

Genes, environment and Oji-Cree type 2 diabetes

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Abstract

The prevalence of type 2 diabetes in Canadian Oji-Cree is among the highest in the world. Our research has uncovered genetic determinants of Oji-Cree type 2 diabetes and related metabolic traits. The most important genetic discovery by far was the private G319S mutation in transcription factor HNF-1 α , encoded by the *HNF1A* gene. *HNF1A* G319S was discovered by candidate gene sequencing and would have been missed using the currently favored strategy of genome-wide scanning. G319S was associated with increased odds of having type 2 diabetes across the whole study sample and in all subgroups, including adolescent Oji-Cree. Furthermore, G319S had specificity and positive predictive value of 97% and 95%, respectively, for developing type 2 diabetes by age 50. The protein bearing the G319S mutation had impaired function *in vitro*. Sigmoidal modeling showed that each dose of the G319S allele accelerated the median age of diabetes onset by about 7 yr. This approach also showed that environment more strongly accelerated the median age-of-onset of Oji-Cree diabetes onset than did G319S, which could have implications for intervention strategies to reduce the burden of this epidemic. There is also evidence for genetic determination of related metabolic traits in the Oji-Cree. © 2003 The Canadian Society of Clinical Chemists. All rights reserved.

Keywords: Type 2 diabetes; Cardiovascular disease; Transcription factors; Risk factors; HNF1A; Aboriginal people

1. Introduction

We have been studying the determinants of diabetes in the Oji-Cree of Northwestern Ontario and Manitoba. The prevalence of type 2 diabetes and impaired glucose tolerance (IGT) in adult Ontario Oji-Cree is ~40%, which is among the highest of any subpopulation in the world and is five times higher than in the general Canadian population [1]. The complications of Oji-Cree type 2 diabetes are anticipated to soon extract a substantial social and economic toll. The high prevalence of diabetes will also challenge health care paradigms, because the ~30,000 Oji-Cree who live on reserves in this region are dispersed across a wide, remote and harsh northern locale. Intervention strategies to prevent or delay the onset of type 2 diabetes and its complications, such as coronary heart disease [2], would be especially important under these circumstances. It is possible that understanding factors involved in the development of type 2 diabetes might help to direct preventive strategies.

One factor is the recent change in the Oji-Cree lifestyle, which has been characterized by an increased intake of dietary fat and decreased level of activity, which have led in turn to obesity and diabetes. Environmental risk factors, such diet, fitness level and physical activity, are significantly associated with Oji-Cree type 2 diabetes [3–6]. However, the very high prevalence of type 2 diabetes in the Oji-Cree suggests that these people may also harbor genetic predisposition.

2. Searching for a type 2 diabetes mutation

We began the search for susceptibility genes for Oji-Cree type 2 diabetes in 1996, with the collaboration of the band council and community members of Sandy Lake, Ontario. Basic demographic attributes of this study sample have been reported [1]. We used two complementary experimental strategies concurrently: positional cloning by linkage analysis and focused candidate gene sequencing. The first of these strategies is the most commonly used approach to detect chromosomal regions harboring disease susceptibility genes. In the positional cloning experiments, sibling pairs

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affected with diabetes were genotyped using a set of DNA markers from across the genome to find chromosomal regions that consistently co-segregated with the diabetes phenotype. Four markers, one each on chromosomes 6, 8, 16, and 22, showed suggestive statistical linkage and association with Oji-Cree type 2 diabetes [7]. None of these corresponded to chromosomal regions linked with type 2 diabetes in other populations. The results suggested that several genetic loci conferred susceptibility to Oji-Cree type 2 diabetes.

In experiments performed in parallel, we used an alternative approach called focused candidate gene sequencing. There are at least 20 candidate genes for type 2 diabetes, based upon the role of the gene products in carbohydrate or insulin metabolism [8]. Genes were also candidates because they harboured mutations that had previously been found to underlie rare familial diabetes syndromes, such as maturity-onset-diabetes-of-the-young (MODY). Although Oji-Cree type 2 diabetes did not resemble MODY, we still felt that it would be desirable to rule out mutations in the MODY1, MODY2, MODY3 and MODY4 genes (with names like *HNF4*, *GK*, *HNF1A* and *IPF4*), and at least would reflect scientific due diligence. Using this approach, we discovered numerous single nucleotide polymorphisms (SNPs) in several candidate genes. However, none of these had the attributes of a possible type 2 diabetes mutation, based on classical criteria such as statistical association of the SNP with diabetes compared to controls.

