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Polimorfismos rs7020673 (G/C) e rs10758593 (A/G) no gene *GLIS3* estão associados com risco para diabetes mellitus tipo 1

Dissertação de Mestrado

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" A Alegria da vida vem de nossos

encontros com novas experiências e, portanto,

não há alegria maior do que ter um horizonte infinitamente mudando,

a cada dia para ter um novo e diferente sol"

Christopher McCandless

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Esta dissertação de mestrado segue o formato proposto pelo Programa de Pós-graduação em Ciências Médicas: Endocrinologia da Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de uma breve introdução sobre o assunto seguida de um artigo original sobre o tema da dissertação.

- Artigo original: "*GLIS3* rs7020673 and rs10758593 polymorphisms interact in the susceptibility for type 1 diabetes mellitus".

SUMÁRIO

LISTA DE ABREVIATURAS

- Introdução:

BACH2	*BTB domain and CNC homolog 2*
DM	Diabete mellitus
DM1	Diabetes mellitus tipo 1
DM2	Diabetes mellitus tipo 2
ERBB3	*Erb-b2 receptor tyrosine kinase*
GLIS3	*Kruppel-like zinc finger protein Gli-similar 3*
GLIS-BS	*GLIS-binding sites*
GWAS	*Genome wide association study*
HLA	*Human leucocyte antigen*
IL27	*Interleukin 27*
IL2RA	*Interleukin 2 receptor subunit alpha*
INS	*Insulin*
Ins2	*Insulin 2* (camundongos)
MafA	*MAF bZIP transcription factor A*
NDH	Diabetes neonatal e hipotireoidismo congênito
NeuroD1	*Neurogenic differentiation 1*
Ngn3	*Neurogenin 3*
ORMDL3	*Phingolipid biosynthesis regulator 3*
Pax6	*Paired box 6*
Pdx	*Pancreatic and duodenal homeobox*
PTPN22	*Protein tyrosine phosphatase, non-receptor type 22*
RC	Razão de chances

RNLS	*Renalase, FAD dependent amine oxidase*
SNPs	*Single nucleotide polymorphisms*
T1DGC	*Type 1 Diabetes Genetics Consortium*

LISTA DE ABREVIATURAS

- Artigo Original:

BMI	Body mass index
BP	Blood pressure
DKD	Diabetic kidney disease
DR	Diabetic retinopathy
FPG	Fasting plasma glucose
GHb	Glycated hemoglobin
GLIS3	*Kruppel-like zinc finger protein Gli-similar 3*
GWAS	Genome wide association study
HAS	Systemic arterial hypertension
HLA	*Human leucocyte antigen*
HWE	Hardy-Weinberg equilibrium
IA	Islet autoimmunity
INS	*Insulin*
Ins2	*Insulin* gene (mouse)
LD	Linkage disequilibrium
NOD	Non-obese diabetic
OR	Odds ratio
SNPs	Single-nucleotide polymorphisms
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus

RESUMO

O diabetes mellitus tipo 1 (DM1) é uma doença multifatorial resultante da destruição autoimune das células-beta pancreáticas por linfócitos T e macrófagos. A autoimunidade contra as células-beta é causada pela complexa interação entre fatores de risco ambientais e genéticos. Entre os fatores genéticos, o *locus HLA* apresenta o maior impacto na suscetibilidade para o DM1. Polimorfismos de troca única (SNPs) em outros 50 genes apresentam um efeito menor no risco para o DM1; entretanto, a combinação de genótipos do *locus HLA* de classe II (DR/DQ) com SNPs nestes outros genes parece melhorar a predição da doença. Dessa forma, a identificação de novos SNPs associados ao DM1 poderá melhorar ainda mais a predição do DM1.

O fator de transcrição *GLI-similar 3* (GLIS3) pertence à subfamília das proteínas de dedo de zinco do tipo Kruppel (*Kruppel-like zinc finger proteins*) e é altamente expresso nas células-beta. Diversos estudos demonstram que GLIS3 tem um papel importante no desenvolvimento das células-beta e também na regulação da expressão do gene da insulina. Recentemente, estudos de varredura do genoma identificaram que o *locus* do gene *GLIS3* está associado com DM1 e marcadores de função das células-beta. No entanto, poucos estudos avaliaram a associação de SNPs neste gene e o DM1 em diferentes populações. Considerando que estudos adicionais são necessários para replicar a associação de variantes no gene *GLIS3* e o DM1, o objetivo do presente estudo foi investigar a associação entre os SNPs rs10758593 (A/G) e rs7020673 (G/C) no gene *GLIS3* e o DM1 em uma população brasileira, ajustando para haplótipos *HLA DR/DQ* de alto risco para esta doença.

As frequências dos polimorfismos rs7020673 e rs10758593 no gene *GLIS3* foram analisadas em 503 pacientes com DM1 (casos) e 442 indivíduos não diabéticos

(controles). Os haplótipos construídos a partir da combinação dos 2 SNPs de interesse no gene *GLIS3* foram inferidos utilizando o programa Phase 2.1, o qual implementa uma estatística Bayesiana. Os haplótipos *HLA DR/DQ* de alto risco para o DM1 foram estimados a partir da combinação de 3 SNPs neste *locus* (rs3104413, rs2854275 e rs9273363), conforme validado em um estudo recente. SNPs no gene *GLIS3* e do *locus HLA DR/DQ* foram genotipados usando-se a técnica de PCR em tempo real.

As frequências genotípicas dos SNPs rs7020673 (G/C) e rs10758593 (A/G) estão em equilíbrio de Hardy-Weinberg nos controles e não diferiram significativamente entre os grupos de estudo. A frequência do alelo C do SNP rs7020673 foi 47,3% em pacientes com DM1 e 45,1% nos indivíduos não diabéticos (p= 0,365), enquanto o alelo A do SNP rs10758593 foi observado em 43,3% dos casos e 41,1% dos controles (p= 0,341). Foram observados 4 haplótipos formados pelos 2 SNPs avaliados nas amostras estudadas. Interessantemente, a presença de ≥3 alelos raros dos SNPs rs7020673 e rs10758593 nos haplótipos foi maior em casos do que controles (6,2% *vs*. 1,6%; p= 0,0001). Essa associação com risco para DM1 manteve-se após ajuste para os haplótipos *HLA-DR/DQ* de alto risco, idade e etnia (RC= 3,684, IC 95% 1,220 – 11,124). Além disso, níveis de hemoglobina glicada foram maiores em pacientes com DM1 com o genótipo A/A do SNP rs10758593 comparado a pacientes portadores do alelo G (p= 0,038).

