Published in final edited form as:

Clin Chim Acta. 2011 April 11; 412(9-10): 785–787. doi:10.1016/j.cca.2010.12.040.

Islet Amyloid Polypeptide Gene Variation (IAPP) and the Risk of Incident Type 2 Diabetes Mellitus: The Women's Genome Health Study

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Abstract

Background—Islet amyloid polypeptide (IAPP) gene variation has recently been implicated in type 2 diabetes mellitus (T2D). However, to date, no prospective epidemiological data are available.

Methods—The association between 10 *IAPP* tag-single nucleotide polymorphisms (tSNPs) and incident T2D was investigated in 22,715 Caucasian participants of the prospective Women's Genome Health Study. All were free of known cardiovascular disease, cancer, and diabetes at baseline. During a 13-year follow-up period, 1,445 participants developed an incident T2D. Multivariable Cox regression analysis was performed to investigate the relationship between genotypes and T2D risk. Haplotype-based analysis was also performed.

Results—No evidence for an association of any of the tSNPs tested or haplotypes thereof with T2D risk.

Conclusions—If corroborated in other large, prospective studies, the present findings further suggest that the *IAPP* gene locus may not be useful predictor for T2D risk assessment.

Keywords

IAPP; Diabetes; Gene; risk factor

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Conflict of interest

None declared

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Introduction

Islet amyloid polypeptide (IAPP) -a 37 amino acid peptide- is a major subunit of amyloid found in insulinomas and pancreatic islet amyloid of patients with type 2 diabetes (T2D) and as such has been associated with the pathogenesis of T2D (1). In addition, there are reports showing that hyperamylinemia and elevated IAPP to insulin ratios are observed in patients with T2D (2). Furthermore, islet amyloid develops as early as in pre-diabetic stages, when individuals may have impaired glucose tolerance (3) or insulin resistance (4). Genetic variation in the *IAPP* gene locus could affect/modify the association of IAPP homeostasis with T2D. Recent studies examining the association of specific *IAPP* genetic variants (MIM: 147940; 12p12.1), including a missense mutation *S20G* (dbSNP rs1800203), and a promoter variant *-132G>A*, with T2D have been conflicting with some showing an association (5,6) while others no evidence for an association (7,8). Most if not all of these previous studies were based on small sample populations and/or case-control design. Furthermore, to date, no prospective epidemiological data are available on examining the importance of the *IAPP* locus as a risk marker for T2D. We thus evaluated the potential association of 10 *IAPP* tagging single nucleotide polymorphisms (tSNPs) with (i) several diabetes-associated factors [baseline glycosylated hemoglobin (HbA1c), C-reactive protein (CRP) levels, and body-mass index (BMI)], and (ii) T2D risk, in a large prospective cohort of 22,715 initially healthy US Caucasian women.

Materials and Methods

Study design

Details of the study design have been previously described (9). In brief, participants in the Women's Genome Health Study (WGHS) –a genetic substudy of the Women's Health Study– included initially healthy North American women aged 45 or older with no previous history of cardiovascular disease, cancer, diabetes or other major chronic illness. A baseline blood sample was collected between 1992 and 1995. All participants gave an informed consent for blood-based analyses related to risks of incident chronic diseases. All study participants were followed up through March 2007 for incident events that were adjudicated by an endpoints committee using standardized criteria and full medical record review. Only confirmed end points were included in this analysis. During a 13-year follow-up period, 1445 newly diagnosed T2D cases out of 22,715 Caucasian participants of the WGHS were identified. Genotyping was performed using a genome-wide Illumina Infinium II assay. The Brigham and Women's Hospital Institutional Review Board for Human Subjects Research approved the study protocol.

Statistical analysis

Genotype frequencies were compared with values predicted by the Hardy-Weinberg equilibrium using the chi-square test with one degree of freedom. Multivariable linear regression analysis, adjusting for age, BMI (not included in the BMI regression model), current smoking status and current (any) hormone use, was performed to assess the relationship of the tSNPs with baseline glycosylated hemoglobin (HbA1c), C-reactive protein (CRP) levels, and BMI. Hazard ratios (HRs) of T2D associated with each of the tSNPs were calculated separately by Cox regression analysis adjusting for age, current smoking status, and further adjusting for BMI, randomized treatment assignment, history of hypertension, and hyperlipidemia, and current (any) hormone use, assuming an additive genetic model. Haplotype estimation and inference were determined by expectation-maximization algorithm. Haplotype blocks were defined using the software Haploview v4.1. In addition, the relationship between haplotypes and T2D was examined by a referent (wild-homozygous) haplotype-based Cox regression analysis, adjusting for the same covariables.