In August 1998, the candidate gene strategy led to the MODY3 gene, namely *HNF1A* encoding hepatocyte nuclear factor (HNF)-1 α , a transcriptional activator of many genes including insulin, albumin, α -1-antitrypsin, and fibrinogen [8]. *HNF1A* is part of the homeobox gene family of transcription factors, has been mapped to chromosome 12q24 and is expressed predominantly in liver and kidney, but also pancreas. We developed reagents to examine the primary DNA sequence of the *HNF1A* gene and then screened genomic DNA from three Oji-Cree diabetic subjects and from nondiabetic controls. We identified ten SNPs using this strategy, of which five were not in coding sequences and two were silent at the amino acid level. Three affected the amino acid sequence, but two of these were seen in both cases and controls. Only one SNP, *HNF1A* G319S was present exclusively in the screened cases [9,10].

Next, we had to construct a case in favor of the argument that *HNF1A* G319S was associated with Oji-Cree type 2 diabetes. An accepted criterion for the etiologic importance of a single amino acid substitution in human disease is the degree of evolutionary conservation of the normal residue. *HNF1A* G319S occurs within the proline II-rich domain of the trans-activation site of HNF-1 α , and alters a normal glycine residue that has been conserved in all species for which DNA sequence data are available, supporting its importance in normal protein function [9].

Another criterion for causation is statistical. We showed that G319S was absent from 990 alleles taken from nondi-

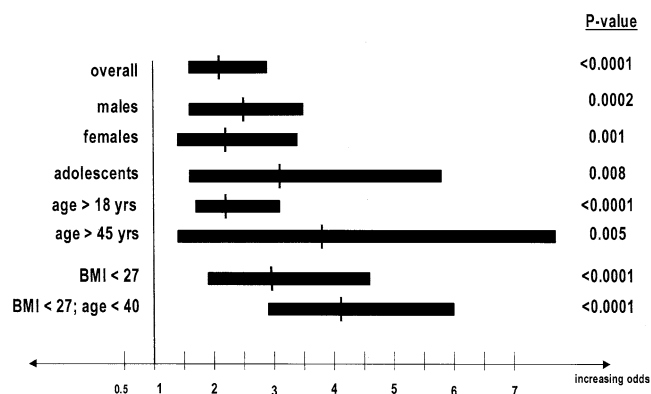


Fig. 1. Heterozygote odds ratios for Oji-Cree type 2 diabetes. Odds ratios and 95% confidence intervals are shown for indicated subgroups according to age, gender and body mass index (BMI). P-values are shown.

abetic subjects from six ethnic groups, suggesting that G319S was private to Oji-Cree [9]. We showed that the G319S allele was more prevalent in diabetic than nondiabetic Oji-Cree (0.21 vs. 0.087, $p = 0.000001$) [9], and had a dosage-relationship with the odds of type 2 diabetes. Compared with G319/G319 homozygotes, S319/S319 homozygotes had an odds ratio of 4.0 (95% CI 2.7–6.0) and S319/G319 heterozygotes had an odds ratio of 2.0 (95% CI 1.4–2.7). Indeed, *HNF1A* G319S heterozygosity was associated with increased odds of having Oji-Cree type diabetes across the whole Sandy Lake sample, and in various subgroups divided by age, gender and body mass (Fig. 1.)

Of the subgroups examined, the association was perhaps strongest in adolescent Oji-Cree: compared with G319/G319 homozygotes, S319/S319 homozygotes had an odds ratio of 120 (95% CI 6–2300) and S319/G319 heterozygotes had an odds ratio of 2.9 (95% CI 1.9–5.8) [11]. Furthermore, *HNF1A* G319S had specificity and positive predictive value of 97% and 95%, respectively, for developing type 2 diabetes by age 50, making it the most specific genetic test yet reported for diabetes in a human population [12]. However, ~60% of Oji-Cree subjects with type 2 diabetes were homozygous for wild type *HNF1A*. This suggests that other genetic and/or environmental factors must be involved in the development of Oji-Cree type 2 diabetes.