Em conclusão, os SNPs rs7020673 e rs10758593 no gene *GLIS3* não parecem estar isoladamente associados ao DM1; porém, haplótipos contendo ≥3 alelos raros desses polimorfismos foram associados com risco para DM1, sugerindo que esses polimorfismos interagem na suscetibilidade para a doença. Além disso, o SNP rs10758593 parece estar associado a um pior controle glicêmico em pacientes com DM1 da nossa população.

ABSTRACT

Type 1 diabetes mellitus (T1DM) is a multifactorial disease resulting from an autoimmune destruction of pancreatic beta-cells by T lymphocytes and macrophages. Autoimmunity against beta-cells is caused by a complex interaction between environmental and genetic risk factors. Among genetic factors, *HLA* locus has shown to improve the greatest impact on susceptibility for T1DM. Single-nucleotide polymorphisms (SNPs) in other 50 genes have minor effects on the risk for T1DM; however, the combination of *HLA* class II (DR/DQ) genotypes and SNPs in these other genes has been show to improve disease prediction. Therefore, the discovery of new SNPs associated with T1DM might improve T1DM prediction.

The transcription factor Gli-similar 3 (GLIS3) is a member of the Krüppel-like zinc finger family, and is highly expressed in beta-cells. Several studies have demonstrated that GLIS3 has a key role in the development of beta-cells and also in the regulation of *insulin* gene expression. Recently, genome wide association studies identified *GLIS3* as being associated with T1DM and markers of beta-cell function. Nevertheless, few studies evaluated the association of SNPs in this gene and T1DM in different populations. Taking into account that additional studies are needed to replicate the association of *GLIS3* variants and T1DM, the aim of this study was to investigate the association of *GLIS3* rs10758593 (A/G) and rs7020673 (G/C) SNPs in a Brazilian population, adjusting for T1DM high-risk *HLA DR/DQ* haplotypes.

Frequencies of *GLIS3* rs7020673 and rs10758593 SNPs were analyzed in 503 T1DM patients (cases) and 442 non-diabetic subjects (controls). Haplotypes constructed from the combination of these SNPs were inferred using Phase 2.1 program, which

implements a Bayesian statistical method. T1DM high-risk *HLA DR/DQ* haplotypes were estimated from a combination of 3 SNPs in this locus (rs3104413, rs2854275 and rs9273363), as validated in a recent study. SNPs in *GLIS3* gene and *HLA DR/DQ* locus were genotyped using Real-Time PCR.

Genotype frequencies of rs7020673 (G/C) and rs10758593 (A/G) SNPs are in Hardy-Weinberg equilibrium in the control sample, and they did not differ significantly between analyzed groups. *GLIS3* rs7020673C allele frequency was 47.3% in T1DM patients and 45.1% in non-diabetic subjects (P= 0.365), while the rs10758593A allele was observed in 43.3% of cases and 41.1% of controls (P= 0.341).

Four haplotypes constituted by combination of the 2 analyzed SNPs were inferred in both samples. Interestingly, frequency of ≥3 minor alleles of rs7020673 and rs10758593 SNPs in haplotypes was higher in cases than controls (6.2% *vs*. 1.6%; P= 0.001). This association with T1DM risk remained after adjustment for high-risk *HLA DR/DQ* haplotypes, age, and ethnicity (OR= 3.684 95% CI 1.220 – 11.124). In addition, glycated hemoglobin levels were higher in T1DM patients with the rs10758593 A/A genotype compared with patients carrying the G allele (P= 0.038).

In conclusion, the rs7020673 and rs10758593 SNPs in *GLIS3* gene do not appear to be individually associated with T1DM; however, presence of ≥3 minor alleles of both SNPs was associated with T1DM risk, suggesting that these SNPs interact in the susceptibility to the disease. Moreover, the *GLIS3* rs10758593 SNP seems to be associated with a worse glycemic control in T1DM patients from our population.

INTRODUÇÃO

- O diabetes mellitus tipo 1 (DM1):

De acordo com a Federação Internacional de Diabetes (IDF) (1), 415 milhões de indivíduos em todo o mundo apresentaram algum tipo de diabetes mellitus (DM) no ano de 2015. Estatísticas mostram que o número de indivíduos afetados continua a aumentar e, se não forem tomadas providências para modificar a trajetória da epidemia, a prevalência poderá chegar a 642 milhões de indivíduos com DM em 2040 (1). O Brasil encontra-se em 4° lugar em um ranking de 10 países com maior prevalência de DM, apresentando atualmente 14 milhões de casos dessa doença. Destes casos, cerca de 5-15% apresentam DM tipo 1 (DM1) (1). Estudos epidemiológicos demonstram que a taxa de incidência do DM1 está crescendo nas últimas décadas em todo o mundo, com estimativa de 86.000 novos casos por ano em crianças menores de 15 anos (1). Isto é preocupante, pois o DM1 é a doença crônica grave mais comum na infância (2, 3).

O DM1 é uma doença crônica de herança multifatorial, sendo resultante da destruição autoimune das células-beta pancreáticas mediada pelos linfócitos T e macrófagos (3-5). A destruição lenta e progressiva das células-beta leva a uma deficiência total na secreção de insulina, deixando os pacientes dependentes de insulina exógena para sobrevivência (3, 5).

O desencadeamento da autoimunidade contra as células-beta é causado pela complexa interação entre fatores de risco ambientais e genéticos (2, 4, 6). Até o momento, diversos estudos de varredura do genoma (*Genome wide association study* – GWAS) identificaram que polimorfismos de troca única (*single-nucleotide polymorphisms* – SNPs) em cerca de 50 genes conferem suscetibilidade ao DM1 (6-9).

A região do *locus HLA* (*Human leucocyte antigen*) apresenta o maior impacto na suscetibilidade para o DM1, com uma Razão de Chances (RC) >7, e explica cerca de 50% da variabilidade genética dessa doença (7, 10). Outros *loci* apresentam um efeito bem menor no risco para o DM1; no entanto, a combinação de genótipos do *locus HLA* de classe II (DR/DQ) com SNPs nestes outros genes parece melhorar a predição da doença (6, 11).

