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ORIGINAL ARTICLE

Robert A. Hegele · Fang Sun · Stewart B. Harris
Carol Anderson · Anthony J.G. Hanley · Bernard Zinman

Genome-wide scanning for type 2 diabetes susceptibility in Canadian Oji-Cree, using 190 microsatellite markers

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Abstract We undertook a genome-wide scan using 190 markers with an average separation of 20 cM in 49 Canadian Oji-Cree sib pairs affected with type 2 diabetes. Four of these markers, one each on chromosomes 6, 8, 16, and 22, showed both suggestive linkage and suggestive association with type 2 diabetes in the Oji-Cree. None of these markers corresponded to any chromosomal region or marker that has so far been linked with type 2 diabetes in other populations. Thus, there might be several genetic loci that confer susceptibility to type 2 diabetes in this study sample. We are following up on these preliminary leads by increasing the density of the markers within these linked and associated regions, and also by increasing the number of study subjects. Also, we found instances in which there were wide disparities between the Oji-Cree and reference Caucasians with respect to marker heterozygosity. This suggests that a particular set of markers for genome-wide scanning will have different informativeness in different ethnic groups. Thus, different marker sets will likely be required for different ethnic groups in order to maximize their information content for linkage calculations.

Key words Complex disease · Carbohydrate · Insulin · Aboriginal populations

R.A. Hegele (✉) · F. Sun · C. Anderson
Blackburn Cardiovascular Genetics Laboratory, John P. Robarts Research Institute 406-100 Perth Drive, London, Ontario, Canada N6A 5K8
Tel. +1-519-663-3461; Fax +1-519-663-3789
e-mail: robert.hegele@rri.on.ca

S.B. Harris
Thames Valley Family Practice Research Unit, University of Western Ontario, London, Ontario, Canada

A.J.G. Hanley · B. Zinman
Samuel Lunenfeld Research Institute, Mount Sinai Hospital, and Departments of Public Health Sciences and Medicine, University of Toronto, Ontario, Canada

Introduction

The prevalence of type 2 diabetes among the Oji-Cree of the Sandy Lake reserve in Northern Ontario is one of the highest in the world (Harris et al. 1997). While this population has had rapid changes in their diet and lifestyle, genetic factors are still considered to play a key role in their susceptibility to type 2 diabetes (Harris et al. 1997). Based upon oral history, most of the contemporary members of the community are descended from about six founding clans, suggesting that type 2 diabetes might have a less complex genetic basis in Oji-Cree than in other populations. We have so far utilized association analysis in order to identify the genetic determinants of intermediate phenotypes in metabolism and vascular disease in the Oji-Cree (Hegele et al. 1996, 1997a–e). Since several genome-wide scans have identified *NIDDM* loci (Hanis et al. 1996, Mahtani et al. 1996, Prochazka et al. 1995) and since linkage analysis can help to define the genetic components of type 2 diabetes (Lander and Schork 1994, Elbein et al. 1994, Ghosh and Schork 1996), we undertook a genome-wide scan in Oji-Cree sib pairs affected with type 2 diabetes.

Methods

Study subjects

The community of Sandy Lake, Ontario, is located about 2000 km northwest of Toronto, in the subarctic boreal forest of central Canada. The community is isolated and is accessible only by air during most of the year. The prevalence of type 2 diabetes in this community is one of the highest in the world (Harris et al. 1997). 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. Since the development of the reservation and residential school systems, the lifestyle has changed radically from physically

active to sedentary. The primary source of food has changed from wildlife with supplementation by roots and berries to processed foods high in animal fats.

Seven hundred and twenty-eight members of this community (72% of the total population) aged above 10 years took part in a type 2 diabetes prevalence study (Harris et al. 1997). All subjects were assessed for a history of physician-diagnosed type 2 diabetes and other clinical attributes, as described (Harris et al. 1997). Concentrations of plasma glucose were determined after an 8- to 12-h fast and 120 min after a 75-g oral glucose tolerance test, as described (Harris et al. 1997). Subjects with physician-diagnosed and/or treated type 2 diabetes or with fasting plasma glucose >11.1 mmol/l were excluded from the oral glucose tolerance test. Subjects who were pregnant had their oral glucose tolerance test deferred until 3 months postpartum. Type 2 diabetes and impaired glucose tolerance were diagnosed using established criteria (Harris et al. 1997). The project was approved by the University of Toronto Ethics Review Committee.