All analyses were carried out using SAS v9.1 package (SAS Institute Inc). A 2-tailed p-value of 0.05 was considered a statistically significant result. Genotyping call rates were >99% per SNP.

Results

The baseline characteristics of the sample population are shown in Table 1. All tSNPs were in Hardy-Weinberg equilibrium (HWE) with p-values >0.070, except rs10743413 (p=0.046). In a multivariable linear regression analysis, no relationship of the tSNPs tested with baseline HbA1c, CRP, or BMI was observed (all uncorrected p-values >0.100; data not shown). Results from the multivariable Cox regression analysis showed no evidence for an association with T2D risk (Table 2). Additional adjustment for baseline HbA1c and CRP levels did not materially change the results (data not shown). Supplementary Data Figure 1 presents the linkage disequilibrium (LD) pattern of the tSNPs tested in the present sample population. The haplotype distribution (defined by Haploview v4.1) is shown in Supplementary Data Table 1. Results from the haplotype-based analysis again showed similar null findings (data not shown).

Discussion

The present large prospective investigation found no evidence for an association of the *IAPP* variants tested and the haplotypes thereof with several T2D-related phenotypes nor with incident T2D.

A recent study by Garin *et al.* observed no relationship between the *IAPP* gene variants rs41275208, rs12306121 or rs5484 with unexplained neonatal (monogenic) diabetes (10). We observed similar null findings for rs12306121 or rs5484 with T2D risk. Although the present study did not investigate rs41275208, this SNP is only 29bp upstream from and in the same haplotype block with rs12306121 (haplotype block 2; Supplementary Data Figure 1). As for the missense mutation *S20G* (rs1800203; not typed in the present study), this SNP also resides within the haplotype block 2. Of note, based on the HapMap information, the linkage disequilibrium block patterns between Europeans, Asians (Chinese/Japanese) and Mexicans were similar.

Strengths of the present study are the overall sample size, the prospective design and the complete long-term follow-up. Nonetheless, some potential limitations of our study require discussion. Our sample population was limited to Caucasian female healthcare professionals from the US. Thus, our results may not be generalizable to other racial/ethnic or socioeconomic groups, geographical regions, or to males. Further information on the *IAPP* variation relating to functional implication was explored via public databases including the NCBI PubMed, the UCSC Genome Bioinformatics-Browser and the Gene Ontology Database. Because of limited information available, no constructive implications can be drawn. Furthermore, the possibility that an uncontrolled (masking) confounders/covariables could account for the present (null) findings with T2D cannot be excluded. In our study, we had the ability to detect, based on the present sample size, assuming 80% power, at an alpha of 0.05, an effect estimate of greater than 1.15 if the minor allele frequency is 0.47, and of greater than 1.25 if the minor allele frequency is 0.07.

The present prospective data from a large cohort of apparently healthy US Caucasian females provide no evidence for an association between the *IAPP* variants tested and T2D risk. If corroborated in other large prospective studies, our data further suggest that the *IAPP* gene locus is not informative for risk assessment of T2D. Furthermore, additional large-

scale studies using comprehensive genome-wide analysis are needed to further elucidate the pathogenesis of T2D.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by grants from the National Institutes of Health HL-096518, HL-043851, HL-080467, and CA-047988; and a Faculty Award supported by the Banco Santander Fund of the *David Rockefeller Center for Latin American Studies* at Harvard University. Collaborative scientific support for genotyping was provided by Amgen, Inc. Special thanks to Alex Parker, PhD, for his expertise and insightful discussions.