Neste contexto, Pociot *et al.* (6) calcularam a contribuição de *loci* não-HLA para a melhora da predição do DM1 usando dados do "*Type 1 Diabetes Genetics Consortium*" (T1DGC) (12, 13). Estes autores demonstraram que a predição do DM1 foi aumentada quando SNPs não-HLA foram incluídos no modelo de predição, isto é, a triagem de 20% da população para SNPs do *loci HLA* identifica 75% dos indivíduos que desenvolverão DM1, enquanto a triagem para SNPs não-HLA + HLA identifica 83% (6). Além disso, Wrinkler *et al.* (11) aplicaram regressão logística multivariada e estatística Bayesiana aos dados do T1DGC sobre genotipagem de 40 SNPs em 4574 casos com DM1 e 1207 controles não diabéticos, mostrando que a aplicação de um modelo de predição com 10 SNPs (HLA *DR/DQ* mais 9 SNPs nos genes *GLIS3*, *PTPN22*, *INS*, *IL2RA*, *ERBB3*, *ORMDL3*, *BACH2*, *IL27* e *RNLS*) melhorou significativamente a predição da doença em crianças de uma coorte prospectiva em comparação com o modelo de predição contendo apenas SNPs do *loci HLA*. Dessa forma, a identificação de novos SNPs associados ao DM1 poderá melhorar ainda mais a predição do DM1 em crianças que apresentam irmãos já afetados por essa doença (10).

- O gene *GLI-Similar 3* (*GLIS3*) e o DM1:

Proteínas *GLI-similar* (GLIS) são fatores de transcrição pertencentes à subfamília das proteínas de dedo de zinco do tipo Kruppel (*Kruppel-like zinc finger proteins)* (14, 15). A subfamília GLIS é composta por 3 membros chamados de GLIS1 a GLIS3. Essas proteínas contêm domínios repressores e ativadores, sugerindo que elas podem funcionar como reguladores positivos ou negativos da transcrição gênica. De fato, GLIS1-3 regulam a expressão gênica através da ligação a sequências de DNA específicas chamadas de sítios de ligação ao GLIS (*GLIS-binding sites* – GLIS-BS), presentes nas regiões promotoras dos genes alvo (16). Durante o desenvolvimento embrionário, *GLIS1-3* são expressos em momentos específicos, possuindo um papel fundamental na organogênese normal (17).

O gene *GLIS3* é expresso em diferentes tecidos, como rins, pituitária, útero, tireóide, mas apresenta expressão aumentada nas células-beta pancreáticas (15, 18, 19). Estudos indicam que GLIS3 tem um papel importante na diferenciação das células do pâncreas, principalmente no desenvolvimento das células-beta (16, 20, 21). Além disso, este fator de transcrição também parece possuir um papel importante na manutenção da massa das células-beta e na regulação do gene da insulina (*INS*) (16, 17, 19, 21, 22).

De acordo com um importante papel do GLIS3 no desenvolvimento e nas funções das células-beta, mutações raras de perda de função nesse gene foram descritas como causadores da síndrome de diabetes neonatal associada com hipotireoidismo congênito, fibrose hepática e rins policísticos (18, 23). Camundongos *knockout* para *Glis3* (*Glis3*$^{-/-}$) desenvolvem um diabetes neonatal letal devido a defeitos na geração de células-beta e na expressão do gene *Ins2* (gene da insulina de camundongos) (19, 21, 24). Isto porque a deficiência de *Glis3* nestes animais causa a diminuição da expressão de alguns fatores de transcrição essenciais para o desenvolvimento da porção endócrina do pâncreas, tais como *Ngn3*, *MafA*, *NeuroD1* e *Pdx1* (16). Já, camundongos adultos

heterozigotos para o *knockout* de *Glis3*$^{-/-}$ mostram uma expressão diminuída de *Ins2*, bem como suscetibilidade aumentada para diabetes induzido por uma dieta rica em gorduras (17). Kang *et al.* (16) analisaram o efeito do *knockdown* (bloqueio *in vitro* do gene usando RNA de interferência) e super-expressão de *Glis3* em uma linhagem de insulinoma de ratos (INS-1 832/13), demostrando que *Glis3* se liga a um GLIS-BS na porção *cis* do gene *Ins2*, modulando diretamente a sua atividade transcricional. Dessa forma, todos esses estudos corroboram o papel do GLIS3 no desenvolvimento e função das células-beta pancreáticas.

- Associação de polimorfismos no gene *GLIS3* com DM1:

Recentemente, estudos de GWAS identificaram a associação entre SNPs no gene *GLIS3* com DM1 (12, 25) e DM tipo 2 (DM2) (26-28), tornando-o um dos poucos genes associados com os dois tipo de DM e o diabetes neonatal (22). SNPs nesse gene também parecem estar associados à glicemia de jejum e função das células-beta (secreção de insulina estimulada por glicose medida pelo HOMA-B) em crianças e adultos (27, 29-32). No entanto, poucos estudos avaliaram a associação de SNPs no gene *GLIS3* e DM1 em diferentes populações. Awata *et al.* (33) relataram que o alelo G do SNP rs140101069 estava associado com proteção para DM1 em uma população japonesa. Já, Kiani *et al.* (34) demonstraram a associação entre o alelo C do SNP rs7020673 com DM1 em uma população Paquistanesa.

Steck *et al.* (35) avaliaram a associação entre SNPs em 20 genes (não-HLA) e o risco para desenvolvimento de autoimunidade contra as ilhotas pancreáticas e progressão para o DM1 no estudo de coorte DAISY (*Diabetes Autoimmunity Study in the Young*). Interessantemente, estes autores observaram que o alelo C do polimorfismo

rs70206373 no gene *GLIS3* foi capaz de predizer a ocorrência de autoimunidade contra as ilhotas, mas não a progressão desta autoimunidade ao DM1 clínico, controlando para história familiar de DM1 e genótipos *HLA DR/DQ* de alto risco para DM1. Sendo assim, são necessários estudos adicionais para confirmar a associação entre SNPs no gene *GLIS3* e o DM1 em diferentes populações.

JUSTIFICATIVA E OBJETIVOS

O DM1 é uma doença crônica que caracteriza um grave problema de saúde pública, uma vez que possui acentuada morbidade e mortalidade e também devido a repercussões econômicas e sociais decorrentes do impacto de suas complicações crônicas, as quais comprometem a qualidade de vida e a produtividade dos indivíduos afetados, além dos elevados custos requeridos para seu tratamento. Sendo assim, a elucidação das bases genéticas e moleculares do DM1 poderá levar à identificação de pacientes que apresentam maior predisposição para o seu desenvolvimento ou um pior prognóstico.

Alguns estudos têm demonstrado que o gene *GLIS3* possui um papel importante na patogênese do DM1 e DM2, estando envolvido no desenvolvimento das células-beta e na regulação da expressão do gene da insulina. Entretanto, até o momento, poucos estudos avaliaram a associação entre SNPs nesse gene e o DM1, sendo que nenhum destes estudos foi realizado no Brasil.

Sendo assim, os objetivos do estudo foram:

Objetivos

Objetivo geral:

- Avaliar a associação entre os SNPs rs7020673 (G/C) e rs10758593 (A/G) no gene *GLIS3* e o DM1.