Finally, we provided evidence at the molecular level that the HNF-1 α G319S-containing protein was defective. HNF-1 α has three main functional domains: a) a dimerization domain; b) a DNA-binding domain; and c) a transactivation domain. Careful *in vitro* studies showed that the mutant was secreted in normal quantities and was not impaired with respect to dimerization or DNA-binding functions. However, the G319S mutant had a significantly diminished ability to transactivate gene expression [13], although the loss of activity was partial, in contrast to *HNF1A* mutations in MODY, which imparted a total loss of function [8].

These observations confirmed that *HNF1A* mutations could cause either of two very distinct phenotypes: Oji-Cree

type 2 diabetes or MODY3. Subjects with Oji-Cree type 2 diabetes are obese, with high plasma insulin and C-peptide concentrations, indicating insulin resistance [13]. In contrast, subjects with MODY3 are lean, with low plasma insulin due to defective secretion [8]. The *in vitro* analysis of transactivation function hints at the possible basis for this *in vivo* difference. The *HNF1A* G319S mutation resulted in diminished, but not absent, residual transcriptional activity of the HNF-1 α protein. Thus, Oji-Cree with the G319S mutation would have relatively lower, but not absent, *in vivo* expression of HNF-1 α -dependent genes, including insulin. The partial loss-of-function of *HNF1A* G319S would require an additional stress to create a diabetes phenotype. Unfortunately, the changes in the lifestyle of the Oji-Cree people over the course of the 20th century have provided more than an adequate stress on the molecular deficiency imparted by *HNF1A* G319S. In particular, the stress of obesity-induced insulin resistance exposed the partial defect in *HNF1A* G319S carriers, causing disease expression [13].

3. Regarding the disparity between candidate gene and positional cloning approaches

The genome scan did not identify the region harboring *HNF1A* as being linked with diabetes [7]. Also, the *HNF1A* mutation, when used directly in sib-pair linkage analysis, was not linked with diabetes [14]. However, *HNF1A* G319S is clearly a disease susceptibility mutation [13]. Why did linkage analysis fail to identify *HNF1A* as a determinant of Oji-Cree type 2 diabetes? The probable etiologic heterogeneity of Oji-Cree type 2 diabetes created a situation in which association analysis was much more sensitive to detect a relationship between *HNF1A* S319 and diabetes than was linkage analysis [14]. The effectiveness of linkage analysis will probably be limited in study samples that have an even greater complexity of genetic background and/or disease etiology. Thus, the absence of linkage does not always mean that a genomic variant is not an important determinant of a complex disease. Furthermore, our experience confirms the value of using several complementary strategies to identify susceptibility genes for a complex disease. The experience with candidate gene sequencing would indicate that the threshold to begin DNA sequencing could be very low, especially with the availability of fairly complete human genome sequence.

4. Using nonlinear functions to understand diabetes onset

We were interested in determining whether genes or environment would be a more important determinant of diabetes onset in the Oji-Cree. For analysis of the dynamics of diabetes onset, all subjects with physician-diagnosed type 2 diabetes were studied. The mean (\pm standard deviation)

present age of these subjects was 44.4 ± 15.2 yr, and the mean age-of-onset of type 2 diabetes was 39.8 ± 12.7 yr. The age-of-onset was plotted on the abscissa against the cumulative proportion of subjects with type 2 diabetes on the ordinate (Fig. 2A). Since this plot appeared sigmoidal, a nonlinear regression analysis, using SAAMII modeling software (SAAM Institute, Seattle, Washington) was performed to determine whether the relationship could be explained by a sigmoidal function of the general form:

