00041 ($\alpha=0.05/121$). Similarly, the p - value for significance for the GWAS and exome chip associations was set at 1.21×10^{-7} after correcting for multiple comparisons based upon the number of SNPs (413,912) investigated.

Results

Demographics

Demographic characteristics of the 506 individuals included in this study are shown in Table 1. Briefly, the average age was 67 years old and just over half of the participants were female (55.3%). The individuals were on average overweight or obese with an average BMI of 31.5kg/m². Approximately 79% of the individuals were affected by T2D with average diabetes duration of 16.6 years. The DHS cohort is broadly representative of T2D-affected patients in the general population: older, relatively obese, and with significant risk factors and history of CVD and kidney function decline.

Heritability

A heritability analysis was performed on AGEs (Table 2). This analysis was run using a variety of covariates. First, with no covariates included, AGEs were not found to be heritable ($h^2 = 0.045$ $p = 0.291$). However, as applicable covariates were added, the heritability estimate became increasingly more significant. When age and sex were included in the analysis as covariates the h^2 increased to 0.084 ($p = 0.167$); addition of BMI increased the heritability estimate further to 0.114 ($p = 0.093$). After adjusting for commonly used covariates including age, sex, BMI and eGFR in model 4, heritability reached statistical significance ($h^2 = 0.317$, $p = 5.75 \times 10^{-4}$). AGE levels were highly heritable in the fully adjusted model when accounting for age, gender, BMI, eGFR, T2D affected status, and current/former smoking status ($h^2 = 0.628$, $p = 8.96 \times 10^{-10}$) (Table 2).

Candidate Genes

We performed an association analysis using 121 SNPs genotyped on the GWAS and exome chips from known AGE receptor genes including, Advanced Glycosylation End Product-Specific Receptor (*AGER*), Dolichyl-Diphosphooligosaccharide-Protein Glycosyltransferase Subunit (non-catalytic) (*DDOST*), Protein Kinase C substrate 80K-H (*PRKCSH*), Galactoside-Binding, Soluble, 3 (*LGALS3*), and Macrophage Scavenger Receptor 1 (*MSRI*) genes. All polymorphic SNPs in the genes (± 20 kb) from the GWAS and exome chips were included in this analysis. In addition to the receptor genes, Metallothionein 1A (*MT1A*) was included based upon prior literature that observed an association with AGE levels [12]. We did not find significant associations of any of the candidate SNPs with AGEs after adjusting for multiple comparisons (threshold $p < 0.00041$) (Table 3). Thirty-three SNPs had a $p < 0.25$ and are shown in Table 3, including 8 variants with a $p < 0.05$. The top SNP was rs1035798 in the *AGER* gene ($p = 0.007$). The next most significant variant was a SNP in the *MT1A* gene, rs7198427 ($p = 0.0099$). *DDOST* contained one SNP with a $p < 0.05$, rs113092523. Top SNPs in other candidate genes did not reach $p < 0.05$ (*MSRI*, rs10503574, $p = 0.052$; *PRKCSH*, rs17426435, $p = 0.054$; and *LGALS3*, rs11125, $p = 0.22$).

Exome-wide Analysis of Exome Chip Genotyping Data

No SNP reached conservative levels of statistical significance (threshold $p < 1.23 \times 10^{-7}$). The top results are shown in Table 4. The most significant SNPs were exonic, missense variants in the Chitinase, acidic (*CHIA*) gene on chromosome 1 (rs41282492, $p = 4.1 \times 10^{-5}$;

rs41282494, $p=4.1\times 10^{-5}$; rs41282496, $p=4.1\times 10^{-5}$) (Table 4). Three additional SNPs had $p < 1.0\times 10^{-4}$; rs10805470 (intergenic, $p=4.9\times 10^{-5}$), rs139025377 (DEAD (Asp-Glu-Ala-Asp) box polypeptide 19A [*DDX19A*], $p=7.4\times 10^{-5}$), and rs147902167 (myomesin 3 [*MYOM3*], $p=8.1\times 10^{-5}$) (Table 4). **Fig. 1** shows a Manhattan plot of the results from the exome chip analysis of AGEs.