Objetivos específicos:

- Comparar a frequência dos SNPs em estudo entre pacientes diabéticos tipo 1 e indivíduos não-diabéticos.

- Avaliar se os SNPS de interesse estão associados a alguma característica clínica ou laboratorial do DM1, como idade de diagnóstico e níveis glicêmicos.

- Avaliar se a associação dos SNPs rs7020673 e rs10758593 no gene *GLIS3* com DM1 é diferente de acordo com a presença dos haplótipos *HLA-DR/DQ* de alto risco para essa doença.

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ARTIGO ORIGINAL

GLIS3 rs7020673 and rs10758593 polymorphisms interact in the susceptibility for type 1 diabetes mellitus

GLIS3 rs7020673 and rs10758593 polymorphisms interact in the susceptibility for type 1 diabetes mellitus

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Short title: _GLIS3_ polymorphisms and T1DM.

Abstract

Background and Aims: The transcription factor Gli-similar 3 (GLIS3) plays a key role in the development and maintenance of pancreatic beta-cells as well as in the regulation of *Insulin* gene expression in adults. Accordingly, genome-wide association studies identified *GLIS3* as a susceptibility locus for type 1 diabetes mellitus (T1DM) and glucose metabolism traits. Therefore, the aim of this study was to investigate the association of the rs10758593 and rs7020673 single-nucleotide polymorphisms (SNPs) in the *GLIS3* gene with T1DM in a Brazilian population.

Material and Methods: Frequencies of the rs7020673 (G/C) and rs10758593 (A/G) SNPs were analyzed in 503 T1DM patients (cases) and in 442 non-diabetic subjects (controls). Haplotypes constructed from the combination of these SNPs were inferred using a Bayesian statistical method.

Results: Genotype and allele frequencies of rs7020673 and rs10758593 SNPs did not differ significantly between case and control groups. However, frequency of ≥3 minor alleles of the analyzed SNPs in haplotypes was higher in T1DM patients compared to non-diabetic subjects (6.2% *vs*. 1.6%; P= 0.001). Presence of ≥3 minor alleles remained independently associated with risk for T1DM after adjustment for T1DM high-risk *HLA DR/DQ* haplotypes, age and ethnicity (OR= 3.684 95% CI 1.220 – 11.124). Moreover, levels of glycated hemoglobin were higher in T1DM patients with rs10758593 A/A genotype than patients carrying the G allele of this SNP (P= 0.038).

Conclusion: Our results indicate that individually the rs7020673 and rs10758593 SNPs are not significantly associated with T1DM but seem to interact in the predisposition for this disease.

Introduction

Type 1 *diabetes mellitus* (T1DM) accounts for 5-15% of all cases of diabetes, and results from a severe autoimmune destruction of pancreatic beta-cells by T lymphocytes and macrophages, leaving patients insulin-dependent for life (1-3). The triggering of autoimmunity against beta-cells is caused by a complex interaction between multiple environmental and genetic risk factors (1). To date, more than 50 genes have been identified to influence the risk of T1DM, with Human Leukocyte Antigen (*HLA*) class II genes showing the greatest impact on susceptibility with an odds ratio (OR) >7 (4-7). Other loci have minor effects on the risk for T1DM; nevertheless, the combination of *HLA* genotypes and non-HLA single-nucleotide SNPs (SNPs) has been shown to aid disease prediction (8, 9). Thus, the discovery of new non-HLA SNPs associated with T1DM might improve the prediction of the disease (10). Moreover, studies have indicated that the effect of non-HLA SNPs on susceptibility to T1DM may be variable according to different *HLA-DR/DQ* haplotypes (4, 11).

Gli-similar 3 (GLIS3) is a member of the Krüppel-like zinc finger family of transcription factors, and is highly expressed in pancreatic beta-cells (12-14). Growing evidence indicates that GLIS3 plays a critical role in pancreatic cell lineage specification, particularly in the development of mature beta-cells (15-17). GLIS3 is also important to beta-cell mass maintenance and regulation of insulin (*INS*) expression in adults (14-16, 18, 19). Accordingly, functional mutations in *GLIS3* cause a rare syndrome of neonatal diabetes and congenital hypothyroidism (NDH) in humans (13), and *Glis3*$^{-/-}$ knockout mice develop lethal neonatal diabetes due to impairment in the generation of beta-cells and *Ins2* expression (15, 16, 20).

Genome-wide association studies (GWAS) identified *GLIS3* as a susceptibility locus for both T1DM (21, 22) and type 2 diabetes mellitus (T2DM) (23-26). *GLIS3* SNPs were also associated with fasting glucose and glucose-stimulated beta-cell function in children and adults (23, 27-30), further corroborating the key role of this transcription factor in human beta-cell development and function. However, only few studies have evaluated the association between *GLIS3* SNPs and T1DM in different populations. Awata *et al*. (31) reported that the low-frequency G allele of the *GLIS3* rs140101069 SNP was strongly associated with protection for T1DM in Japanese. Kiani *et al*. (32) showed a significant association between *GLIS3* rs7020673C allele and risk for T1DM in a Pakistani population. Interestingly, Steck *et al.* (10) showed that *GLIS3* rs7020673C allele was associated with islet autoimmunity, adjusting for family history and T1DM high-risk *HLA-DR3/4* haplotype, in the The Diabetes Autoimmunity Study in the Young (DAISY) prospective cohort. However, these authors were not able to find an association between *GLIS3* rs7020673 SNP and progression from islet autoimmunity to T1DM.

Therefore, taking into account that additional studies are required to replicate the association between *GLIS3* SNPs and T1DM in different populations, we investigated whether the rs10758593 and rs7020673 SNPs in the *GLIS3* gene are associated with T1DM in subjects from a Brazilian population, adjusting for T1DM high-risk *HLA DR/DQ* haplotypes.

Materials and methods

Study participants, phenotype measurements, and laboratory analyses

This case-control study was designed in accordance with STROBE and STREGA guidelines for reporting of genetic association studies (33, 34). The case sample consisted of 503 unrelated T1DM patients recruited from the outpatient clinic at Hospital de Clínicas de Porto Alegre (Rio Grande do Sul, Brazil) between January 2005 and December 2013. According to American Diabetes Association guidelines, patients were considered to have T1DM if they had been diagnosed with hyperglycemia before the age of 30 years, required insulin for glycemic control within 1 year of diagnosis, and this treatment could not be interrupted thereafter (3). The non-diabetic group (controls) comprised 442 healthy blood donors recruited from the same hospital, and who did not have diabetes or family history of this disease. Only subjects with glycated hemoglobin (GHb) <5.7% were included in the control group (3). The ethnic group was defined based on self-classification, and frequencies of non-white subjects in each sample were as follows: 7.9% of non-white patients in the T1DM group and 14.3% of non-white subjects in the non-diabetic group (P= 0.002).

