Genes and Environment in Type 2 Diabetes and Atherosclerosis in Aboriginal Canadians

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The incidence of coronary heart disease (CHD) among aboriginal people in northern Ontario has tripled over the past 20 years. This is inextricably linked to the remarkably high prevalence of type 2 diabetes in these native communities. Approximately 40% of the Oji-Cree of northern Ontario have typical obesity-related type 2 diabetes, which represents a drastic increase from virtually unreportable levels 50 years ago. The Oji-Cree have a private mutation in the *HNF1A* gene, namely G319S, which is absent from other ethnic groups and aboriginal populations. The most compelling reasons that *HNF1A* S319 is a diabetes-susceptibility allele are its consistent statistical association with the presence and severity of diabetes. Also, *HNF1A* S319 has specificity and positive predictive values of 97% and 95%, respectively, for the development of diabetes in the Oji-Cree by 50 years of age. This makes the *HNF1A* G319S genotype the most specific predictive genetic test for diabetes in any human population. *HNF1A* S319 has all the attributes of a thrifty allele in the Oji-Cree. It is possible that the recent increase in CHD in the aboriginal people of northern Ontario is the result of the expression of diabetes susceptibility due to *HNF1A* S319 as a consequence of rapid changes in environment and lifestyle.

Introduction

Similar to other aboriginal groups around the world, the rapid cultural changes currently faced by aboriginal Canadian populations are anticipated to accelerate the development of "diseases of westernization," such as atherosclerosis and diabetes [1–4]. Proof of this tendency came through the recent documentation that the incidence of coronary heart disease (CHD) in the aboriginal population in northern Ontario has tripled over the last 20 years (Fig. 1), and is now fourfold more prevalent than in the general population of Ontario [5]. A factor that is inextricably linked to this CHD epidemic is the remarkably high prevalence of type 2 diabetes in native communities [6]. For example, the Oji-Cree people of northern Ontario have a prevalence of type 2 diabetes of approximately 40%, which is the third highest for any population in the world [7], and is more than six times higher than the prevalence of type 2 diabetes in the general Canadian population. It is somewhat remarkable that diabetes was virtually unknown as a medical diagnosis in these people as little as 60 years ago [6,7]. The rapid change in lifestyle is seen to be of primary importance in the recent diabetes epidemic. However, the remarkably high population prevalence of type 2 diabetes suggests that there may be some endogenous susceptibility among the Oji-Cree.

The Oji-Cree of Sandy Lake

The search for genetic determinants of type 2 diabetes and related metabolic traits in the Oji-Cree of Northern Ontario began in 1997. The Sandy Lake reserve is located at the 55th parallel of latitude, just east of the Manitoba border (Fig. 2). The geographically isolated reserve is below the timberline and within the subarctic boreal forest. It is accessible only by air during most of the year. Historically, the ancestors of the contemporary residents of this region lived a nomadic, hunting-gathering subsistence typical of other Algonkian-speaking peoples of the northeastern subarctic. The present language has attributes of both Ojibwe and Cree, and the community members define themselves as Oji-Cree.

Since the introduction of the reservation and residential school systems, the lifestyle of these people has changed drastically, from one that had been characterized by a high level of physical activity to one that is presently very sedentary. Most contemporary members of the reserve live in winterized cottages, with indoor plumbing and electricity. Many community members have snowmobiles, automobiles, and motorized boats. Most residents have televisions. The main food source for the community has changed from wildlife with roots and berries to animal fat-rich processed foods, which are supplied by a company store.

