Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

Evaluation of *DLG2* as a positional candidate for disposition index in African-Americans from the IRAS family study

Nicholette D. Palmer [a,b,c], *Josyf C. Mychaleckyj* [f], *Carl. D. Langefeld* [d], *Julie T. Ziegler* [d], *Adrienne. H. Williams* [d], *Michael. Bryer-Ash* [g], *Donald. W. Bowden* [a,b,c,e,*]

[a] *Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC, United States*
[b] *Center for Human Genomics, Wake Forest University School of Medicine, Winston-Salem, NC, United States*
[c] *Diabetes Research Center, Wake Forest University School of Medicine, Winston-Salem, NC, United States*
[d] *Biostatistical Sciences, Wake Forest University School of Medicine, Winston-Salem, NC, United States*
[e] *Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, United States*
[f] *Center for Public Health Genomics, University of Virginia, Charlottesville, VA, United States*
[g] *Section on Endocrinology and Diabetes, University of Oklahoma, Oklahoma City, OK, United States*

ARTICLE INFO

Article history:
Received 9 April 2009
Received in revised form
23 September 2009
Accepted 26 October 2009
Published on line 20 November 2009

Keywords:
African-Americans
Glucose Homeostasis
Disposition Index
SNPs
Linkage
Association

ABSTRACT

Aims: Evaluate discs large homolog 2 (*DLG2*) as a positional candidate gene for disposition index (DI) in the Insulin Resistance Atherosclerosis Family Study (IRAS-FS) African-American sample.

Methods: SNPs ($n = 193$) were selected for genotyping in 580 African-American individuals using a modified tagging algorithm. Follow-up genotyping was carried out within regions associated with DI. A subset of highly associated, uncorrelated SNPs was used as covariates in the linkage analysis to assess their contribution to linkage.

Results: Evidence of association with DI was observed at the *DLG2* locus (admixture-adjusted $P = 0.050–8.7 \times 10^{-5}$) with additional signals observed in follow-up genotyping of 17 SNPs ($P = 0.033–0.0012$). Inclusion of highly associated, uncorrelated SNPs as covariates in the linkage analysis explained linkage at the *DLG2* locus (90.8 cM) and reduced the maximal LOD score (72.0 cM) from 4.37 to 3.71.

Conclusions: Evidence of association and an observed contribution to evidence for linkage to DI was observed for SNPs in *DLG2* genotyped on the African-American individuals from the IRAS-FS. Although not the only gene in the region, these results suggest that variation at the *DLG2* locus contributes to maintenance of glucose homeostasis through regulation of insulin sensitivity and β-cell function as measured by DI.

1. Introduction

Maintenance of glucose homeostasis encompasses both peripheral insulin sensitivity and β-cell function. It has been widely observed that insulin-resistant subjects have markedly increased insulin secretory function compared with insulin-sensitive subjects. This compensatory relationship or negative feedback loop has been quantified experimentally and demonstrated to function as a hyperbolic relationship between insulin sensitivity and β-cell function [1]. The disposition index (DI) quantifies the relationship between insulin sensitivity (S_I) and pancreatic β-cell function (AIR);

* *Corresponding author at*: Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC 27157, United States. Tel.: +1 336 713 7500; fax: +1 336 713 7566.
E-mail address: dbowden@wfubmc.edu (Donald. W. Bowden).
0168-8227/$ – see front matter
doi:10.1016/j.diabres.2009.10.015

DI = S_IxAIR [2]. Type 2 diabetes (T2D) is characterized by the failure of this compensatory relationship and reduced DI is a strong predictor of T2D [3]. Through investigation of the relationship between S_I and AIR quantified by DI, we aim to identify molecular mechanism(s) that detrimentally modulate glucose homeostasis.

In a genome-wide scan based on 284 nondiabetic African-Americans from 21 pedigrees recruited by the Insulin Resistance Atherosclerosis Family Study (IRAS-FS), evidence for linkage to DI on chromosome 11q was observed with a LOD score of 3.21 at 81.0 cM flanked by markers D11S2371 and D11S2002 [4]. Following fine mapping with microsatellite markers in the initial family sample (Set 1) and in an independent set of 214 African-American subjects in 21 pedigrees (Set 2), the resulting linkage signal increased to a LOD score of 4.80 at 80.0 cM near marker D11S937. Suggestive evidence for linkage to acute insulin response (AIR) at two separate locations flanking the DI peak (64.0 cM, LOD 2.77, flanked by markers D11S4076 and D11S981; and 85.0 cM, LOD 2.54, flanked by markers D11S4172 and D11S2002) was also observed, but no evidence of linkage to the insulin sensitivity index (S_I) [5]. The goal of this study was to evaluate the *DLG2* locus as a positional candidate gene for DI and assess its contribution to the observed linkage signal.