A standard questionnaire was used to collect information on age, age at T1DM diagnosis, T1DM duration and medications in use, and all patients underwent physical and laboratory evaluations, as previous described (35, 36). Serum and plasma samples were taken after 12 h of fasting for laboratory analyses (35, 36).

Genotyping

DNA was extracted from peripheral blood leucocytes using a standardized salting-out procedure (37). The rs7020673 (G/C) and rs10758593 (A/G) SNPs in the *GLIS3* gene were genotyped using primers and probes contained in specific Human Custom TaqMan Genotyping Assays 40x (Thermo Fisher Scientific, Foster City, CA, USA). Reactions were conducted in 384-well plates, in a total 5 μl reaction volume, using 2 ng of

genomic DNA, TaqMan Genotyping Master Mix 1x (Thermo Fisher Scientific), and Custom TaqMan Genotyping Assay 1x. Plates were then positioned in a real-time PCR thermal cycler (ViiA7 Real Time PCR System; Thermo Fisher Scientific) and heated for 10 min at 95 °C, followed by 50 cycles of 95 °C for 15 s and 60 °C for 1 min. Common SNPs of interest were selected based on previous studies that reported associations of them with T1DM (21, 22, 32) or IA (10).

Since T1DM *HLA DR/DQ* haplotypes may influence the association of non-HLA SNPs with T1DM (4, 11), we also analyzed frequencies of high-risk *HLA* haplotypes in case and control samples. For this, 3 SNPs of the *HLA* class II loci (rs3104413, rs2854275 and rs9273363) were genotyped in all samples using specific Custom TaqMan Genotyping Assays 40x (Thermo Fisher Scientific), as described above. A recent study showed that these SNPs can predict *HLA DR/DQ* haplotypes relevant to T1DM with an accuracy >99% (4). Thus, using the method proposed by Nguyen *et al.* (4), we calculated frequencies of the following *HLA DR/DQ* haplotypes: high-risk haplotypes (*DR4/DQ8* or *DR3/DR4-DQ8* or *DR3/DR3*), intermediate-risk haplotype (*DR3/DRx*), and low-risk haplotypes (*DRx/DRx* or *DR4/DQ7*), where x can be different non-risk alleles.

Statistical analyses

Allele frequencies were determined by gene counting, and departures from the Hardy–Weinberg equilibrium (HWE) were verified using χ^2-tests. Allele and genotype frequencies were compared between groups of subjects using χ^2-tests. Linkage disequilibrium (LD) between rs10758593 (A/G) and rs7020673 (G/C) SNPs was calculated using Lewontin's D' |D'| and r^2 (38). Haplotypes constructed from the combination of the two analyzed SNPs and their frequencies were inferred using Phase

2.1 program (Seattle, WA, USA), which implements a Bayesian statistical method (39). We also used this program to compare the distributions of different *GLIS3* haplotypes between T1DM patients and non-diabetic subjects through permutation analysis of 10,000 replicates (39).

Clinical and laboratory characteristics were compared between groups by using unpaired Student's t-test, ANOVA or χ^2, as appropriate. The magnitude of the association of different genotypes or haplotypes with T1DM was estimated using odds ratio (OR) tests with 95% CI, adjusting for age, ethnicity and T1DM high-risk *HLA DR/DQ* haplotypes in logistic regression analysis.

Power calculations (www.openepi.com) showed that this study has a power of approximately 80% at a significance level of 0.05 to detect an OR of 1.5 or higher (for the presence of the minor alleles of each SNP). All analyses were performed with the SPSS 18.0 software (SPSS, Chicago, IL). P values <0.05 were considered significant.

Results

Sample description

The main clinical and laboratory characteristics of T1DM patients and non-diabetic subjects included in this study are shown in **Table 1.** In brief, mean age and mean body mass index (BMI) were higher in the non-diabetic group compared with T1DM patients (P< 0.0001). As already commented, non-white subjects comprised 7.9% of the T1DM group and 14.3% of non-diabetic subjects (P= 0.002). As expected, mean GHb, prevalence of arterial hypertension (HAS) and frequencies of high-risk *HLA DR/DQ* haplotypes (*DR4/DQ8*, *DR3/DR4-DQ8* or *DR3/DR3*) were higher in T1DM patients than controls (P <0.0001). Mean age at T1DM diagnosis was 16.7 ± 10.0 years, and

29.0% of T1DM patients had diabetic kidney disease (DKD), and 44.9% had diabetic retinopathy (DR).

Genotype and allele distributions

Genotype distributions of *GLIS3* rs7020673 (G/C) and rs10758593 (A/G) SNPs were in agreement with those predicted by HWE in non-diabetic subjects (all P ≥0.05). Frequencies of *GLIS3* minor alleles in white and non-white subjects were as follows: 71.4% *vs*. 62.4% for the rs7020673C allele (P= 0.079), and 67.7% *vs*. 64.7% for the rs10758593A allele (P= 0.613), considering a dominant model of inheritance.

Genotype and allele frequencies of the rs7020673 and rs10758593 SNPs in T1DM patients and non-diabetic subjects are illustrated in **Table 2**. *GLIS3* rs7020673C allele frequency was 47.3% in T1DM patients and 45.1% in the non-diabetic group (P= 0.365), while the rs10758593A allele was present in 43.3% of cases and 41.1% of controls (P= 0.341). Genotypes of rs7020673 and rs10758593 SNPs did not differ significantly between case and control groups (P= 0.468 and P= 0.279, respectively). In addition, frequencies of both SNPs did not differ significantly between groups under dominant (**Table 2**), recessive or additive inheritance models (data not shown). Of note, presence of rs7020673C and rs10758593A minor alleles (dominant model) remained not associated with T1DM after adjustment for high-risk *HLA DR-DQ* haplotypes, age and ethnicity (OR= 0.890, 95% CI 0.580 – 1.364; P= 0.592; and OR= 1.393, 95% CI 0.914 – 2.12; P= 0.123, respectively).

Haplotype analysis

Frequencies of different haplotypes produced by the combination of *GLIS3* rs7020673 and rs10758593 SNPs in T1DM patients and non-diabetic subjects are shown in **Table**

3. Four haplotypes were inferred in both samples, and permutation analysis showed that distributions of these haplotypes were different between case and control groups (P = 0.011), with Ht4 (C A) being more frequent in T1DM patients compared to controls (4.1% *vs*. 1.6%; residual P <0.01). It is noteworthy that the two SNPs of interest are in partial LD in our population (|D'| = 0.88 and r^2= 0.49).