$$y = x^b / (x^b + a^b) \quad (1)$$

Parameter a is equivalent to t_{50} , or the age at which half of subjects had become diabetic. Parameter b, also called the Hill constant, is a direct index of the sigmoidicity of the function that describes this relationship. To determine whether there was a relationship between the age-of-onset of type 2 diabetes and the *HNF1A* genotype, subjects were stratified by genotype (Fig. 2B). These curves were also satisfactorily modeled using sigmoidal functions (each $r^2 > 0.9$ and $p < 0.00001$), as shown in Table 1. The defining parameters of these curves were compared. For parameter a, or t_{50} , all three pairwise between-genotype comparisons indicated significant differences (each $p < 0.00001$). The findings were consistent with the gene-dosage relationship suggested by the linear statistics in the initial population survey [9]. Subjects without G319S developed diabetes with median age ~ 41 yr. One dose of the G319S accelerated the median disease by ~ 7 yr and a second dose of the G319S further accelerated disease onset by ~ 7 yr. The stresses of reserve life that have resulted in the type 2 diabetes epidemic take their toll on all individuals. However, subjects with *HNF1A* G319S cannot mount a satisfactory insulin secretory response. The consequence of the molecular defect can be seen as a clinically relevant difference in age-of-onset. The earlier onset of diabetes prolongs the duration of disease in carriers, resulting in more time for the development of diabetes complications.

Next, to determine whether the dynamics of conversion from health to disease differed according to the era in which the individuals were born, subjects were stratified by year of birth before or after 1935. Similar plotting and nonlinear regression analyses were performed for the two age-defined cohorts (Fig. 2C). These two curves were also both satisfactorily modeled using sigmoidal functions (each $r^2 > 0.9$ and $p < 0.00001$), as shown in Table 1. The defining parameters of each curve were compared. Parameter a, or t_{50} , was different with $p < 10^{-21}$. This indicated that subjects born in 1935 and later had a different pattern of disease onset compared with those born before 1935. Despite variation in medical practice over this time period, the differences in the curve parameters likely reflect a true difference in age-of-onset. This is because there was almost no overlap between the two curves: no subject born before 1935 was diagnosed with diabetes before the fifth decade of life, whereas the majority of subjects born since 1935 were

