The Prevalence of the *HNF-1α* G319S Mutation in Canadian Aboriginal Youth With Type 2 Diabetes

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OBJECTIVE — To investigate the prevalence of the unique *HNF-1α* G319S mutation in a population of aboriginal youth with type 2 diabetes and to describe the relationship between clinical and historical characteristics and the presence or absence of the *HNF-1α* G319S mutation.

RESEARCH DESIGN AND METHODS — Participating youth were genotyped for the G319S mutation of the HNF-1α gene. Clinical, laboratory, and historical data were collected via chart review (blinded to genotype results). Comparison data were derived from another study involving young nondiabetic pregnant aboriginal women.

RESULTS — A total of 51 youth seen sequentially in a type 2 diabetes clinic participated in this study. Of these, 21 (41.2%) had at least one copy of the mutant allele. The allele frequency in the study population was 0.29 (95% CI 0.20–0.38), which was significantly different from the allele frequency of 0.13 in the comparison population ($\chi^2 = 6.78$, $P = 0.009$). The frequency of the homozygous mutation (S319/S319) was 0.18. Mean BMI was significantly lower ($P = 0.002$), mean HbA$_{1c}$ was significantly higher ($P = 0.02$), and acanthosis nigricans was significantly less frequent ($P = 0.004$) in those with the mutation compared with the wild type. Mean insulin levels were lower and insulin sensitivity (assessed by homeostasis model assessment [HOMA]) was greater in the homozygote group compared with the wild-type group ($P = 0.002$ and $P = 0.0007$, respectively). A dose-dependent gradient was observed for these characteristics.

CONCLUSIONS — These data support the association between the *HNF-1α* G319S mutation and early-onset type 2 diabetes in this population. Those with the mutation lacked clinical characteristics of insulin resistance (e.g., obesity and acanthosis nigricans) and had lower insulin levels, suggesting that an insulin-secretory and/or -production defect plays an important role in the development of diabetes in this group. Further investigation of the pathophysiology of the S319 homo- and heterozygote is needed because it may impact treatment and/or prevention of this disease.

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T ype 2 diabetes in children and youth is recognized as a new and serious health concern with a marked predilection for specific ethnic groups (1,2). In some Canadian aboriginal populations, type 2 diabetes in youth is more common than type 1 diabetes in the general pediatric population (2). Of the 141 children and youth with type 2 diabetes who were seen at the Children's Hospital of Manitoba between 1986 and 1999, 138 (97.9%) were of aboriginal origin. This propensity for a specific subpopulation group suggests that genetic factors may be involved in the development of type 2 diabetes in this population.

Hepatic nuclear factor-1α (HNF-1α) is one of the transcription factors involved in the transcription of hepatic genes including albumin, α-1-antitrypsin, apolipoproteins, and several clotting factors (3). It is also expressed in pancreatic β-cells (4), kidney, and intestine (5). Heterogeneous mutations of the HNF-1α gene have been identified to cause one form of transcription-related diabetes or maturity-onset diabetes of the young type 3 (MODY 3) (6–8). More recently, small numbers of individuals with typical insulin-resistant type 2 diabetes have been described with mutations of the HNF-1α gene, suggesting co-inheritance of type 2 diabetes susceptibility genes and transcription factor–related diabetes (9,10).

A previously undescribed polymorphism in the HNF-1α gene has been identified in the Oji-Cree of Sandy Lake, Ontario, Canada involving a substitution of serine for glycine at codon 319 (*HNF-1α* G319S) (10). This mutation was positively associated with diabetes in this population; this group has one of the highest prevalence rates of type 2 diabetes in the world (10,11). The diabetic phenotype in this community is of typical insulin-resistant type 2 diabetes. Of the population with diabetes, those with the identified mutation were characterized by a lower BMI (10) and, in women, a younger age of onset (12). Women with the mutation had the onset of diabetes in the third and fourth decade of life compared with the fifth decade in those without the mutation.

Identification of the factors involved in the development of youth-onset type 2 diabetes in the aboriginal population may better direct our efforts to develop therapeutic and preventative strategies specific to this population. This study was undertaken to investigate the prevalence of the unique *HNF-1α* G319S mutation in the population of aboriginal youth with type 2 diabetes seen at the Winnipeg Children's Hospital, Winnipeg, Canada. A secondary objective was to investigate the relationship between clinical and historical characteristics and the presence or absence of the *HNF-1α* G319S mutation.