Considering the low frequency of Ht4 (C A), we further analyzed haplotype frequencies dichotomizing samples to only two groups of polymorphic combinations: 1) subjects carrying ≤2 minor alleles of the rs7020673 and rs10758593 SNPs, and 2) subjects carrying ≥3 minor alleles (**Table 2**). Frequency of ≥3 minor alleles of the two analyzed SNPs was higher in T1DM patients compared to non-diabetic subjects (6.2% *vs*. 1.6%; P= 0.001). Of note, excluding non-white subjects from the samples did not significantly change this association (P= 0.003). In addition, frequency of ≥3 minor alleles remained independently associated with risk for T1DM after adjustment for T1DM high-risk *HLA DR/DQ* haplotypes, age, and ethnicity (OR= 3.684 95% CI 1.220 – 11.124; P= 0.021), suggesting that rs7020673 and rs10758593 SNPs interact in the susceptibility for T1DM.

Comparison of clinical and laboratory characteristics of T1DM among different genotypes of GLIS3 SNPs

In an exploratory analysis, clinical and laboratory characteristics related to T1DM were compared among different genotypes of *GLIS3* rs7020673 and rs10758593 SNPs and are shown in **Table 4**. Age, gender, ethnicity, age at T1DM diagnosis, GHb, fasting plasma glucose (FPG), body mass index (BMI), systolic and diastolic blood pressure (BP), and prevalence of DR and DKD did not differ significantly among genotypes of the rs7020673 SNP. The same characteristics were similar among genotypes of the

rs10758593 SNP, with exception of mean GHb, which was higher in T1DM patients who were homozygous for the rs10758593 A/A genotype compared with G allele carriers (P= 0.038). In non-diabetic subjects, mean GHb was similar among different genotypes of the two analyzed SNP (P >0.05) (data not shown).

Of note, clinical and laboratory characteristics of T1DM were similar between T1DM patients carrying ≥3 minor alleles of the rs7020673 and rs10758593 SNPs and patients carrying ≤2 minor alleles of these SNPs (all P >0.05; **Supplementary Table 1**).

Discussion

The transcription factor GLIS3 plays a critical role in the generation of pancreatic beta-cells and regulation of *INS* gene expression (14-18, 40). Nevertheless, to date, only few studies have evaluated the association between *GLIS3* SNPs and T1DM. Thus, for the first time, we investigated the association of *GLIS3* rs7020673 and rs10758593 SNPs with T1DM in a Brazilian population of mixed ethnicity. Although, *GLIS3* rs7020673 and rs10758593 SNPs were not individually associated with T1DM, presence of ≥3 minor alleles of both SNPs conferred an increased risk for this disease adjusting for T1DM high-risk *HLA DR/DQ* haplotypes, age and ethnicity. Furthermore, T1DM patients homozygous for the rs10758593 A/A genotype showed higher levels of GHb compared with G allele carriers.

Barret *et al.* (21) performed a GWAS combined with a meta-analysis of two previously published studies from European populations from Great-Britain and Denmark, and showed that *GLIS3* rs7020673 SNP was associated with protection for T1DM (OR= 0.88, 95% CI 0.83-0.93). Steck *et al.* (10) was not able to find any association between the rs7020673 SNP and T1DM in a non-Hispanic prospective

cohort from USA, but they showed that the C allele was significantly associated with protection against the development of IA [Hazard Ratio (HR)= 0.65, 95% CI 0.45-0.93) after adjustment for co-variables. In contrast, this SNP was reported as being associated with risk for T1DM in a Pakistani population (32) as well as susceptibility for rheumatoid arthritis in the same population (41). These controversial results may be explained by different ethnic groups that were analyzed. To date, only one study investigated if the rs10758593 SNP was associated with T1DM. In a follow-up analysis of GWAS data, Grant *et al.* (22) observed that the A allele of this SNP was associated with risk for T1DM (OR= 1.131, 5% CI 1.074 – 1.190) in five cohorts from USA. Further studies are necessary to replicate the association between *GLIS3* SNPs and T1DM and to assess their functional role on *GLIS3* expression.

In the present study, rs7020673C and rs10758593A alleles were significantly associated with risk for T1DM only when combined in haplotypes, suggesting that these *GLIS3* SNPs interact in the susceptibility for the disease. However, we cannot exclude an individual association of these SNPs with T1DM since we only had statistical power to detect OR ≥1.5. Therefore, the rs7020673 and rs10758593 SNPs could have weaker effects on T1DM risk in our population.

Of note, Wrinkler *et al.* (9) applied multivariate logistic regression and Bayesian feature selection to the Type 1 Diabetes Genetics Consortium (T1DGC) database with genotyping of *HLA* plus 40 SNPs in 4574 cases and 1207 controls. The application of a 10 SNPs model (HLA *DR/DQ* plus 9 SNPs from the *GLIS3*, *PTPN22*, *INS*, *IL2RA*, *ERBB3*, *ORMDL3*, *BACH2*, *IL27* and *RNLS* genes) to prospectively followed children significantly improved the prediction of T1DM over that provided by high-risk *HLA DR/DQ* alone.

The exact mechanisms by which SNPs in the *GLIS3* gene might contribute to T1DM pathogenesis remain to be explored. There is a hypothesis that GLIS3 may be a T1DM autoantigen (2, 31) since *GLIS3* mRNA is moderately expressed in the human thymus (12). Hence, *GLIS3* risk alleles that cause decreased *GLIS3* expression in the thymus could lead to T1DM autoimmunity through GLIS3-reactive T-cells that would escape negative selection. In addition, diabetogenic T-cell response after beta-cell apoptosis could be enhanced by *GLIS3* risk alleles (31).

In this context, Nogueira *et al.* (19) showed that *GLIS3* knockdown increased human and rat beta-cell apoptosis under basal condition and also sensitized these cells to apoptosis induced by pro-inflammatory cytokines or palmitate, which contribute to beta-cell loss in T1DM and T2DM, respectively. This increase in apoptosis was due to the activation of the intrinsic apoptotic pathway through alternative splicing of the pro-apoptotic BH3-only protein Bim, favoring the expression of its most pro-apoptotic isoform, Bim Small (BimS). Bim is known to contribute to cytokine- (42), virus- (43) and high-glucose-induced beta-cell death (44). Interestingly, Dooley *et al.* (45) identified that *Glis3* is a new genetic component of diabetes susceptibility in non-obese diabetic (NOD) mice, with a decrease in *Glis3* expression leading to immune-independent beta-cell apoptosis and senescence through activation of the endoplasmic reticulum stress.