Genetic analysis

Each study subject was placed into 1 of 88 kindreds, which ranged in size from 2 to more than 60 individuals. A total of 119 subjects had type 2 diabetes. A total of 16 sibships had 2 or more affected sibs with type 2 diabetes. There were 1, 4, 3, and 1 sibships that had, respectively, 6, 4, 3, and 2 affected sibs with type 2 diabetes who also had a sufficient quantity of DNA for a genome-wide scan. These 33 subjects affected with type 2 diabetes comprised a total of 49 affected sib pairs and was the sample for the genome-wide scan. A total of 80 unrelated normal non-diabetic control subjects were used to determine reference allele frequencies for this population. These normal control subjects were selected based upon age above 40 years, body mass index (BMI) above 27 kg/m^2, the absence of a history of diabetes, normal fasting blood sugar levels, and a normal glucose tolerance test result.

Genotyping was performed on an ABI 377 automated sequencer (Perkin Elmer, Applied Biosystems Division, Foster City, CA, USA) using the Weber/CHLC screening set version 8A (Research Genetics, Huntsville, AL, USA), with an average genetic distance of ~20 cM between markers. Affected sib pair linkage analysis was performed using the SIBPAL (version 2.8) subroutine of SAGE (version 2.2 [see Acknowledgments]), with correction for multiple sib pairs in sibships. Allele frequencies for final identity-by-descent (IBD) calculations were derived from at least 40 Oji-Cree subjects and up to 80 normal adult Oji-Cree. Because of the relatively wide marker separation, single point linkage analysis was performed. A nominal $P < 0.01$ was taken to be suggestive for linkage.

Association analysis was then performed with those markers that were found to show suggestive linkage. Allele frequencies of the most prevalent allele for each of the six suggestively linked markers were compared in 31 unrelated affected subjects and 80 normal controls with $2 \times 2 \chi^2$ analysis (one degree of freedom). A nominal $P < 0.05$ was taken to be suggestive for association for these six markers.

Results

Study sample

The baseline clinical and biochemical features of the 31 affected sib pairs with type 2 diabetes were as follows: 48% females; age, 51.6 ± 12.2 years; BMI, 28.5 ± 4.8 kg/m^2; fasting plasma glucose, 10.8 ± 5.0 mmol/l. The baseline clinical and biochemical features of the 80 unrelated normal non-diabetic control subjects were as follows: 55% females; age, 43.1 ± 12.5 years; BMI, 28.7 ± 5.2 kg/m^2; fasting plasma glucose, 5.5 ± 0.5 mmol/l.

Marker heterozygosity

We had heterozygosity estimates from all 80 normal adult Oji-Cree for 31 markers. We plotted the observed marker heterozygosity in the Oji-Cree against the reported marker heterozygosity in Caucasians (Weber/CHLC screening set version 8A Research Genetics, Huntsville, AL, USA). The results are shown in Fig. 1. For all but one of the 31 markers, the observed marker heterozygosity in the Oji-Cree was lower than in Caucasians (Fig. 1). A regression line with the following characteristics described the relationship between the marker heterozygosities in Caucasians and Oji-Cree:

Caucasian marker heterozygosity = 0.711 + 0.099 (Oji-Cree marker heterozygosity)

The r^2 for the regression line was 0.072 ($P < 0.15$). Thus, there was a trend for direct relationship between a Caucasian marker heterozygosity and Oji-Cree marker heterozygosity. We also observed instances in which there were wide disparities between the two populations with respect to marker heterozygosity. For example, there were markers that were highly informative in Caucasians, with heterozygosity >0.8, but were relatively uninformative in Oji-Cree, with heterozygosity ~0.2 (Fig. 1). In contrast, no marker was more informative in the Oji-Cree than in Caucasians; all but one had lower heterozygosity (Fig. 1). This indicates that for different ethnic groups, there are important differences in marker heterozygosity. It further emphasizes that marker allele frequencies from different ethnic groups cannot be used interchangeably for linkage analysis.