References

1. Hoppener JW, Ahren B, Lips CJ. Islet amyloid and type 2 diabetes mellitus. *N Engl J Med* 2000;343:411–9. [PubMed: 10933741]
2. Zheng X, Ren W, Zhang S, et al. Serum levels of proamylin and amylin in normal subjects and patients with impaired glucose regulation and type 2 diabetes mellitus. *Acta Diabetol* 2010;47:265–70. [PubMed: 20509034]
3. Kahn SE, Verchere CB, Andrikopoulos S, et al. Reduced amylin release is a characteristic of impaired glucose tolerance and type 2 diabetes in Japanese Americans. *Diabetes* 1998;47:640–5. [PubMed: 9568698]
4. Gulli G, Rossetti L, DeFronzo RA. Hyperamylinemia is associated with hyperinsulinemia in the glucose-tolerant, insulin-resistant offspring of two Mexican-American non-insulin-dependent diabetic parents. *Metabolism* 1997;46:1157–61. [PubMed: 9322799]
5. Novials A, Rojas I, Casamitjana R, Usac EF, Gomis R. A novel mutation in islet amyloid polypeptide (IAPP) gene promoter is associated with Type II diabetes mellitus. *Diabetologia* 2001;44:1064–5. [PubMed: 11484088]
6. Seino S. S20G mutation of the amylin gene is associated with Type II diabetes in Japanese. Study Group of Comprehensive Analysis of Genetic Factors in Diabetes Mellitus. *Diabetologia* 2001;44:906–9. [PubMed: 11508277]
7. Garcia-Gonzalez CL, Montoya-Fuentes H, Padilla-Rosas M, Sanchez-Corona J. Amylin S20G mutation in Mexican population. *Diabetes Res Clin Pract* 2007;76:146–8. [PubMed: 16950544]
8. Hayakawa T, Nagai Y, Ando H, et al. S20G mutation of the amylin gene in Japanese patients with type 2 diabetes. *Diabetes Res Clin Pract* 2000;49:195–7. [PubMed: 10963832]
9. Ridker PM, Chasman DI, Zee RY, et al. Rationale, design, and methodology of the Women's Genome Health Study: a genome-wide association study of more than 25,000 initially healthy american women. *Clin Chem* 2008;54:249–55. [PubMed: 18070814]
10. Garin I, Martinez R, de las Heras J, Perez-Nanclares G, Castano L, Perez de Nanclares G. Mutations in MAFA and IAPP are not a common cause of monogenic diabetes. *Diabet Med* 2009;26:746–8. [PubMed: 19573128]

Table 1

Baseline characteristics.

Variable	N=22,715
Age, years	52 (48, 58)
Body-mass index, kg/m ²	24.80 (22.40, 28.28)
History of hyperlipidemia $\geq$ 240 mg/dL, %	29.29
History of hypertension $\geq$ 140/90 mmHg, %	23.72
Smoking status	
current	11.62
past	37.42
never	50.96
Current hormone use, %	44.15
Aspirin use, %	49.82
Beta-carotene use, %	49.81
Vitamin E use, %	50.13
HbA1c, %	4.98 (4.083, 5.16)

Data are median and interquartile range for continuous, and percentages for categorical variables.

Table 2

Cox regression analysis of incident T2D.

SNP	Position	MA	MAF	HR	95%CI	P ^{Uncorrected}
rs10743413	21398340	G	0.1383	0.993	0.892–1.105	0.8936
rs10841798	21403463	C	0.0781	1.008	0.880–1.155	0.9089
rs2045938	21411618	A	0.2738	0.964	0.887–1.048	0.3947
rs7137767	21416872	C	0.4481	0.947	0.879–1.020	0.1496
rs12306121	21417738	G	0.1887	0.928	0.842–1.023	0.1333
rs12300126	21418149	G	0.1884	0.929	0.843–1.024	0.1359
rs5484	21422703	A	0.1818	0.926	0.839–1.022	0.1266
rs2417977	21424434	G	0.2599	0.989	0.909–1.076	0.8013
rs12818993	21433213	A	0.0842	1.009	0.884–1.151	0.8962
rs4762822	21446004	G	0.4687	0.999	0.925–1.078	0.9760

Adjusted for age, body-mass index, randomized treatment assignment, history of hypertension, hyperlipidemia, current smoking, and current (any) hormone use.

MA, minor allele; MAF, minor allele frequency; HR, hazard ratio; CI, Confidence interval.