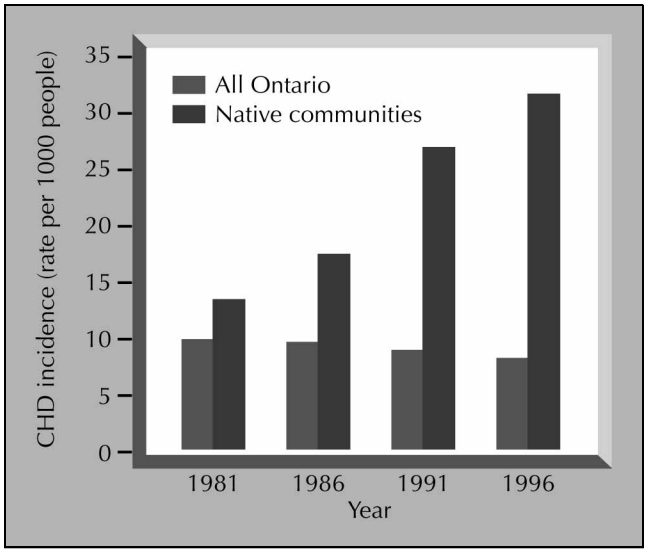

Figure 1. Coronary heart disease (CHD) events per 1000 people in Ontario. (*Adapted from* Shah *et al*. [5]).

As part of the studies of the genetic determinants of type 2 diabetes, medical histories were obtained and physical examinations were performed on 728 volunteers, aged 10 to 80 years. The represented about 72% of the community; the high participation rate reflected the interest and degree of importance that the community placed on the problem of diabetes. Fasting blood samples were obtained for measurement of biochemical traits and for determination of genotypes [7,8]. Descriptions of the clinical, biochemical, and genetic attributes of these patients have been published [7,8].

Searching for a Type 2 Diabetes Mutation in the Oji-Cree

The most commonly used approach to detect chromosomal regions harboring susceptibility genes for type 2 diabetes can be referred to as "orthodox genetic mapping" or "positional cloning." In this approach, family units with affected members, such as affected sibling pairs, are evaluated with informative, evenly spaced markers from across the genome to identify chromosomal regions that consistently segregate with disease. This approach was used to find linked loci for type 2 diabetes in Sandy Lake; specifically, a genome-wide scan using 190 random genomic markers was carried out in 49 Oji-Cree sibling pairs affected with type 2 diabetes [8].

The positional cloning experiments indicated that four genomic markers, one each on chromosomes 6, 8, 16, and 22, showed suggestive linkage and association with type 2 diabetes in the Oji-Cree [8]. None of these markers corresponded to any chromosomal region that had been previously linked with type 2 diabetes in other populations. The results suggested that several genetic loci conferred susceptibility to type 2 diabetes in this study sample. The traditional approach to follow-up on these preliminary leads is to increase the density of the markers within these linked and associated regions, and also to increase the number of patients in the study.

In parallel experiments, an alternative approach was used to search for diabetes-susceptibility genes in the Sandy Lake Oji-Cree, namely "candidate gene sequencing." There were at least 20 candidate genes for type 2 diabetes based upon the known role of their products in carbohydrate or insulin metabolism. Genes were also candidates because they harbored mutations that had previously been found to underlie rare familial diabetes syndromes, such as maturity onset diabetes of the young (MODY) [9]. In fact, the typical clinical picture of MODY (*ie*, lean adults with deficient insulin secretion) was the opposite of the type 2 diabetes in Sandy Lake (*ie*, obese adults and adolescents with hyperinsulinemia). Although Oji-Cree type 2 diabetes did not resemble MODY, it was felt to be desirable to rule out mutations in the *MODY1*, *MODY2*, *MODY3* and MODY4 genes (*HNF4*, *GK*, *HNF1A*, and *IPF4*, respectively) as being associated with type 2 diabetes. This approach lead to the discovery of numerous single nucleotide polymorphisms (SNPs) in several candidate genes in Sandy Lake. The initial criteria for considering a new SNP to be a disease mutation were statistical, such an increased frequency in diabetic patients compared with nondiabetic control patients.

Discovery of *HNF1A* G319S

In August 1998, the candidate gene approach lead to the MODY3 gene (Online Mendelian Inheritance in Man [OMIM; http://www.ncbi.nlm.nih.gov/Omim/] 600496), namely *HNF1A* or *TCF1* (OMIM 142410). *HNF1A* encodes hepatic nuclear factor (HNF)-1α, a transcriptional activator of many genes, including albumin, α-1-antitrypsin, and fibrinogen [9]. *HNF1A* is part of the homeobox gene family, has been mapped to chromosome 12q24, and is expressed predominantly in the liver and kidneys [9]. Interestingly, the genome scan had indicated that *HNF1A* was not a strong positional candidate for diabetes [8]. However, the rationale to sequence *HNF1A* was to rule this gene out as a possible genetic determinant, not because the Oji-Cree had MODY.

