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Research paper

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 (≈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 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.

1. Introduction

Advanced glycation end products (AGEs) are a diverse, heterogenous 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) (Singh et al., 2014). AGE formation is accelerated in T2D-affected individuals due to increased concentration of circulating glucose (Nowotny et al., 2015). Previous investigations in diabetic cohorts have associated AGEs with cardiovascular outcomes (Hanssen et al., 2015), diabetic nephropathy (Beisswenger et al., 2013), retinopathy (Choudhuri et al., 2013), and neuropathy (Juranek et al., 2013). In addition, AGEs have been shown to be associated with a number of clinical measures and behaviors including estimated glomerular filtration rate (eGFR) (Nin et al., 2011), endothelial dysfunction (Van Eupen et al., 2013), body mass index (BMI), and smoking (Goh & Cooper, 2008; Nin et al., 2011; Tanaka et al., 2013).

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

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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levels. AGEs were previously reported to be heritable (Leslie et al., 2003) 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 (Bansal et al., 2013; Giacconi et al., 2014; Jang et al., 2007). 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 (Bowden et al., 2010, 2008).

2. Materials and methods

2.1. 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 (Bowden et al., 2010; Bowden et al., 2008).

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.

2.2. 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.

2.3. 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 (Cox et al., 2014). 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 ± 0.0001% (mean ± 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.

Table 1
Demographic characteristics of the Diabetes Heart Study participants.

	All	T2D	Non-T2D
N	506	399	107
Age (years) ± SD	67.7 ± 9.0	68.0 ± 8.7	66.5 ± 9.8
Female (%)	280 (55.3)	210 (52.6)	70 (65.4)
Body mass index (m^2/kg) ± SD	31.5 ± 6.6	32.3 ± 6.6	28.5 ± 5.6
T2D affected (%)	399 (78.9)	399 (100)	0 (0.0)
T2D duration (years) ± SD	16.6 ± 6.6	16.6 ± 6.6	N/A
Coronary artery calcification ± SD	1101 ± 2140	1245 ± 2215	561 ± 1737
Carotid artery calcification ± SD	226 ± 597	252 ± 626	131 ± 464
Abdominal aortic calcification ± SD	7922 ± 11,912	8648 ± 12,139	5175 ± 10,636
Carotid intima-media thickness ± SD	0.67 ± 0.13	0.67 ± 0.13	0.63 ± 0.10
Prior history of CVD (%)	164 (32.4)	149 (37.3)	15 (14.0)
Cholesterol ± SD	180.1 ± 47.9	180.1 ± 47.9	180.1 ± 47.9
High density lipoprotein ± SD	41.5 ± 12.2	41.5 ± 12.2	41.5 ± 12.2
Low density lipoprotein ± SD	99.5 ± 34.7	99.5 ± 34.7	99.5 ± 34.7
Triglycerides ± SD	198.8 ± 142.2	198.8 ± 142.2	198.8 ± 142.2
Pulse pressure (mm Hg) ± SD	61.1 ± 16.1	62.7 ± 16.1	55.1 ± 14.7
Hypertension (%)	440 (87.0)	363 (91.0)	77 (72.0)
Estimated glomerular filtration rate ± SD	69.2 ± 19.6	69.5 ± 20.5	68.3 ± 15.8
Albumin/creatine ratio ± SD	77.4 ± 465.7	94.2 ± 522.7	14.2 ± 22.7
Fasting glucose ± SD	133.8 ± 49.5	143.6 ± 51.3	97.2 ± 10.3
HbA1c ± SD	7.11 ± 1.34	7.45 ± 1.30	5.85 ± 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 ± SD	40.7 ± 20.2	41.1 ± 21.3	49.2 ± 15.3

SD: standard deviation, T2D: type 2 diabetes.