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Table 1—*Genotype and allele frequencies*

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Genotype	Study participants (n = 51)	Comparison population (n = 109)	Comparison population from B region (n = 68)	Nondiabetic (n = 334)*	Diabetic (n = 117)*	Diabetic <25 years of age (n = 16)†
G319/G319	0.569	0.771	0.680	0.829	0.624	0.437
G319/S319	0.255	0.193	0.260	0.168	0.333	0.437
S319/S319	0.176	0.037	0.059	0.003	0.043	0.125
Allele						
S319	0.294	0.133	0.188	0.087	0.209	0.343
	(P < 0.001)					

*See ref. 10; †see ref. 15.

RESEARCH DESIGN AND METHODS

Study subjects

All patients with type 2 diabetes of self-declared aboriginal origin seen sequentially at the Children's Hospital of Manitoba, Winnipeg, Canada between April and August 2000 were invited to participate in this study. All participants were under the age of 18 years. The Children's Hospital of Manitoba is a pediatric tertiary care facility that provides services to the province of Manitoba and Northwestern Ontario (catchment population ~1.5 million people). Written informed consent was obtained from the parent or guardian and assent was obtained from the child. The Human Research Ethics Committee of the University of Manitoba approved the study protocol.

Study design

Blood for genetic analysis (~5 cm^3) was drawn when blood work for clinical care purposes was performed. DNA was extracted by standard methods, and genotyping for the *HNF-1α* G319S polymorphism was performed as previously described (10).

Clinical and historical data were gathered on each participant using chart review by an observer blinded to the genetic analysis. The data collected included age at diagnosis, current BMI, current HbA$_{1c}$, family history of diabetes, history of maternal diabetes, community of origin, current acanthosis nigricans, and mode of presentation of diabetes. Insulin sensitivity was assessed using the homeostasis model assessment (HOMA) (13,14).

Comparison population

A total of 109 pregnant young aboriginal women who were having blood taken for *HNF-1α* G319S analysis for an unrelated study served as a comparison population (A. Fisher, C.R. Greenberg, S. Taback, P. Smith, unpublished data); 41 of these women were from a remote community ("A") in Central Manitoba and 68 were from two remote communities ("B" region) in northeastern Manitoba. Community A is a Cree First Nation Community; community B is an Oji-Cree community. These groups are linguistically and culturally distinct aboriginal populations. None of the comparison subjects were known to have diabetes.

Statistical analysis

Much of the data collected was analyzed with descriptive statistics. Between-group differences in genotype and allele frequencies were compared using the χ^2 test for proportions employing Yates' correction; 95% CIs were also calculated. The Student's *t* test was used to test for differences between means for normally distributed continuous variables (BMI, HbA$_{1c}$, age at diagnosis, insulin levels, and HOMA). Fisher's exact test for proportions was used to compare acanthosis nigricans and symptoms at presentation in those with and without the mutation.

Definitions

The wild-type genotype is defined as G319/G319, the heterozygote state as G319/S319, and the homozygote state as S319/S319.

RESULTS

Frequency of the S319 allele

A total of 51 of a possible 52 subjects agreed to participate in this study. Of the 102 alleles typed, 30 mutant alleles were found, giving an allele frequency of 0.294 (95% CI 0.206–0.382) (Table 1). This is significantly different from the allele frequency of 0.133 in the comparison population (χ^2 = 11.76, $P < 0.001$). Among our study participants, a frequency of 0.176 was found for the S319/S319 homozygous genotype, which is higher than that which has been previously reported (10,15).

Regional origin

Twenty-one participants in this study had at least one copy of the mutant allele (41.2%). Of the 51 participants, 18 were from the B region communities. Of these 18, 14 (77.8%) had at least one copy of the mutant allele. Nine of 14 were homozygous for the mutant allele (all homozygotes were from the B region communities). Therefore, the frequency of the mutant allele in youth from the "B" region was 0.639, and the frequency of the S319/S319 genotype in participants from this region was 0.500 (9 of 18). Of the seven other individuals with the mutant allele, three were from different remote communities in Northwestern Ontario and one each were from four other remote communities in Northern Manitoba.