Genomic DNA-amplification primers were developed for the 10 exons, promoter, and 3'-untranslated regions of the *HNF1A* gene. Genomic DNA from three diabetic Oji-Cree patients with body mass index (BMI) of less than 30 kg/m^2 and aged less than 50 years, each of whom had type 2 diabetes for more than 10 years, were screened. Genomic DNA from three nondiabetic Oji-Cree patients, each of whom had BMI greater than 30 kg/m^2 and aged more than 60 years, was also screened. Ten *HNF1A* SNPs were found using this strategy (Table 1). Of these, five were not in coding sequences and two were silent at the amino acid level, meaning that they were unlikely to have functional

Figure 2. Map of North America showing the location of the Sandy Lake reserve.

Table 1. HNF1A single nucleotide polymorphisms detected in Sandy Lake Oji-Cree with type 2 diabetes

Exon	Position	DNA change	Amino acid change*
1	Codon 17	CTC-->CTG	Silent (Leu17)
1	Codon 27	ATC-->CTC	Ile27>Leu
4	Codon 319	GGT-->AGT	Gly319>Ser
7	Codon 459	CTG-->TTG	Silent (Leu459)
7	Codon 487	AAC-->AGC	Asn487>Ser

*Nucleotide positions of noncoding sequence changes were 734G>A, 2504C>T, 4969A>G, 5411T>C, and 6201T>C.

relevance. Three other SNPs were missense alterations affecting the amino acid sequence, of which two were polymorphic in both cases and control patients. Only one missense mutation, *HNF1A* G319S, was present exclusively in the screened cases [10•]. *HNF1A* S319/S319 homozygous patients and S319/G319 heterozygous patients, respectively, had significant odds ratios for type 2 diabetes of 4.0 and 2.0, respectively, compared with G319/G319 homozygous patients. Furthermore, *HNF1A* G319S was absent from more than 1000 alleles taken from patients representing six other ethnic groups, suggesting that it was a private SNP for Oji-Cree [10•]. There are many other examples of ethnic group-specific disease mutations, and all results to date would indicate that *HNF1A* G319S in aboriginal type 2 diabetes is one such mutation. Another well-defined aboriginal group with a high diabetes prevalence, the Pima of Arizona, had no mutation in *HNF1A* [11].

The most compelling reasons that *HNF1A* G319S was a disease mutation were its consistent statistical association with the disease itself [10•], with the severity of the disease and its superior ability to act as a predictive test for disease susceptibility [12•]. The age-specific diagnostic performance of *HNF1A* S319 for diabetes in Oji-Cree is shown in Figure 3. For Oji-Cree patients aged 50 years and above, S319 had specificity and positive predictive value of 97% and 95%, respectively [12•]. This makes it the most specific genetic test for type 2 diabetes yet described in any population [12•]. The sensitivity and negative predictive value of S319 for diabetes were somewhat lower, which is consistent with the existence of other etiologic factors for diabetes in these people [12•].

The cellular basis for the development of type 2 diabetes in Oji-Cree patients with *HNF1A* S319 is not yet established; however, some promising hints at loss of in vitro function have been found (Triggs-Raine, unpublished data). Most previously reported mutations in *HNF1A* have been observed with MODY3, which typically occurs in lean, younger adults and is associated with defective