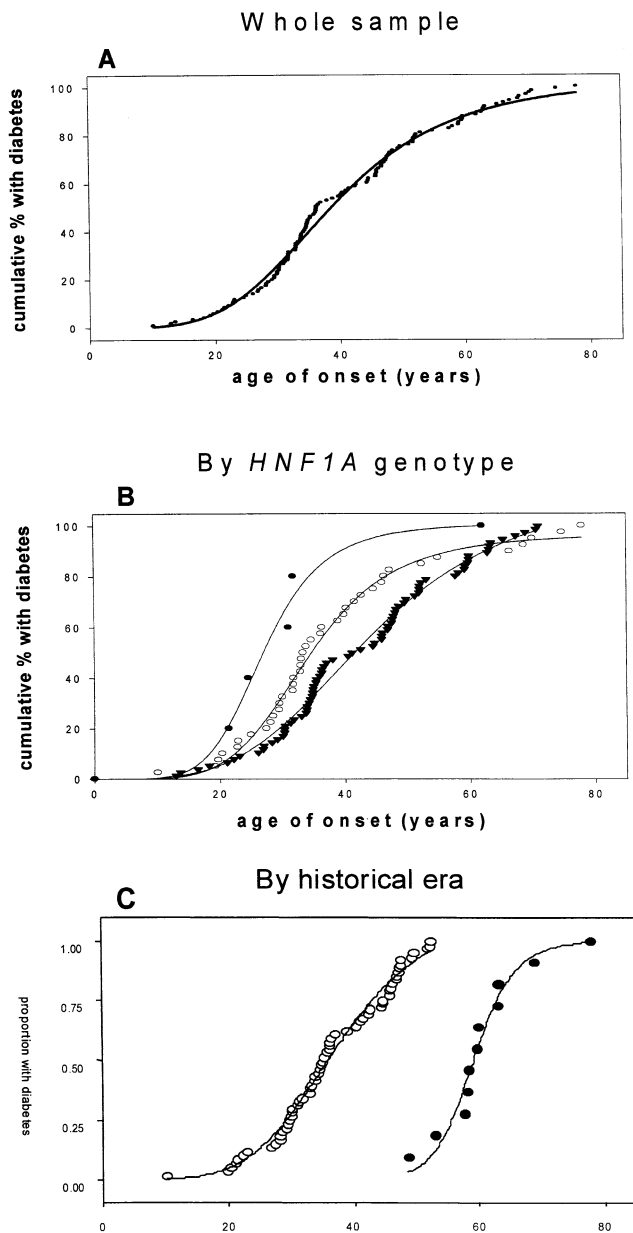


Fig. 2. Dynamics of conversion to diabetes in the Sandy Lake Oji-Cree. (A) Cumulative proportion of subjects from Sandy Lake with type 2 diabetes plotted against age-of-onset. Solid line represents regression line with parameters as shown in Table 1. (B) Cumulative proportion of subjects from Sandy Lake with type 2 diabetes plotted against age-of-onset. Solid circles are subjects with *HNF1A* S319/S319 genotype, open circles are subjects with S319/G319 genotype and triangles are subjects with G319/G319 genotype. Solid lines represent regression lines with parameters as shown in Table 1. (C) Cumulative proportion of subjects from Sandy Lake with type 2 diabetes plotted against age-of-onset. Solid circles are subjects born before 1935 and open circles are subjects born in 1935 and later. Solid lines represent regression lines with parameters as shown in Table 1. Fig. 2A and 2B reproduced from "HNF-1 G319S, a transactivation-deficient mutant, is associated with altered dynamics of diabetes onset in an Oji-Cree community" by Barbara L. Triggs-Raine, Robert D. Kirkpatrick, Sherrie L. Kelly, Lisa D. Norquay, Peter A. Cattini, Kazuya Yamagata, Anthony J. G. Hanley, Bernard Zinman, Stewart B. Harris, P. Hugh Barrett, and Robert A. Hegele; Published in *Proc Natl Acad Sci USA* 99: 4614 to 4619, 2002. Triggs-Raine et al. Copyright 2002 National Academy of Sciences USA and used with permission

diagnosed with diabetes before the third decade of life. The results were similar when 1945 was used as the threshold for age-of-onset (data not shown).

Sigmoidal relationships are common in biology, and denote a changing tendency for an outcome depending upon contextual changes and accumulation of outcome determinants. Pharmacodynamics provides the most appropriate analogy with the age-of-onset dynamics for Oji-Cree type 2 diabetes. The ED50 from pharmacodynamic models, or the effective drug dose that produces a specified effect in 50% of individuals, would be an analogue of t_{50} of the sigmoidal curves. The conversion from health to disease reflects both individual susceptibility and the cumulative "dose" of acquired factors. The burden of acquired factors in turn would depend upon both the intensity and duration of exposure. Age-of-onset is a direct index of exposure duration and is thus one determinant of the cumulative "dose" of acquired factors. The leftward shift in t_{50} among subjects born in 1935 and later could reflect either increased susceptibility, or a more rapid accumulation of acquired determinants, or a combination of both. In the case of the curves stratified by genotype, the leftward shift would reflect increased susceptibility related to the mutation. In contrast, for the curves stratified by age, the leftward shift would not reflect endogenous differences, since a major change in the Oji-Cree genome is unlikely to have occurred since 1935. In contrast, sociological and cultural changes have been rapid since over this time, seemingly coincident with the time frame encompassing the leftward shift in t_{50} . Also, Oji-Cree women tend to reach milestones of obesity at younger ages, imparting a gender-specificity to the relationship between obesity, disease onset and genetics [15]. Other studies, such as prospective interventions, might confirm a mechanistic role for such factors.

The above findings extended the applicability of sigmoidal models to the mathematical description of disease onset in a closed human community. Mathematical modeling permits examination of events, processes or systems that cannot be directly evaluated due to constraints imposed by temporality, magnitude, physical location or complexity. They allow for indirect visualization of otherwise imperceptible molecular events. The findings from Sandy Lake indicate that, under appropriate circumstances, the use of descriptive mathematical functions can be extended to biologic data collected from higher levels of organization, such as closed human communities.