As already mentioned, *Glis3*$^{-/-}$ knockout mice develop neonatal diabetes due to impairment in the generation of beta-cells and *Ins2* gene expression (15, 16, 20). These mice also show decreased expression of several key transcription factors necessary for the endocrine development of the pancreas, such as Ngn3, MafA, NeuroD1, and Pdx1 (15). Additionally, heterozygous *Glis*$^{+/-}$ adult mice have defective *Ins2* secretion and increased susceptibility to high-fat diet (HFD)-induced diabetes (18). In vitro blockade

or overexpression of *Glis3* in rat insulinoma INS-1 832/13 cells showed that this transcription factor binds to two Glis-binding sites in the rat *Ins2* promoter, regulating its expression (14, 15). Glis3 also interacts with Pdx1, MafA and NeuroD1 further increasing *Ins2* promoter activity (14). In agreement with these studies, different SNPs in the *GLIS3* gene have been reported as being associated with FPG and beta-cell function in children and adults (23, 27-30) as well as with T2DM susceptibility (23-26). None of these studies evaluated the rs7020673 and rs10758593 SNPs. Here, the rs10758593 A/A genotype was associated with increased levels of GHb in T1DM patients, which is in agreement with a role of GLIS3 in beta-cell function.

The aforementioned studies corroborate the key role of GLIS3 in beta-cell development, maintenance and function, and strongly point *GLIS3* as a convincing example of a gene predisposing for both T1DM and T2DM. In fact, the *GLIS3* region is the only locus showing association with both forms of diabetes, glucose metabolism traits, and neonatal diabetes (19). Therefore, we hypothesized that subjects carrying ≥3 minor alleles of the rs7020673 and rs10758593 SNPs might show decreased *GLIS3* expression in beta-cells and thymus, which would trigger increased cytokine induced-beta-cell apoptosis and impairment in glucose-stimulated insulin expression, predisposing for T1DM. Further studies are necessary to replicate the association of these polymorphisms with T1DM in different populations and to evaluate their effects on *GLIS3* expression and beta-cell fate.

This study has a couple of limitations. First, we cannot rule out the possibility of population stratification bias when analyzing our samples, even though frequencies of *GLIS3* minor alleles were similar in white and non-white subjects, the results were adjusted for ethnicity, and the exclusion of non-white subjects from the whole sample did not change the observed results, thus reducing the risk of false-positive/negative

associations due to this bias. Second, we cannot exclude the possibility of a type II error when evaluating the individual association between the rs7020673 and rs10758593 SNPs and T1DM. We had more than an 80% power ($\alpha = 0.05$) to detect an OR of 1.5 or higher for the analyzed SNPs. However, we cannot exclude the possibility that these two SNPs would be individually associated with T1DM with lower ORs.

In conclusion, our results indicate that individually the rs7020673 and rs10758593 SNPs are not significantly associated with T1DM but seem to interact in the predisposition for this disease since the presence of ≥3 minor alleles of both polymorphisms were increased in T1DM patients. Importantly, this association remained after adjustment for high-risk *HLA DR/DQ* haplotypes, indicating that the rs7020673 and rs10758593 SNPs are independent risk factors for T1DM. Moreover, the *GLIS3* rs10758593 SNP seems to be associated with a worse glycemic control in our population.

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Declaration of interest

The authors declare no conflict of interest.

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Table 1. Characteristics of T1DM patients (cases) and non-diabetic subjects (controls) included in this case-control study

Characteristic	**Cases (n= 503)**	**Controls (n= 442)**	**P value**
Age (years)	34.0 ± 12.0	38.2 ± 10.1	<0.0001
Gender (% males)	52.7	56.1	0.350
Ethnicity (% non-white)	7.9	14.3	0.002
Age at diagnosis (years)	16.7 ± 10.0	-	-
GHb (%)	8.7 ± 2.0	5.3 ± 0.2	<0.0001
BMI (Kg/m^2)	23.8 ± 3.5	26.8 ± 4.8	<0.0001
Hypertension (%)	29.8	5.4	<0.0001
DKD (%)	29.0	-	-
DR (%)	44.9	-	-
T1DM high-risk *HLA DR/DQ* haplotypes (%)*	57.1	17.5	0.0001

BMI: Body mass index; DKD: diabetic kidney disease; DR: diabetic retinopathy; GHb: glycated hemoglobin; P-value was computed using χ^2 tests or Student t-test, as appropriated. * T1DM high-risk *HLA DR/DQ* haplotypes: *DR4/DQ8*, *DR3/DR4-DQ8* or *DR3/DR3*.

Table 2. Genotype and allele frequencies of rs7020673 (G/C) and rs10758593 (A/G) SNPs in the *GLIS3* gene in patients with type 1 diabetes mellitus (cases) and non-diabetic subjects (controls)

	Cases	**Controls**	**Unadjusted OR (95% CI)/ P***	**Adjusted OR (95% CI) / P†**
rs7020673 SNP	n= 501	n= 440		
Genotype				
G/G	140 (27.9)	139 (31.6)	1	1
G/C	248 (49.5)	205 (46.6)	1.201 (0.891 – 1.619) / 0.229	0.944 (0.600 – 1.483) / 0.801
C/C	113 (22.6)	96 (21.8)	1.169 (0.816 – 1.674) / 0.395	0.772 (0.441 – 1.354) / 0.367
Allele				
G	0.527	0.549	0.365	-
C	0.473	0.451		
Dominant model				
G/G	140 (27.9)	139 (31.6)	1	1
G/C – C/C	361 (72.1)	301 (68.4)	1.191 (0.900 – 1.576) / 0.222	0.890 (0.580 – 1.364) / 0.592

rs10758593 SNP	n= 503	n= 442		
Genotype				
G/G	155 (30.8)	156 (35.3)	1	1
A/G	261 (51.9)	208 (47.1)	1.263 (0.947 – 1.683) / 0.111	1.396 (0.895 – 2.17) / 0.142
A/A	87 (17.3)	78 (17.6)	1.123 (0.769 – 1.638) / 0.549	1.382 (0.780 – 2.45) / 0.268
Allele				
G	0.567	0.589	0.341	-
A	0.433	0.411		
Dominant model				
G/G	155 (30.8)	156 (35.3)	1	1
A/A – A/G	348 (69.2)	286 (64.7)	1.225 (0.993 – 1.607) / 0.144	1.393 (0.914 – 2.12) / 0.123
***GLIS3* haplotypes**	n= 485	n= 434		
< 2 risk alleles	455 (93.8)	427 (98.4)	1	1
> 3 risk alleles	30 (6.2)	7 (1.6)	4.022 (1.748 – 9.253) / 0.001	3.684 (1.220 – 11.124) / 0.021

Data are presented as number (%) or proportion. * P values were computed by χ^2 tests comparing T1DM patients and non-diabetic subjects or univariate logistic regression analysis. † Adjusted OR (95% CI) / P values were obtained from logistic regression analyses adjusting for age, ethnicity and *HLA* risk haplotypes.