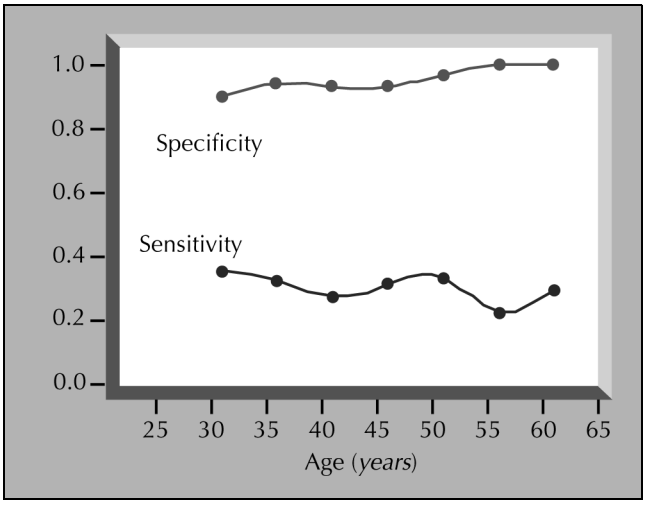

Figure 3. Diagnostic performance of *HNF1A* G319S in diabetes prediction according to age. The figure shows a very high diagnostic specificity of *HNF1A* G319S for diabetes across all ages. The relatively lower sensitivity reflects the presence of other causative factors for diabetes, some of which might be genetic.

insulin secretion from pancreatic β-cells. As mentioned, type 2 diabetes in the Oji-Cree appears to be a typical form of the phenotype, associated with obesity and hyperinsulinemia. This suggests that *HNF1A* has pleiotropic effects on insulin and carbohydrate metabolism, with some mutations leading to an insulin-secretion defect producing MODY3, but with other defects, such as S319, contributing to a more typical obesity-related insulin-resistant type 2 diabetes phenotype, as seen in the Oji-Cree.

Disparity Between Association and Linkage Analysis

It was notable that the genome scan did not identify the region harboring *HNF1A* as being linked with diabetes [8]. Also, the *HNF1A* G319S mutation, when used directly in sibling-pair linkage analysis, was not significantly linked with diabetes [13]. However, *HNF1A* G319S was very strongly associated with diabetes, predicted the clinical severity of diabetes, and performed well as a diagnostic predictive test for diabetes in the Oji-Cree [10•,12•]. Despite the failure of linkage analysis to identify *HNF1A* as a determinant of type 2 diabetes, G319S has an important pathogenic role in Oji-Cree diabetes, based upon the highly suggestive association studies. The probable etiologic heterogeneity of type 2 diabetes in the Oji-Cree created a situation in which association analysis was much more sensitive to detect a relationship between *HNF1A* S319 and diabetes than was linkage analysis [13]. The sensitivity of linkage analysis will probably be even more limited in study samples that have a greater complexity of genetic background or disease etiology. Thus, the absence of significant linkage does not always mean that a genomic variant is not an important determinant of a complex disease. The threshold to begin sequencing of candidate genes could be very low indeed, especially because the human genome project will very soon result in the positioning of most genes and expressed-sequence tags at specific chromosomal sites.

HNF1A and Youth-onset Type 2 Diabetes in the Oji-Cree

A new form of diabetes, namely youth-onset type 2 diabetes (Y2DM) has emerged as a significant public health concern in several ethnic groups around the world [14–19]. Y2DM is more common in some aboriginal communities than is type 1 diabetes in the general pediatric population [14–19]. Aboriginal Canadians with Y2DM are typically nonketotic, obese female adolescents or young adults [14–19]. The phenotype is typical type 2 diabetes, associated with obesity and hyperinsulinemia. It is distinct from MODY [14–19]. Most clinicians have assumed that the Y2DM phenotype has a component of genetic susceptibility [14–19]. The relationship between *HNF1A* S319 and Y2DM was studied in 317 adolescent Oji-Cree from Sandy Lake [20]. The frequency of *HNF1A* S319 was 0.343 and 0.086 in patients with Y2DM and normoglycemic patients, respectively (P=0.00005). *HNF1A* S319/S319 homozygous patients and S319/G319 heterozygous patients had significant odds ratios for Y2DM of 119 and 2.9, respectively, compared with patients with the *HNF1A* G319/G319 genotype. Thus, *HNF1A* S319 was strongly associated with Y2DM in the Oji-Cree, and can be considered to be a contributing factor for this remarkable phenotype [20].