Clinical and laboratory characteristics

Homozygotes had a significantly lower BMI at diagnosis and at study entry than those without the mutation (at diagnosis

Table 2—Clinical and laboratory characteristics by genotype

Genotype	BMI (kg/m^2) at diagnosis	BMI (kg/m^2) at study entry	Acanthosis nigricans	Mean age at diagnosis (years)	HbA$_{1c}$ (%)	Fasting insulin (pmol/l)	HOMA
GG (G319/G319)	32.13 (28.79–35.52)	31.08 (28.86–33.3)	80.0	12.73 (12.05–13.41)	8.13 (7.17–9.09)	202 (142–261)	11.43 (8.21–14.66)
GS (G319/S319)	28.65 (26.12–31.18)	28.86 (25.98–31.74)	66.6	12.29 (11.09–13.49)	10.18 (7.93–12.43)	142 (92–193)	9.72 (5.26–14.19)
SS (S319/S319)	22.88 (20.21–25.55)	22.43 (19.51–25.35)	22.2	13.56 (12.04–15.04)	10.44 (8.35–12.53)	62 (46–79)	4.70 (3.32–6.69)

Data are means (95% CI) or %.

mean BMI in homozygotes 22.88 ± 4.09 and 32.12 ± 8.47 kg/m^2 in the wild type, $P < 0.0002$; at study entry, 22.43 ± 4.48 and 31.08 ± 6.20 kg/m^2, respectively, $P = 0.002$) (Table 2). The BMI of the heterozygotes was intermediate between the homozygote and wild type at diagnosis and at study entry. (Mean age at diagnosis and at study entry did not differ significantly; thus, we have not reported age-standardized BMI.) Mean HbA$_{1c}$ also differed between the groups significantly. The mean HbA$_{1c}$ in the group with the mutant allele was 10.30 ± 3.46%; in those without it was 8.13 ± 2.52% ($P = 0.001$) (Table 3). Acanthosis nigricans, a marker of insulin resistance, was more frequently found among those with the wild-type genotype compared with those either heterozygous or homozygous for the S319 mutation (80.0 vs. 47.6%, $P = 0.06$ nonsignificant). This meets statistical significance when comparing the wild-type to the homozygous mutant (S319/S319) (80 vs. 22%, $P = 0.004$). Fasting insulin levels were significantly lower in those homozygous for the S319 mutation compared with those with the wild type ($P = 0.002$). Insulin sensitivity, assessed using the surrogate HOMA, indicated a significantly greater degree of insulin resistance in the wild-type compared with the homozygous S319 mutation (as indicated by a higher HOMA score) ($P = 0.0006$).

For each of these characteristics (mean BMI, mean HbA$_{1c}$, acanthosis nigricans, fasting insulin, and HOMA), the heterozygous genotype (G319/S319) was intermediate between the wild-type (G319/G319) and homozygous mutant (S319/S319) genotypes (Table 2).

The age of diagnosis of diabetes did not differ significantly between those with the mutation and those without (12.72 ± 1.94 and 12.61 ± 2.2 years, respectively).

A history of maternal diabetes during pregnancy was found in 48 and 50% of those with the mutation and those without, respectively. One individual in the group without the mutation was adopted; thus, family information was available for 29 of 30 of these subjects. A positive family history of diabetes (first-degree relative) was also similar in the two groups: 18 of 21 (86%) and 23 of 29 (82%) for those with and those without the mutation.

Classic symptoms of hyperglycemia were significantly more frequent in those with the wild-type allele (13 of 29, 44.8% [data not available for one participant]). Only two individuals with the mutation had symptoms of hyperglycemia at presentation (2 of 21, 9.5%; $P = 0.01$).

CONCLUSIONS

Allele and genotype frequency
The increased frequency of the S319 allele found in this study supports the positive association proposed by Hegele et al. (10) between this mutation and type 2 diabetes. The allele frequency in published data from the Sandy Lake First Nation in Northwestern Ontario in whom the G319S mutation was first identified is 0.087 and 0.209 in the nondiabetic and diabetic populations, respectively (10). The S319 mutation was found in 40% of those with diabetes in a population with a high prevalence of type 2 diabetes.

The Sandy Lake report involved participants with a mean age of onset of diabetes in the fifth decade of life. Our study involved a younger group of subjects who were diagnosed with diabetes before 18 years of age. Of note, homozygote S319/S319 individuals in the Sandy Lake study had the youngest age of diagnosis of diabetes compared with G319/G319 and G319/S319 individuals. The Sandy Lake study included 16 individuals between 10 and 25 years of age with diabetes (15). The allele frequency within this group was 0.34, similar to 0.29 found in this study. This suggests that the mutant allele is associated not only with type 2 diabetes but also with onset of the disease at a younger age.