Analysis of Genome-wide Association Study

There were no SNPs from the GWAS that reached statistical significance (threshold $p < 1.23\times 10^{-7}$). The most significant SNP associated with AGE levels was rs17054480 ($p=7.77\times 10^{-7}$), an intronic SNP in the cytoskeletal associated protein (*PALLD*) gene on chromosome 4 (Table 5). There were 3 SNPs with $p < 1\times 10^{-5}$ surrounding the Niemann-Pick disease, type C2 (*NCP2*) and iron-sulfur cluster assembly 2 (*ISCA2*) genes on chromosome 14 (rs11159086, $p=2.70\times 10^{-6}$; rs4899514, $p=4.18\times 10^{-6}$; rs1029701, $p=9.11\times 10^{-6}$) (Table 5). Four additional SNPs showed nominal association with $p < 1\times 10^{-5}$ including rs4454866 in the F-box Protein 33 (*FBXO33*) gene ($p=5.07\times 10^{-6}$), rs716316 in the MACRO domain containing 2 (*MACROD2*) gene ($p=7.53\times 10^{-6}$), rs679597 ($p=8.42\times 10^{-6}$), and rs6765857 ($p=9.32\times 10^{-6}$) in the zinc finger and BTB domain containing 38 (*ZBTB38*) gene (Table 5). There were 52 additional SNPs that were nominally associated with a $p < 1\times 10^{-4}$ (Table S1). **Fig. 2** shows a Manhattan plot of the results from the GWAS analysis of AGEs.

Discussion

In this study we evaluated heritability and genetic association between polymorphisms in specific genes and genome wide data with AGEs in a sample with a high prevalence of diabetes. Only one other study has calculated heritability for AGE levels and that for a specific type of AGE, N ϵ -carboxymethyl lysine (CML) which was highly heritable ($h^2 = 0.74$) [10]. In the study, Leslie et al. observed a higher correlation of CML levels in monozygotic twins compared to dizygotic twins. Importantly, heritability of fasting glucose and HbA1c did not explain the CML heritability. The results of the current study are consistent with this; in the fully adjusted model we showed that circulating AGE levels are highly heritable ($h^2 = 0.628$), suggesting that there is a substantial genetic component to not just CML levels, but also total AGEs quantitated by ELISA.

Most of the prior genetic association studies for AGE levels have focused on a few candidate genes, most notably the receptor for advanced glycation end products (RAGE) gene (*AGER*) on chromosome 6. Several previous studies have shown evidence of an association between polymorphisms in *AGER* and circulating AGE levels [11, 13]. Bansal et al. found that the serine allele of rs2070600 (Gly82Ser) and rs1800625 (C allele) were associated with increased AGEs [11]. Jang et al., on the other hand, found that the individuals with the G/G genotype at rs2070600 had increased levels of AGEs [13]. There are additional AGE receptors including AGE-R1 or Oligosaccharyl Transferase-4 coded by the *DDOST* (Dolichyl- Diphosphooligosaccharide-Protein Glycosyltransferase Subunit (non-catalytic)) gene, AGE-R2 (80 K-H phosphoprotein (Protein kinase C substrate)) which is coded by the Protein Kinase C substrate 80K-H (*PRKCSH*) gene, AGE-R3 (Galectin-3) coded by the

Galactoside-Binding, Soluble, 3 (*LGALS3*) gene, and macrophage scavenger receptors type I and type II (SR-A) coded by the Macrophage Scavenger Receptor 1 (*MSR1*) gene, none of which, to our knowledge, have shown genetic associations with circulating AGE levels. In addition to *AGER*, Metallothionein 1A (*MT1A*) contains a SNP (rs8052394) that was previously found to be associated with AGE levels [12]; individuals with the G allele had increased levels of AGEs compared to individuals with the AA genotype. Significantly, to date, there are no GWAS or exome chip studies investigating circulating AGE levels.