Table 3. Haplotypes of the *GLIS3* gene in patients with type 1 diabetes mellitus (cases) and non-diabetic subjects (controls)

	Cases	**Controls**	**Total sample**	**P***
Haplotypes	n= 970	n= 868		
Ht1 (G G)	0.135	0.149	0.142	0.011
Ht2 (G A)	0.391	0.398	0.394	
Ht3 (C G)	0.433	0.437	0.435	
Ht4 (C A) †	0.041	0.016	0.029	

Data are presented as proportion. n, number of chromosomes. The first letter of haplotypes refers to the rs7020673 SNP and the second letter to the rs10758593 SNP. *P value was obtained by permutation test for comparisons of haplotype frequencies between groups. †Adjusted residuals that deviated from expected values (P <0.01).

Table 4. Clinical and laboratory characteristics of T1DM patients, broken down by presence of different rs7020673 and rs10758593 *GLIS3* SNPs

	***GLIS3* rs7020673 (G/C)**				***GLIS3* rs10758593 (G/C)**			
	G/G (n= 140)	**G/C (n= 248)**	**C/C (n= 113)**	**P***	**A/A (n= 87)**	**A/G (n= 261)**	**G/G (n= 155)**	**P***
Age (years)	37.2 ± 12.7	36.0 ± 13.1	34.5 ± 12.4	0.455	35.2 ± 12.5	36.7 ± 12.8	35.7 ± 13.0	0.726
Gender (% males)	51.8	49.5	59.6	0.267	50.7	51.2	56.2	0.630
Ethnicity (% non-white)	10.1	7.7	5.5	0.412	6.9	8.5	7.3	0.849
Age at diagnosis (years)	17.9 ± 11.5	16.6 ± 8.9	15.2 ± 10.1	0.332	14.5 ± 9.8	17.9 ± 9.5	15.9 ± 10.7	0.104
GHb (%)	8.6 ± 1.9	8.9 ± 2.1	8.2 ± 2.0	0.072	8.8 ± 2.2	8.9 ± 2.0	8.2 ± 2.0	0.038
FPG (mg/dl)	171.0 ± 91.8	186.8 ± 116.3	167.8 ± 110.6	0.358	178.3 ± 93.5	183.9 ± 113.1	168.6 ± 108.7	0.533
BMI (kg/m^2)	24.1 ± 4.0	24.0 ± 13.6	23.2 ± 3.1	0.391	24.2 ± 4.4	23.8 ± 3.4	23.7 ± 3.3	0.766
SBP (mmHg)	124.4 ± 17.6	119.6 ± 19.1	122.4 ± 17.9	0.152	124.4 ± 16.7	119.2 ± 19.0	123.7 ± 18.2	0.073
DBP (mmHg)	78.6 ± 11.3	77.4 ± 11.1	78.7 ± 12.6	0.599	78.4 ± 11.3	77.0 ± 11.3	79.1 ± 11.7	0.334
DKD (%)	37.7	25.2	27.8	0.215	32.4	27.5	29.7	0.834
DR (%)	45.4	45.4	40.2	0.716	45.6	44.8	44.7	0.993

Data are mean – standard deviation (SD) or %. *P values are according to chi-squared test or analysis of variance (ANOVA), as appropriate. BMI: body mass index; DBP: diastolic blood pressure; DKD: diabetic kidney disease; DR: diabetic retinopathy; FPG: fasting plasma glucose; GHb: glycated hemoglobin; SBP: systolic blood pressure.

Supplementary Table 1. Clinical and laboratory characteristics of T1DM patients broken down by presence of ≥3 minor alleles of the rs10758593 (A/G) and rs7020673 (G/C) polymorphisms in the haplotypes

	***GLIS3* rs7020673 (G/C) – 10758593 (A/G) SNPs**		
	≤ 2 minor alleles (n = 455)	**≥ 3 minor alleles (n = 30)**	**P value***
Age (years)	36.0 ± 12.9	35.4 ± 11.6	0.828
Gender (% males)	52.3	31.6	0.125
Ethnicity (% non-white)	8.0	6.2	0.999
Age at diagnosis (years)	16.8 ± 10.2	16.0 ± 10.4	0.768
GHb (%)	8.7 ± 2.1	9.1 ± 1.9	0.411
FPG (mg/dl)	178.0 ± 107.3	175.8 ± 137.5	0.940
BMI (kg/m^2)	23.8 ± 3.6	23.5 ± 3.5	0.757
SBP (mmHg)	121.5 ± 18.8	121.8 ± 14.7	0.949
DBP (mmHg)	78.1 ± 11.5	75.0 ± 8.5	0.280
DKD (%)	30.4	30.0	0.980
DR (%)	44.0	46.7	0.999

BMI: Body mass index; DBP: diastolic blood pressure; DKD: diabetic kidney disease; DR: diabetic retinopathy; GHb: glycated hemoglobin; SBP: systolic blood pressure. *P-value was computed using χ^2 tests or Student t-test, as appropriated.

CONCLUSÕES GERAIS

Os resultados do presente estudo indicam que, isoladamente, os polimorfismos rs7020673 (G/C) e rs10758593 (A/G) no gene *GLIS3* não estão associados com risco para o DM1 em indivíduos do Sul do Brasil. Quatro haplótipos constituídos por estes dois SNPs foram inferidos nas amostras estudadas e o haplótipos Ht4 (C A) mostrou-se aumentado nos pacientes com DM1. Interessantemente, haplótipos contendo ≥3 alelos raros dos dois SNPs estudados foram associados com risco para DM1. Essa associação manteve-se após ajuste para os haplótipos *HLA-DR/DQ* de alto risco para DM1, idade e etnia, sugerindo que os SNPs rs7020673 e rs10758593 interagem na suscetibilidade para a doença. Além disso, pacientes com DM1 homozigotos para o genótipo A/A do polimorfismo rs10758593 apresentaram aumento dos níveis de hemoglobina glicada, indicando que este SNP está associado a um pior controle glicêmico em pacientes com DM1 da nossa população. Estudos adicionais são necessários para elucidar o papel desses polimorfismos na expressão do gene *GLIS3*, bem como no desenvolvimento do DM1 e metabolismo da glicose.