Linkage analysis

The results of the 20 cM genome scan with 190 markers are summarized in Fig. 2. We found six markers for chromosomal regions that showed suggestive linkage with type 2 diabetes in the Oji-Cree based on the a-priori nominal level of significance. Each of these had a z score above 2 and a nominal level of significance of <0.01. The details of the markers are presented in Table 1.

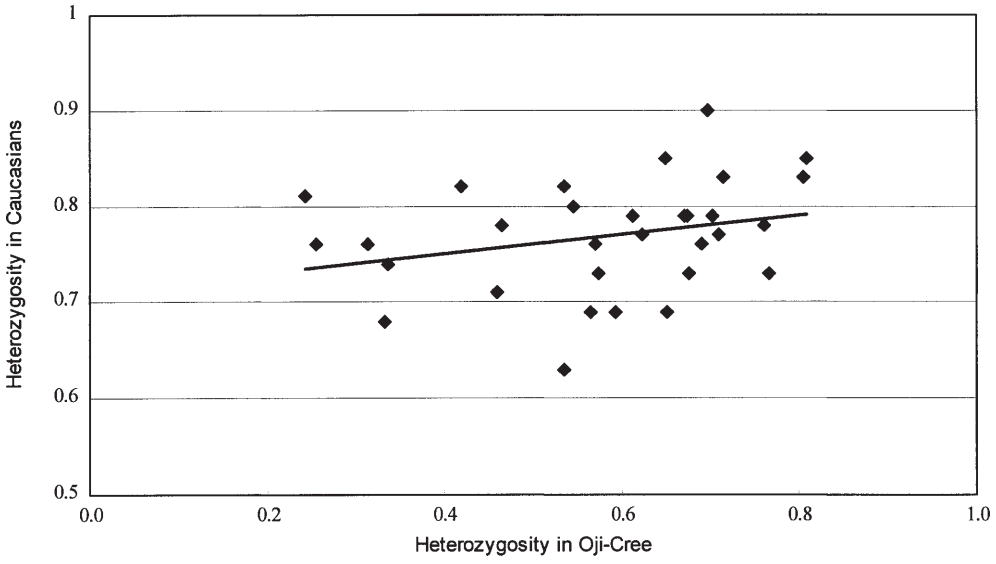

Fig. 1 Heterozygosity in Oji-Cree and reported Caucasian populations. Plot of marker heterozygosities in reference Caucasians on the *ordinate* versus Oji-Cree on the *abscissa* for the 31 markers for which there was heterozygosity information from 80 normal Oji-Cree subjects. The *dark line* represents the regression line, defined as $y = 0.711 + 0.099x$. The r^2 for the regression line was 0.072 ($P < 0.15$)