5. Testing other candidate genes for Oji-Cree type 2 diabetes

Because *HNF1A* G319S was present in only ~40% of subjects with Oji-Cree type 2 diabetes, there must be other genetic determinants in this population. Over the past few years, common variants in several candidate genes have been proposed as being associated with type 2 diabetes [8].

Table 1
Sigmoidal age-of-onset dynamics in Oji-Cree type 2 diabetes

A) by HNF1A genotype				
	all subjects	S319/S319	S319/G319	G319/G319
present age (years)	44.4 ± 15.2	39.3 ± 16.3	42.2 ± 15.2	45.7 ± 15.1
serum insulin (pmol/L)	178 ± 15	177 ± 51	183 ± 39	177 ± 13
a (t ₅₀ in years)	38.5 ± 0.11	26.7 ± 0.72	34.7 ± 0.18	40.8 ± 0.21
b (Hill-constant)	4.3 ± 0.1	5.7 ± 1.0	4.7 ± 0.1	4.5 ± 0.1
adjusted model r ²	0.99	0.92	0.99	0.98
model P-value	<0.00001	<0.00001	<0.00001	<0.00001
B) by historical era				
	born before 1935		born 1935 and later	
a (t ₅₀ in years)	59.2 ± 0.34		35.6 ± 0.16	
b (Hill constant)	18.0 ± 0.26		6.03 ± 0.17	
adjusted model r ²	0.99		0.98	
model P-value	<0.00001		<0.00001	

These include common variants in the hereditary hemochromatosis gene [16], and in the genes encoding calpain-10 and plasma glycoprotein PC-1 [17] and peroxisome proliferator activated receptor- γ (18). Each of these was studied in Sandy Lake. The main genetic variant associated with hemochromatosis was almost totally absent from the Oji-Cree, and was not associated with the presence of type 2 diabetes [16]. The proposed causative variants in calpain-10 and plasma glycoprotein PC-1 were present at relatively high frequency, but were not significant determinants of Oji-Cree type 2 diabetes [17]. Finally, the peroxisome proliferator activated receptor- γ allele suggested to be associated with protection from type 2 diabetes in other populations was significantly associated with the presence of Oji-Cree type 2 diabetes [18]. The most charitable interpretation of these results is that these proposed common diabetes susceptibility alleles have context-dependent and population-specific associations, underscoring the challenges in human genetic studies of complex traits. Recently, we showed that a variant in the *PTP1B* gene encoding protein tyrosine phosphatase 1B was associated with protection from diabetes and impaired glucose tolerance in the Oji-Cree [19].

6. Genetic determinants of other traits in the Oji-Cree

Over the last six years, we have studied several other metabolic traits in the Oji-Cree. The positive associations are summarized in Table 2. We have found that while *HNF1A* G319S is an important determinant of diabetes susceptibility, common variants in other genes appear to be associated with variation in both fasting and post prandial plasma glucose [20–23]. Associations with plasma lipoproteins have also been extensively studied, and the totality of the evidence indicates that phenotype-genotype associations appear to be more numerous and more significant in adolescents than in adults [24–29]. This could mean that the accumulation of environmental influences on plasma li-

poprotein traits with time obscures the identification of potential genetic associations. Interestingly, there were no associations of lipoproteins with variants in promoters for hepatic lipase and 7- α hydroxylase [30,31]. We also found that there were associations between blood pressure and variants in the genes encoding angiotensinogen [32] and the G-protein β 3 subunit [33]. Variation in the gene encoding angiotensinogen was also associated with indices of renal function [34–37]. There was no association between blood pressure and the alleles of genes encoding α -adducin [38]. Of the genes tested for association with obesity, those encoding intestinal fatty acid binding protein [39] and nuclear lamin A/C [40] were associated, but the one encoding the β 3 adrenergic receptor and the G-protein β 3 subunit were not [33,41]. Another way of summarizing all these analyses was to simply tally the “deleterious alleles” for atherosclerosis related phenotypes [20–43]. This indicated when compared with average Caucasian allele frequencies, Oji-Cree have an excess of “deleterious alleles” [44], although the clinical relevance of this profile is uncertain.