In the present study we investigated potential associations with SNPs in and near multiple AGE receptor protein genes and genes suspected of influencing AGE levels. Several nominal associations were observed. Of particular interest is the association ($p = 0.0073$) of AGE levels with SNP rs1035798 located in *AGER*. Although the function of this SNP is unknown [20], recent studies observed rs1035798 to be associated with small-vessel disease [21] and cardiovascular death [22]. RAGE is a member of the immunoglobulin superfamily and is the best characterized of the known and suspected AGE receptors [23]. AGE interactions with RAGE mediate diverse cellular functions, possibly functioning as a promotion factor for pathologic conditions [24]. Significantly less is known of the other AGE receptors, but it is suggested that they function more so in AGE clearance and detoxification versus transduction of intracellular signaling [25-27].

With an appreciation of the limited power due to a small sample size, we further expanded our investigation to a full GWAS and exome chip study. The top hit SNP was an intronic SNP in the *PALLD* gene (rs17054480, $p = 7.77 \times 10^{-7}$) (Table 4). *PALLD* codes for an actin associated protein, palladin, involved in cytoskeleton morphology [28]. Specifically, palladin is involved in the control of the cell shape, adhesion, and contraction [29] and may play a role in cardiovascular pathology [30]. Other nominal associations highlighted the *NPC2* and *ISCA2* genes, involved in cholesterol transport [31, 32] and the maturation of mitochondrial sulfur proteins [33], respectively.

Further studies need to be performed to investigate AGEs in more depth. This study investigated global AGE levels; however, AGEs are a diverse set of molecules. Directly measuring these individual molecules may lead to an increased understanding of AGE genetics. In conclusion, we found that AGEs are a highly heritable trait, but were unable to identify any specific genetic components of circulating AGE levels. We saw nominal associations between SNPs in candidate genes and AGE levels, but no SNP from either the GWAS or exome chip reached statistical significance. Additional studies with increased sample size and using a more sophisticated measure of AGEs may yield stronger results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DHS	Diabetes heart study
AGE	Advanced Glycation end product
ELISA	Enzyme linked immunosorbant assay
T2D	Type 2 diabetes
SNP	Single nucleotide polymorphism
GWAS	Genome Wide Association Study
eGFR	Estimated glomerular filtration rate
HbA1c	Glycosylated hemoglobin
SOLAR	Sequential Oligogenic Linkage Analysis
BMI	Body mass index
CVD	Cardiovascular disease
RAGE	Receptor of advanced glycation end products

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Highlights

- We performed genetic associations with advanced glycation end products.
- Advanced glycation end products were found to be highly heritable.
- Genetic analysis of candidate genes showed evidence of genetic influences on AGEs.
- GWAS and exome chip analysis showed nominal association with AGEs.

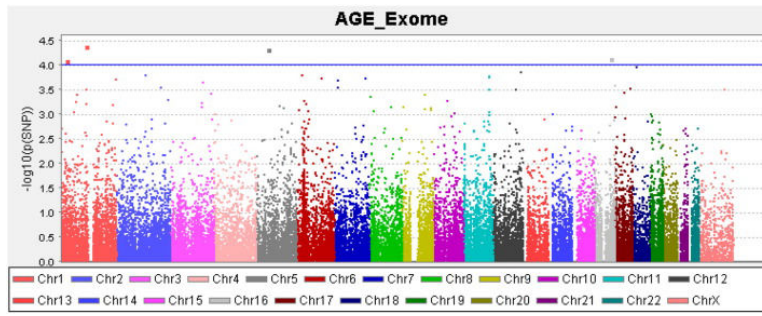


Figure 1.
Manhattan Plot of Exome Chip Association Results for Advanced Glycation End Product Levels