2. Subjects

Study design, recruitment and phenotyping for the IRAS-FS have been described in detail [6]. Briefly, the IRAS-FS is a multi-center study designed to identify the genetic determinants of quantitative measures of glucose homeostasis and adiposity in African-Americans and Hispanic Americans. A clinical examination was performed that included an interview, a frequently sampled intravenous glucose tolerance test (FSIGT), anthropometric measurements, and blood collection. The Institutional Review Board of the clinical and analysis sites approved the study protocol and all study participants provided their written informed consent. Measures of glucose homeostasis included those from the FSIGT using the reduced sampling protocol [7–9] calculated by mathematical modeling methods (MINMOD) [10]: insulin sensitivity (S_I), acute insulin response and disposition index. Individuals with a self-reported diagnosis of diabetes or fasting glucose>126 mg/dL, were excluded from this analysis. This analysis includes 580 African-American individuals from 42 pedigrees. Distributions of the primary phenotypes are listed in Table 1.

Table 1 – Demographic summary of IRAS-FS African-American participants.

	African-Americans		
	n	Mean ± SD	Median
Subjects	580		
Demographics			
Age (years)	580	42.9 ± 14.0	41.5
Female gender (%)	344	59.2%	
BMI (kg/m^2)	575	30.0 ± 6.8	29.0
Glucose homeostasis			
S_I ($\times 10^{-5}$ min^{-1}/[pmol/L])	500	1.63 ± 1.17	1.41
AIR (pmol/L)	499	1005.7 ± 826.2	771.5
DI ($S_I \times$ AIR; $\times 10^{-5}$ min^{-1})	499	1425.7 ± 1269.2	1151.5
Fasting glucose (mg/dL)	512	94.7 ± 9.7	93.0

3. Materials and methods

3.1. Genotyping

SNPs were chosen for genotyping within the *DLG2* gene (longest annotated transcript; Chr11:82843701-85015962, NCBI Build 36.1 hg18) using a modified tagging algorithm. SNPs were identified for genotyping based on binning Illumina-designable SNPs according to a threshold linkage disequilibrium score (r^2) [11]. This algorithm specifically tagged SNPs (as opposed to haplotypes) and was agnostic towards haplotype block structure, although larger bins were likely to encompass haplotype block regions. Genotypic data from the Yoruba (YRI) population of the International HapMap project [12] was used as the best ancestral model for the IRAS-FS African-American population. The risk with use of YRI data alone is that African-American allele frequencies will differ from the ancestral population. Caucasians could be used to estimate the Caucasian ancestry limit, but it is not certain that the simple genetic drift model of ancestral allele frequency spectrum is applicable or useful in this case. A total of 193 SNPs were selected for typing at the *DLG2* locus on the Illumina BeadArray system at the Centers for Inherited Disease Research (CIDR).

Seventeen additional SNPs which captured additional variation in *DLG2* were selected for follow-up using the Tagger program [19] of Haploview. This genotyping was performed on the Sequenom MassArray Genotyping System [13]. Blind duplicates were included to evaluate genotyping accuracy.

3.2. Statistical analysis

Each SNP was examined for Mendelian inconsistencies using PedCheck [14]. Genotypes inconsistent with Mendelian inheritance were converted to missing. Maximum likelihood estimates of allele frequencies were computed using the largest set of unrelated African-American individuals (n = 58), genotypes were tested for departures from Hardy–Weinberg proportions and LD structure was evaluated using Haploview 4.0 [15], using the block definition from Gabriel et al. [16]. To evaluate coverage, *DLG2* genotypes (Chr11:82843701-85015962) from the HapMap YRI population of the International HapMap project were evaluated. Specifically, using the Tagger program [19] of Haploview the amount of common variation captured was assessed by forced inclusion of SNPs typed and calculation of r^2 across the interval using a minor allele frequency (MAF) threshold of 0.05 and the aggressive tagging algorithm.

To test for association between individual SNPs and the quantitative phenotype DI, variance component analysis was performed as implemented in SOLAR [17]. When necessary,