Clinical Utility of *HNF1A* Genotyping for Diabetes in Aboriginal Canadians

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OBJECTIVE— To determine the diagnostic performance characteristics of *HNF1A* genotyping for diabetes and impaired glucose tolerance (IGT) in Canadian Oji-Cree Indians.

RESEARCH DESIGN AND METHODS— We studied all Oji-Cree subjects ≥50 years of age (96 subjects) who had participated in a community-wide prevalence survey for type 2 diabetes. Subjects were classified either as having "disease," which included type 2 diabetes and IGT, or not. All subjects were genotyped for the *HNF1A* G319S mutation.

RESULTS— The prevalence of disease in this group was 65.7%, of whom 71.4% had type 2 diabetes. For a carrier of *HNF1A* S319, the specificity, sensitivity, and positive and negative predictive values were 97.0, 30.1, 95.0, and 42.1%, respectively. When the pretest disease prevalence was accounted for, the probability of disease after a positive test was 97.2%, and the probability of disease after a negative test was 42.2%. The values were very similar for the subgroup of subjects with type 2 diabetes alone.

CONCLUSIONS— The *HNF1A* genotype appears to be the most specific genetic test yet reported for the prediction of a common multifactorial disease by applying present-day standards of clinical epidemiology in molecular genetics. A positive test result had particular diagnostic value in the Oji-Cree: a subject with *HNF1A* S319 was virtually certain of having diabetes or IGT by 50 years of age. In contrast, a subject without *HNF1A* S319 had a reduced risk compared with the age-specific prevalence but was not totally risk-free. Because *HNF1A* S319 was not the only predisposing factor for diabetes in the Oji-Cree, subjects without *HNF1A* S319 were still at some risk for diabetes or IGT.

Diabetes Care 23:775–778, 2000

The prevalence of type 2 diabetes and impaired glucose tolerance (IGT) in adult Ontario Oji-Cree Indians approaches 40%, which is the third highest of any subpopulation in the world and is ~5 times higher than the prevalence observed in the general Canadian population (1). The complications of type 2 diabetes in the Oji-Cree are anticipated to soon extract a substantial social and economic toll. The high prevalence of diabetes will also challenge existing health care delivery paradigms because the ~30,000 Oji-Cree who live on reservations in northwestern Ontario and in Manitoba, Canada, are dispersed across a wide, remote, and harsh area. Intervention strategies to prevent or delay the onset of type 2 diabetes and its complications would include the modification of diet and lifestyle in high-risk subjects because diet and lifestyle appear to contribute to the expression of type 2 diabetes susceptibility in the Oji-Cree (1).

We recently identified a private *HNF1A* mutation, G319S, that was very strongly associated with type 2 diabetes in the Oji-Cree (2,3). The *HNF1A* S319 allele was present in >40% of the Oji-Cree who had diabetes and was associated with an earlier age at onset of diabetes (2,3), adolescent-onset type 2 diabetes (4), and changes in plasma lipoproteins (5). *HNF1A* S319 carriers had a phenotype that resembled typical type 2 diabetes and not maturity-onset diabetes of the young, which can also result from *HNF1A* mutations (2–5).

Whatever the mechanistic basis for its association with type 2 diabetes, the *HNF1A* G319S genotype appears to be potentially useful as a predictive test for type 2 diabetes in the Oji-Cree. The availability of such a predictive test would be important because it may help to identify high-risk subjects at the presymptomatic stage who may benefit from intervention strategies. In the present study, we evaluated the *HNF1A* genotype as a clinical test for the prediction of type 2 diabetes in the Oji-Cree of northern Ontario. We evaluated Oji-Cree residents of the Sandy Lake reservation who are ≥50 years of age because the onset of type 2 diabetes and IGT is clearly related to age, and most susceptible subjects should have manifested either disease by that age.

RESEARCH DESIGN AND METHODS

Study subjects

The community of Sandy Lake, Ontario, is located ~2,000 km northwest of Toronto in the subarctic boreal forest of central Canada. Community members ≥50 years old and not known to be related who had participated in the Sandy Lake Health and Diabetes Project (1) were studied for the present report. Approximately 72% of eligible subjects had volunteered to participate in this community-wide diabetes prevalence study. Subjects with physician-diagnosed diabetes and/or those who were taking oral hypoglycemic agents and/or insulin were excluded from the oral glucose tolerance test (OGTT). All others provided a fasting blood sample and then received the 75-g

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Received for publication 17 November 1999 and accepted in revised form 10 February 2000.