In our study, the mutant allele was found most frequently in participants from a remote northeastern region (region B) of Manitoba (allele frequency = 0.639). These are Oji-Cree communities with close geographic, cultural, and linguistic links to the community of Sandy Lake, Ontario. These communities are of

Table 3—Clinical characteristics by presence or absence of mutant allele

	Mutant allele (G319/S319 or S319/S319)	Wild type (G319/G319)	P
Age at diagnosis (years)	12.65 (11.71–13.63)	12.73 (12.05–13.41)	NS
BMI (kg/m^2)	26.10 (23.41–28.37)	31.08 (28.86–33.30)	0.006
HbA$_{1c}$ (%)	10.30 (9.14–12.16)	8.13 (7.17–9.09)	0.001
Presence of acanthosis nigricans	47.6	80.0	NS

Data are means (95% CI) or %.

the same linguistic group, and it is likely that they share a common genetic heritage. Of the seven individuals with the mutant allele who were not from one of the B region communities, three were from other Oji-Cree communities in Northwestern Ontario, including one from Sandy Lake, suggesting that this mutation is specific to this population.

Clinical and laboratory characteristics

Participants with the wild-type genotype (G319/G319) were more likely to have symptoms of hyperglycemia at presentation (44.8 vs. 9.5%). The lack of symptomatology in S319 heterozygotes and homozygotes may increase the time between onset and diagnosis of diabetes. This may have a negative impact on the course of the disease, as diabetes complications are influenced by both glycemic control and duration of disease.

The lower average BMI (at diagnosis and study entry) and decreased frequency of acanthosis nigricans suggest that insulin resistance is not the major pathologic factor in the development of diabetes in the subpopulation with the G319S mutation. This is supported by the lower fasting insulin levels and lower HOMA score (indicating less insulin resistance) in this population. Evidence of decreased production and secretion of insulin in the $HNF-1\alpha$ $(-/-)$ knockout mouse lends support to this proposed mechanism (3).

The observation in our group of a gene dose-dependent gradient in the homozygote, heterozygote, and wild-type genotypes for BMI, acanthosis nigricans, fasting insulin, and HOMA differs from the well-described monogenic autosomal-dominant transcription factor diabetes (MODY 3). In addition, the onset of severe hyperglycemia in adolescence that is gene dose dependent in our population also differs from the pattern described in MODY 3 (16). MODY 3 has not been described within a population with a high prevalence of insulin-resistant type 2 diabetes or as a gene dose-dependent condition.

The participants in this study come from a population that has an increased prevalence of type 2 diabetes compared with the nonaboriginal population (18.9 vs. 4.54% of those aged 20–79 years) (17). We suggest that the clinical phenotype of those with the G319S mutation may result from the inheritance of an $HNF-1\alpha$ mutation from either one or both parents compounded by the inheritance of genes predisposing to type 2 diabetes. The concept of "co-inheritance" or "double gene dose" has been previously proposed by Tack et al. (18). Further evaluation will require detailed genetic analysis of family members.

Recent data has demonstrated the functional consequences of the $HNF-1\alpha$ G319S mutation. In an in vitro model, the transactivation potential of the HNF-1α gene was decreased by 54%, with no effect on DNA binding or stability (19). This group observed disease in those with an obese, insulin-resistant phenotype, and this observation suggests that the defect is modest and the additional stress of obesity-associated insulin resistance is necessary to expose the defect resulting in disease expression. Based on their previous works, the authors suggest that the defect is modest and has no clinical consequences in the young, lean Oji-Cree (10). Our experience does not support this. We hypothesize that the mutation and the co-inheritance of other type 2 diabetes susceptibility genes combine to create disease.

Diabetes control was significantly worse in those with the mutant allele, as evidenced by a higher average HbA$_{1c}$ in this group. This finding requires further investigation, but may reflect a difference in the pathophysiology of diabetes in those with the G319S mutation. It is possible that lifestyle interventions, including exercise and weight control, aimed at improving insulin sensitivity are not adequate in this disease and that a more aggressive use of insulin or pharmacologic agents that promote insulin secretion would have benefit.

In summary, these data support an association between the G319S allele and youth-onset diabetes in this population lacking clinical and laboratory characteristics of insulin resistance. This suggests that an insulin-secretory and/or -production defect plays an important role in the development of diabetes in this group. Recent in vitro data lend support to this suggestion. We hypothesize that the clinical phenotype of those either hetero- or homozygous for the G319S mutation results from the co-inheritance of the G319S mutation and genes predisposing to type 2 diabetes. The pathophysiology of diabetes in this population needs further investigation in order to develop and implement appropriate treatment strategies.

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