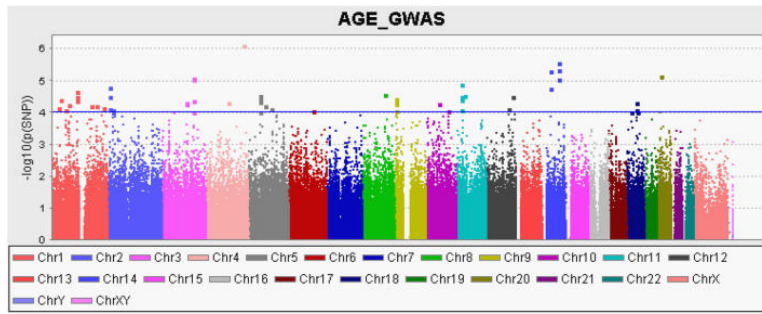


Figure 2.
Manhattan Plot of Genome-Wide Association Results For Advanced Glycation End Product Levels

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Table 1

Demographics characteristics of the Diabetes Heart Study participants

	All	T2D	Non-T2D
N	506	399	107
Age (years) \pm SD	67.7 \pm 9.0	68.0 \pm 8.7	66.5 \pm 9.8
Female (%)	280 (55.3)	210 (52.6)	70 (65.4)
Body Mass Index (m ² /kg) \pm SD	31.5 \pm 6.6	32.3 \pm 6.6	28.5 \pm 5.6
T2D Affected (%)	399 (78.9)	399 (100)	0 (0.0)
T2D Duration (years) \pm SD	16.6 \pm 6.6	16.6 \pm 6.6	N/A
Coronary Artery Calcification \pm SD	1101 \pm 2140	1245 \pm 2215	561 \pm 1737
Carotid Artery Calcification \pm SD	226 \pm 597	252 \pm 626	131 \pm 464
Abdominal Aortic Calcification \pm SD	7922 \pm 11912	8648 \pm 12139	5175 \pm 10636
Carotid Intima-Media Thickness \pm SD	0.67 \pm 0.13	0.67 \pm 0.13	0.63 \pm 0.10
Prior History of CVD (%)	164 (32.4)	149 (37.3)	15 (14.0)
Cholesterol \pm SD	180.1 \pm 47.9	180.1 \pm 47.9	180.1 \pm 47.9
High Density Lipoprotein \pm SD	41.5 \pm 12.2	41.5 \pm 12.2	41.5 \pm 12.2
Low Density Lipoprotein \pm SD	99.5 \pm 34.7	99.5 \pm 34.7	99.5 \pm 34.7
Triglycerides \pm SD	198.8 \pm 142.2	198.8 \pm 142.2	198.8 \pm 142.2
Pulse Pressure (mm Hg) \pm SD	61.1 \pm 16.1	62.7 \pm 16.1	55.1 \pm 14.7
Hypertension (%)	440 (87.0)	363 (91.0)	77 (72.0)
Estimated Glomerular Filtration Rate \pm SD	69.2 \pm 19.6	69.5 \pm 20.5	68.3 \pm 15.8
Albumin/Creatine Ratio \pm SD	77.4 \pm 465.7	94.2 \pm 522.7	14.2 \pm 22.7
Fasting Glucose \pm SD	133.8 \pm 49.5	143.6 \pm 51.3	97.2 \pm 10.3
HbA1c \pm SD	7.11 \pm 1.34	7.45 \pm 1.30	5.85 \pm 0.31
Insulin Use (%)	148 (29.2)	147 (36.8)	1 (0.09)
Diabetes Medication Use (%)	327 (64.6)	323 (81.0)	4 (3.7)
Statin Use (%)	235 (46.4)	200 (50.1)	35 (32.7)
Lipid Medication Use (%)	240 (47.4)	204 (51.1)	36 (33.6)
Blood Pressure Medication Use (%)	363 (71.7)	307 (76.9)	56 (52.3)
History of Smoking (%)	276 (54.5)	220 (55.1)	56 (52.3)
Advanced Glycation End Products \pm SD	40.7 \pm 20.2	41.1 \pm 21.3	49.2 \pm 15.3