7. Clinical relevance

These findings in the Oji-Cree provide one of the first –if not the very first –example of a strong genetic determinant of type 2 diabetes that is relatively common in a human subpopulation and may allow for prediction of disease onset. There is presently no comparable test for genetic susceptibility to type 2 diabetes for any other aboriginal group in the world. Clinicians working with the Oji-Cree and especially with type 2 diabetic adolescents now include molecular diagnostic testing for *HNF1A* G319S as part of the overall clinical assessment [45]. More broadly, the actual causative gene –*HNF1A* –is a relatively minor genetic determinant of diabetes in the general population. However, in the United Kingdom, studies of the MODY population are beginning to reveal clinical benefit to knowing the

Table 2
Summary of positive genetic associations in Oji-Cree

trait	gene and marker	comments	reference(s)
fasting plasma glucose	<i>PON2</i> A148G	diabetic subjects only	20,21
	<i>NAT2</i> C282T	non-diabetic subjects only	22
post-prandial glucose	<i>PPP1R3</i> deletion	diabetic subjects only	23
plasma lipoproteins			
HDL cholesterol	<i>HNF1A</i> G319S	non-diabetic subjects only	24
LDL cholesterol	<i>APOE</i> isoforms		25
	<i>PON1</i> R192Q	adolescents only	26
	<i>PON2</i> A148G		27
triglycerides	<i>APOC3</i> promoter		25
	<i>FABP2A54T</i>	adolescents only	26
	mtDNA D-loop		28
	<i>PON1</i> L55M	diabetic subjects only	29
systolic blood pressure	<i>AGT</i> M235T		32
	<i>GNB3</i> C825T		33
serum creatinine	<i>AGT</i> M235T		34
serum urea	<i>AGT</i> M235T		34
microalbuminuria	<i>AGT</i> M235T	diabetic subjects only	35
obesity	<i>FABP2A54T</i>	BMI and percent body fat	39
	<i>LMNA</i> C1908T	BMI and WHR	40

Abbreviations: *PON2*, gene encoding paraoxonase-2; *NAT2*, gene encoding N-acetyltransferase-1; *PPP1R3*, gene encoding protein phosphatase-1 regulatory subunit 3A; *HNF1A*, gene encoding hepatocyte transcription factor-1 alpha; *APOE*, gene encoding apolipoprotein E; *PON1*, gene encoding paraoxonase-1; *APOC3*, gene encoding apolipoprotein CIII; *FABP2*, gene encoding intestinal fatty acid binding protein; mtDNA, mitochondrial DNA; *AGT*, gene encoding angiotensinogen; *LMNA*, gene encoding nuclear lamin A/C; BMI body mass index; WHR ratio of waist-to-hip circumference.

precise molecular diagnosis - for instance *HNF1A*-related MODY vs. other forms of MODY –when evaluating attributes such as disease severity or response to medications [46]. These examples provide a glimpse of the potential clinical utility of molecular testing for *HNF1A* specifically but probably for a spectrum of diabetes susceptibility genes eventually.

8. Conclusions

Our research has shown that the high prevalence of diabetes in Sandy Lake is related to both genetic and environmental factors. The population-specific *HNF1A* G319S mutation clearly confers susceptibility to Oji-Cree type 2 diabetes. However, two generations ago, the diagnosis of diabetes was virtually unknown among these people. While the mutation must have been present in the past, its effects were thus neutral at worst. Why is diabetes such a problem now? The answer lies in the changed environment, as indicated in Fig. 2 and in the corresponding analyses. The community as a whole has had a massive shift in body mass index, which has been due to decreased activity and to increased intake of total calories, saturated fat and sugar. This has brought about diet-induced obesity, a phenotype that may have its own genetic component, and this has resulted, in turn, in hyperinsulinemia and insulin resistance. Subjects with one or two doses of the transactivation deficient *HNF1A* G319S mutant develop diabetes at earlier ages

in a dose-dependent manner. However, mathematical models suggest that most of the burden of earlier onset of type 2 diabetes is related to environmental factors rather than *HNF1A* G319S. The implication of this interpretation is that changes in environment, at the level of lifestyle, could overturn the genetic susceptibility, probably rapidly and in a “low-tech” manner. The effectiveness of intervention strategies directed at lifestyle is now being prospectively evaluated.

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