Abbreviations: IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Clinical and biochemical attributes of Oji-Cree ≥50 years of age*

	Subjects with diabetes or IGT	Subjects without diabetes	*P*
All subjects/women (*n*)	63/36	33/14	—
Age (years)	61.7 ± 8.5	59.8 ± 8.4	0.02
BMI (kg/m^2)	30.1 ± 4.9	27.3 ± 4.8	0.0003
Fasting glucose (mmol/l)	9.3 ± 4.8	5.5 ± 0.5	<0.0001
Serum insulin (U/l)	153 ± 86	118 ± 65	<0.0001
Serum C-peptide (nmol/l)	0.90 ± 0.39	0.80 ± 0.42	<0.0001
Carriers of *HNF1A* S319 (%)	30.1	3.0	<0.0001

Data are *n*, means ± SEM, or %.

OGTT followed by a second blood sample after 120 min for plasma glucose determination. Type 2 diabetes, IGT, and normal glucose tolerance were diagnosed using established pre-1997 criteria (1). For analyses of diagnostic performance of the *HNF1A* genotype, we studied grouped subjects with definite type 2 diabetes together with subjects with definite normal glucose tolerance according to these established criteria (6,7). The project was approved by the University of Toronto Ethics Review Committee and the Sandy Lake First Nations Band Council.

Biochemical and genetic analyses

Concentrations of fasting and postchallenge plasma glucose, fasting serum insulin, and fasting C-peptide were determined as described (1). DNA was extracted as described, and *HNF1A* G319S was genotyped as described (2,3). This method was found to yield reproducible results with 100% concordance between the results of analyses performed on samples obtained from 80 individuals that were run on 2 different days with blinding of the technologist and the interpreter of genotypes. A DNA sequence–proven standard was run with each set of reactions.

Statistical analyses

SAS Version 6.12 statistical software was used for all statistical comparisons (8). Between-group differences in allele and genotype frequencies were compared using χ^2 analysis and a 2-tailed Fisher's exact test, respectively. Estimates of the relative risk of type 2 diabetes between genotypes were determined using odds ratios with the Mantel-Haenszel method. For the purpose of analysis of performance characteristics, subjects were classified either as carriers or noncarriers of *HNF1A* S319. Standard formulas were used to calculate sensitivity, specificity, positive predictive value, negative predictive value, and the probability of diabetes before and after a positive or a negative test (9).

RESULTS

Baseline attributes of the study sample

A total of 96 Oji-Cree subjects ≥50 years of age were included in this analysis, of whom 27 (28.1%) had a previous medical diagnosis of diabetes, 18 (18.8%) were newly diagnosed with diabetes, 18 (18.8%) had IGT, and 33 (34.3%) were normoglycemic. For subsequent analyses of the clinical utility of the genetic testing, subjects with normoglycemia were compared against the group of subjects with abnormal glycemia, which consisted of subjects with diabetes and IGT. The clinical attributes of these subjects are shown in Table 1. Subjects in the disease group had a higher mean age and higher BMI, serum glucose, insulin, and C-peptide levels.

Two subjects, both with diabetes, were homozygotes for *HNF1A* S319/S319. A total of 18 subjects were heterozygotes for *HNF1A* S319/G319. The remainder of the subjects were homozygotes for *HNF1A* G319/G319. For the purpose of evaluating clinical utility, both *HNF1A* S319/S319 homozygotes and S319/G319 heterozygotes were considered to be *HNF1A* S319 carriers and were studied together. In a separate post hoc analysis of the subgroup of Oji-Cree subjects with diabetes, no difference was evident in mean concentrations of either fasting serum insulin (154 ± 99 vs. 159 ± 96 pmol/l) or serum C-peptide (0.96 ± 0.49 vs. 0.89 ± 0.41 nmol/l) among *HNF1A* S319 carriers versus noncarriers.