SD: Standard Deviation, T2D: Type 2 Diabetes

Table 2

Heritability Analysis of Advanced Glycation End Products

Covariates	h^2 (SE)	p-value
None	0.045 (0.084)	0.291
Age, Sex	0.084 (0.090)	0.167
Age, Sex, BMI	0.114 (0.090)	0.093
Age, Sex, BMI, eGFR	0.317 (0.110)	5.75×10^{-4}
Age, Sex, BMI, eGFR, T2D, Smoking	0.628 (0.112)	8.96×10^{-10}

Bold – indicates statistical significance; BMI: Body Mass Index, eGFR: Estimated Glomerular Filtration Rate, T2D: Type 2 Diabetes

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Table 3

Association Results for Advanced Glycation Endproduct Receptor Candidate Variants with Advanced Glycation Endproduct Levels

SNP	Source	Gene/Nearest Gene	MAF	CHR	Position	p-value	$\beta \pm SE$
rs1035798	GWAS	<i>AGER</i>	0.235	6	32,151,222	0.0073	-0.089±0.033
rs7198427	GWAS	<i>MT1A</i>	0.086	16	56,685,080	0.0099	0.130±0.049
rs6499850	GWAS	<i>MT1A</i>	0.095	16	56,687,638	0.014	-0.120±0.048
rs204995	Exome	<i>AGER</i>	0.291	6	32,154,285	0.022	0.076±0.033
rs7197489	GWAS	<i>MT1A</i>	0.181	16	56,683,993	0.031	-0.077±0.036
rs204993	Exome	<i>AGER</i>	0.001	6	32,155,581	0.045	0.064±0.032
rs113092523	Exome	<i>DDOST</i>	0.001	1	20,971,057	0.045	0.850±0.420
rs3130284	Exome	<i>AGER</i>	0.003	6	32,140,487	0.047	0.067±0.035
rs10503574	GWAS	<i>MSR1</i>	0.036	8	15,984,590	0.052	-0.151±0.077
rs1871574	GWAS	<i>MSR1</i>	0.018	8	15,975,574	0.053	0.209±0.108
rs17426435	Exome	<i>PRKCSH</i>	0.061	19	11,546,269	0.054	0.110±0.056
rs3134946	Exome	<i>AGER</i>	0.209	6	32,145,993	0.054	0.067±0.035
rs3134947	Exome	<i>AGER</i>	0.001	6	32,145,205	0.054	0.067±0.035
rs3096697	Exome	<i>AGER</i>	0.197	6	32,134,510	0.054	0.067±0.035
rs204992	Exome	<i>AGER</i>	0.002	6	32,156,908	0.062	0.063±0.034
rs176095	Exome	<i>AGER</i>	0.001	6	32,158,319	0.070	0.061±0.034
rs414836	GWAS	<i>MSR1</i>	0.065	8	16,045,585	0.087	-0.096±0.056
rs204994	Exome	<i>AGER</i>	0.255	6	32,154,998	0.093	0.057±0.034
rs17677443	GWAS	<i>MSR1</i>	0.060	8	16,029,070	0.094	0.099±0.059
rs11786960	GWAS	<i>MSR1</i>	0.461	8	16,010,949	0.096	-0.046±0.027
rs142241539	Exome	<i>AGER</i>	0.001	6	32,130,655	0.10	-0.690±0.420
rs3130349	Exome	<i>AGER</i>	0.184	6	32,147,696	0.12	0.059±0.038
rs448578	GWAS	<i>MSR1</i>	0.066	8	16,042,900	0.13	0.085±0.056
rs6530943	GWAS	<i>MSR1</i>	0.090	8	15,973,590	0.14	-0.074±0.050
rs10103856	GWAS	<i>MSR1</i>	0.065	8	16,048,551	0.16	-0.079±0.056
rs640742	GWAS	<i>DDOST</i>	0.412	1	20,984,554	0.17	0.041±0.029
rs501257	Exome	<i>PRKCSH</i>	0.140	19	11,536,575	0.17	0.055±0.040
rs149461273	Exome	<i>AGER</i>	0.001	6	32,155,917	0.18	0.560±0.420
rs111334879	Exome	<i>DDOST</i>	0.001	1	20,981,976	0.20	-0.360±0.280
rs204989	GWAS	<i>AGER</i>	0.222	6	32,161,852	0.20	0.045±-0.035
rs204990	GWAS	<i>AGER</i>	0.222	6	32,161,430	0.20	0.045±0.035
rs204991	Exome	<i>AGER</i>	0.225	6	32,161,366	0.20	0.044±0.035
rs11125	Exome	<i>LGALS3</i>	0.098	14	55,611,839	0.22	-0.059±0.048