Other Candidate Genes for Type 2 Diabetes in the Oji-Cree

Although *HNF1A* G319S was strongly associated with type 2 diabetes, the majority of diabetic patients did not have this variant, suggesting that there might have been other genetic determinants of diabetes susceptibility. In the course of sequencing other candidate genes in diabetic patients who were homozygous for *HNF1A* G319/G319, some of them were found to have the *PPARG* A12 variant [21]. After genotyping *PPARG* in all adults, *PPARG* A12 was strongly associated with type 2 diabetes [21]. This directly conflicts with another report in white patients, suggesting that *PPARG* A12 protects against type 2 diabetes [22], indicating that disease associations can vary between studies and populations. In the Oji-Cree, the associations of both *HNF1A* S319 and *PPARG* A12 with type 2 diabetes confirms the etiologic complexity of the phenotype, because at least two genes were involved in determining susceptibility to the disease in these people [21]. In contrast, the hereditary hemochromatosis *HFE* C282Y mutation was not associated with the type 2 diabetes seen in the Oji-Cree [23].

Conclusions

The high prevalence of both CHD and diabetes in Sandy Lake appears to be related to genetic and environmental factors. The *HNF1A* G319S diabetes mutation has all the attributes of a thrifty allele in the Oji-Cree. Carrying this mutation was associated with a greater than 95% risk of developing diabetes by the age of 50 years. It is very likely that genetic susceptibility to type 2 diabetes, mediated in part by *HNF1A* S319 in the context of the rapid changes in environment and lifestyle, underlies the diabetes epidemic [24].

Two generations ago, diabetes was a virtually unknown diagnosis among the Oji-Cree, according to clinicians who worked at Sandy Lake. Why is diabetes such a problem now? The answer surely lies in the changed environment. The community has had a massive shift in body mass index, which has been due to decreased activity and to increased intake of total calories, particularly saturated fat and sugar. The superimposition of this environmental change upon the genetic predisposition to diabetes has resulted in the observed epidemic rise in disease prevalence.

Is *HNF1A* S319 an allele of the thrifty genotype? According to the thrifty genotype hypothesis [25], native North Americans evolved a complement of genetic variants that would allow survival in a harsh environment characterized by marked excursions in energy intake, sometimes described as "feast or famine." The hypothesis proposed that variant gene products both efficiently utilized caloric energy for cellular metabolism and enabled the easy storage of excess energy for future use [25].

Although such energy metabolism might have been advantageous to natives in the context of a feast or famine pattern of subsistence, recent cultural changes have created a context of "feast and more feast" for some native groups. Thus, the genetic propensity to more efficiently utilize caloric energy and to easily store the excess, which were advantageous in the context of feast or famine, would be deleterious in the context of feast and more feast. *HNF1A* S319 was probably neutral in the past, but is now strikingly associated with a deleterious phenotype in the present day context of obesity, inactivity, and a high-saturated fat diet in the Sandy Lake natives.

Assuming that the allele and genotype frequencies in Sandy Lake reflect those of the approximately 16,000 aboriginal residents of northern Ontario, there may be 200 S319/S319 homozygous people and 3000 S319/G319 heterozygous people. Therefore, the S319 allele may contribute considerably to morbidity related to diabetes and its complications, both in absolute and relative terms, amongst these aboriginal people.

What can be done? Even before knowledge of the *HNF1A* S319 allele, efforts were being made to change the Oji-Cree lifestyle. The idea was to balance energy intake and expenditure in a manner that would faithfully match the ancestral Oji-Cree lifestyle. Education efforts have been most intensively focused on school-aged children. Although it is unrealistic to expect that today's Oji-Cree would choose to reverse history and live again as their ancestors did, it might be possible to creatively reconstitute aspects of the ancestral lifestyle. For example, advice may include an increase in daily activity and using contemporary dietary components to mirror a more traditional diet. Such preventive measures would be even more essential in carriers of the mutant allele. This concept could form the basis of a prospective study to evaluate the effectiveness of lifestyle modification that also takes advantage of genetic information.

Acknowledgments

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