The overall prevalence of diabetes and IGT in Sandy Lake Oji-Cree who were ≥50 years of age was 65.7% (95% CI 56.0–75.2). The formulations used to calculate the diagnostic performance indices of the *HNF1A* genotype are shown in Table 2. In general, strikingly high levels of specificity, positive predictive value, positive likelihood ratio, and probability of being affected given a positive test for *HNF1A* S319 carrier status were evident. In contrast, the values for sensitivity, negative predictive value, negative likelihood ratio, and probability of being affected given a negative test for *HNF1A* S319 carrier status were more modest.

The diagnostic performance variables were also calculated after excluding subjects with IGT because not all subjects with IGT proceed to develop type 2 diabetes. This allowed for assessment of the genotype performance variables when considering a narrower, more severe phenotype. Exclusion of subjects with IGT left a subgroup of 33 normoglycemic subjects and 45 subjects with either previously or newly diagnosed diabetes who were ≥50 years of age. The overall prevalence of diabetes alone in this subgroup was 57.7% (46.7–68.7). For the detection of diabetes, *HNF1A* S319 carrier status had a sensitivity of 33.3% (20.0–43.7), a specificity of 97.0% (91.2–99.9), a positive predictive value of 93.8% (88.5–99.1), a negative predictive value of 51.6% (40.6–62.7), a likelihood ratio of a positive test result of 11.0 (2.3–437), a likelihood ratio of a negative test result of 0.687 (0.617–0.800), a probability of diabetes after a positive test (from Baye's equation) of 95.3% (90.5–99.9), and a probability of diabetes after a negative test (from Baye's equation) of 34.0% (23.5–44.5). Similar to the situation in which subjects with diabetes and subjects with IGT were included, strikingly high levels of specificity, positive predictive value, positive likelihood ratio, and probability of being affected given a positive test for *HNF1A* S319 carrier status were evident. In contrast, the values for sensitivity, negative predictive value, negative likelihood ratio, and probability of being affected given a negative test for *HNF1A* S319 carrier status were more modest.

CONCLUSIONS

— The results indicate that *HNF1A* genotype is very specific for marking an Oji-Cree subject ≥50 years of age as having diabetes or IGT. Because the genotype is invariant from birth, the results suggest that the *HNF1A* genotype may be useful for predicting diabetes and IGT in Oji-Cree by the time they are 50 years of age. A positive test result in particular had remarkable diagnostic value. A subject who was positive for *HNF1A* S319 was virtually certain (95%) of having dia-

betes or IGT by 50 years of age, which provided a significant increment over the age-specific prevalence of diabetes or IGT (~66%). This indicated that the *HNF1A* S319 test was superior to inferring the risk of disease from age-specific prevalence data. In contrast, a subject who was negative for *HNF1A* S319 was at reduced risk for diabetes or IGT but was not completely risk-free. The risk of diabetes or IGT if S319 was negative (~40%) was markedly lower than the risk inferred from simple age-specific prevalence data but was not 0. This was not surprising because we had previously shown that many diabetic Oji-Cree were homozygous for the wild type *HNF1A* G319/G319 genotype (2–5). Therefore, *HNF1A* S319 was not the only predisposing factor for diabetes in the Oji-Cree, and its absence did not preclude the development of diabetes. Once the other factors for diabetes susceptibility in the Oji-Cree are determined, they may be useful diagnostic adjuncts to the *HNF1A* genotype.

The reports of applications of molecular genetic analyses in clinical diagnosis are sometimes deficient regarding the standards from the underlying principles of clinical epidemiology (10). The suggested standards include reproducibility of the test, objectivity of test interpretation, delineation of the case group, adequacy of spectrum in the case group, delineation of the comparison group, adequacy of the comparison group, and quantitative summary of results (10). Each standard has been satisfied in this analysis of *HNF1A* genotype and diabetes in Oji-Cree ≥50 years of age.