Results listed only for associations with a p-value < 0.25, MAF: Minor Allele Frequency, CHR: Chromosome, SE: Standard Error.

Table 4

Most Significant Association Results from Exome Chip Analysis for Advanced Glycation Endproduct Levels

SNP	Gene/Nearest Gene	AA Change	MAF	CHR	Position	p-value	$\beta \pm SE$
rs41282492	<i>CHIA</i>	N45D	0.121	1	111854889	4.10×10^{-5}	0.179 \pm 0.043
rs41282494	<i>CHIA</i>	D47N	0.121	1	111854895	4.10×10^{-5}	0.179 \pm 0.043
rs41282496	<i>CHIA</i>	R61M	0.121	1	111854938	4.10×10^{-5}	0.179 \pm 0.043
rs10805470	<i>ESM1</i>	Intergenic	0.384	5	54187813	4.90×10^{-5}	-0.118 \pm 0.029
rs139025377	<i>DDX19A</i>	K96R	0.006	16	70398987	7.40×10^{-5}	-0.784 \pm 0.197
rs147902167	<i>MYOM3</i>	I434V	0.015	1	24417419	8.10×10^{-5}	-0.439 \pm 0.110

AA: Amino Acid, MAF: Minor Allele Frequency, CHR: Chromosome, SE: Standard Error.

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Table 5

Most Significant Association Results of Genome-wide Association Study for Advanced Glycation Endproduct Levels

SNP	Gene/Nearest Gene	AA Change	MAF	CHR	Position	p-value	$\beta \pm SE$
rs17054480	<i>PALLD</i>	Intronic	0.053	4	169608790	7.77×10^{-7}	-0.312 ± 0.062
rs11159086	<i>ISCA2/NPC2</i>	Intergenic	0.141	14	74962275	2.70×10^{-6}	-0.180 ± 0.038
rs4899514	<i>NPC2/ISCA2</i>	Intergenic	0.139	14	74940170	4.81×10^{-6}	0.177 ± 0.038
rs4454866	<i>FBXO33</i>	Intergenic	0.052	14	40676918	5.07×10^{-6}	0.281 ± 0.061
rs716316	<i>MACROD2</i>	Intronic	0.347	20	14908741	7.53×10^{-6}	0.129 ± 0.028
rs6795197	<i>ZBTB38</i>	Intronic	0.412	3	141047072	8.42×10^{-6}	-0.131 ± 0.029
rs1029701	<i>NPC2/ISCA2</i>	Intergenic	0.150	14	74940815	9.11×10^{-6}	-0.161 ± 0.036
rs6765857	<i>ZBTB38</i>	Intronic	0.420	3	141050805	9.32×10^{-6}	0.130 ± 0.029

AA: Amino Acid, MAF: Minor Allele Frequency, CHR: Chromosome, SE: Standard Error.