First, the genotyping method was standardized against DNA sequence–proven reference samples. Furthermore, complete concordance of the results occurred with blinding of the technologist and interpreter of genotypes from 80 samples assayed on 2 separate days. This satisfied the standards of reproducibility and objectivity. Second, the cases and control subjects included nonrelated Oji-Cree ≥50 years of age only, and the clinical diagnosis was uniform and based on strict American Diabetes Association criteria (6,7). This satisfied the standards of delineation of cases and control subjects. Subjects with type 2 diabetes or IGT were included in the case group. This satisfied the standard of adequacy of disease spectrum in cases. Furthermore, the findings were the same when a more narrow definition of disease was used by excluding subjects with IGT, which suggests that the results were not susceptible to spectrum bias (10). The control subjects were all members of the same ethnic group, were matched regarding minimum age, and were clearly nondiabetic from the point of view of glycemia (Table 1), which satisfied the standard of adequacy of control subjects. Finally, our results were expressed using traditional measures of diagnostic test performance as shown in Table 2. Thus, the results presented herein at least match, and probably exceed, standards of clinical epidemiology used in most current molecular genetics research.

Table 2—*Performance characteristics of* HNF1A *G319S genotype in Oji-Cree ≥50 years of age*

HNF1A S319 genotype	Diabetes or IGT		
	Present	Absent	Total
Positive	19	1	20
	(a)	(b)	(a + b)
Negative	44	32	76
	(c)	(d)	(c + d)
Total	63	33	96
	(a + c)	(b + d)	(a + b + c +d)

Prevalence: (a + c)/(a + b + c + d) = 63/96 = 65.7%; sensitivity: a/(a + c) = 19/63 = 30.1% (18.8–41.4); specificity = d/(b + d) = 32/33 = 97.0% (91.2–99.9); positive predictive value: a/(a + b) = 19/20 = 95.0% (85.4–99.9); negative predictive value: d/(c +d) = 32/76 = 42.1% (31.0–53.2); likelihood ratio of a positive test result: sensitivity/(1 − specificity) = [a/(a +c)]/[b/(b +d)] = 10.0 (2.1–414); likelihood ratio of a negative test result: (1 − sensitivity)/specificity = [c/(a + c)]/[d/(b + d)] = 0.720 (0.643–0.813); probability(affected) after a positive test (from Baye's equation): 97.2% (93.9–99.9); probability(affected) after a negative test (from Baye's equation): 42.2% (32.3–52.1).

The results from the Oji-Cree emphasize that population-specific susceptibility alleles likely exist for complex diseases such as diabetes. The *HNF1A* G319S genotyping assay would have no clinical utility in populations other than the Oji-Cree because this mutation is absent in all other ethnic groups (4,5). Thus, the development of a panel of genetic tests to predict the risk of diabetes will need to account for ethnicity, at least in the case of certain susceptibility alleles such as S319.

Because DNA is invariant from birth, in appropriate instances, the *HNF1A* G319S genotype likely can presymptomatically identify a subject who would be virtually certain of having diabetes or IGT by 50 years of age. Having the ability to identify susceptible individuals at a young age could enable the design of a protocol or intervention strategy to delay or prevent the onset of the disease. The very high specificity and positive predictive value for S319 also means that this information may be used to help improve motivation or compliance regarding traditional intervention strategies such as diet and exercise.

Thus, the *HNF1A* genotype appears to be the most specific genetic test yet reported for the prediction of a common multifactorial disease when applying present-day standards of clinical epidemiology in molecular genetics. The results also indicate that a genetic marker can have clinical utility in the absence of a complete understanding of the basic science underlying its association with disease. We strongly suspect that the *HNF1A* S319 allele contributes to the more typical picture of type 2 diabetes that is associated with obesity, hyperinsulinemia, and insulin resistance that is seen in the Oji-Cree. However, even in the absence of insight into the causative mechanism, the genotype appears to have diagnostic utility that could help identify Oji-Cree subjects who are at risk many years before the onset of diabetes or IGT.

Acknowledgments — This work was supported by grants from the National Institutes of Health (DK-44597-01), the Ontario Ministry of Health (04307), the Medical Research Council of Canada (MT-13430), the Canadian Diabetes Association (in honor of Rheta Maude Gilbert), and the Blackburn Group. R.A.H. is a Career Investigator of the Heart and Stroke Foundation of Ontario. S.B.H. is a Career Investigator of the Ontario Ministry of Health. A.J.G.H. is supported by Health Canada through a National Health Research and Development Program Research Training Award.

We acknowledge the chief and council of the community of Sandy Lake, the Sandy Lake community surveyors, the Sandy Lake nurses, the staff of the University of Toronto Sioux Lookout Program, and the Department of Clinical Epidemiology of the Samuel Lunenfeld Research Institute.

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