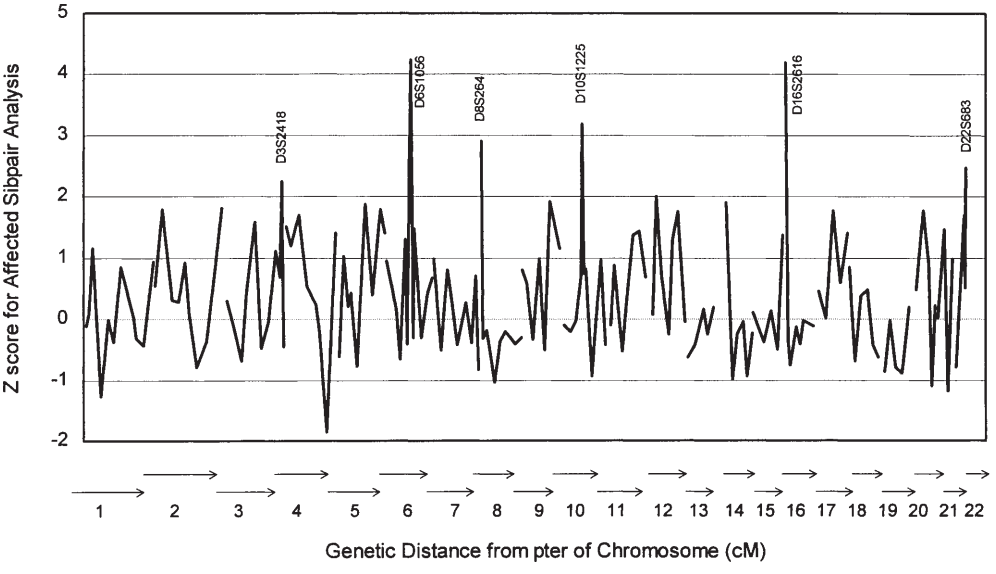

Fig. 2 Genome scan for Sandy Lake native diabetic study. Summary of genome scan in 49 Oji-Cree sib pairs affected with type 2 diabetes. The *ordinate* represents the z score from the linkage analysis. The *abscissa* represents the genetic distance in centi-Morgans along the linearized genome, divided by chromosome, as indicated. Each chromosome is oriented as pter-qter, with the *arrowheads* representing qter. The *six markers* with the highest z scores suggesting linkage are indicated *above the plot*

Table 1 Summary of markers with suggestive linkage to type 2 diabetes in 49 affected Oji-Cree sib pairs

Marker name	Heterozygosity	Mean + SD P(IBD)	Z score	*P* value
D3S2418	0.46	0.53 ± 0.10	2.26	0.01
D6S1056	0.65	0.65 ± 0.22	4.24	<0.0001
D8S264	0.81	0.60 ± 0.19	2.91	0.003
D10S1225	0.69	0.58 ± 0.17	3.19	0.001
D16S2616	0.65	0.58 ± 0.12	4.20	<0.0001
D22S683	0.70	0.59 ± 0.19	2.48	0.009

Heterozygosity for these six markers was determined from 80 normal adult Oji-Cree subjects.
P(IBD), The probability of identical-by-descent alleles in the affected sib pairs

Table 2 Summary of association analysis performed in 31 affected Oji-Cree subjects with type 2 diabetes and 80 unaffected Oji-Cree controls

	Frequency of most prevalent allele			
Marker name	Affected	Unaffected	χ^2	*P* value
D3S2418	0.64	0.64	0.00	NS (0.99)
D6S1056	0.26	0.10	8.00	0.0048
D8S264	0.45	0.21	11.7	0.0008
D10S1225	0.35	0.29	0.44	NS (0.52)
D16S2616	0.30	0.17	3.86	0.05
D22S683	0.37	0.19	6.30	0.013

NS, Not significant

Association analysis

The results of the association analysis for the six markers from Table 1 are shown in Table 2. For two of these, namely, *D3S2418* and *D10S1225*, there was no difference in the frequency of the most common allele, or for any other allele, between cases and controls. For each of the four remaining markers, namely, *D6S1056*, *D8S264*, *D16S2616*,

and *D22S683*, there was a significantly higher frequency of the most common allele in cases compared with controls. However, the frequency of the most common allele for each of these four markers was always <0.50. Therefore, there was no common allele for any of these markers that was present in a majority of the Oji-Cree subjects who were affected with type 2 diabetes.

Discussion

In this preliminary, low-density genome-wide scan in Oji-Cree sib pairs affected with type 2 diabetes, we observed suggestive linkages with six markers and suggestive associations with four of these. The four markers that showed both suggestive linkage and association with type 2 diabetes in the Oji-Cree were *D6S1056*, *D8S264*, *D16S2616*, and *D22S683*. None of these markers corresponded to any chromosomal region or marker so far shown to be linked with type 2 diabetes in other populations (Hanis et al. 1996, Mahtani et al. 1996, Prochazka et al. 1995). Furthermore, the loci in our study did not overlap with loci linked to maturity onset diabetes of the young, namely, *MODY1*, *MODY2*, or *MODY3* (Velho and Froguel 1998). The chromosome 6 marker *D6S1056* occurs within an interval that is more centromeric than type 1 diabetes loci *IDDM5* and *IDDM8* (Hanis et al. 1996, Mahtani et al. 1996, Prochazka et al. 1995). Furthermore, markers from within the intervals that have been linked to *IDDM5* and *IDDM8* were not linked with type 2 diabetes in the Oji-Cree. Thus, our results suggest that there may be some new loci for type 2 diabetes in the Oji-Cree. Furthermore, these results are consistent with the idea that type 2 diabetes is genetically heterogeneous, and that there are likely to be several susceptibility genes, which vary between populations.

There was no single marker that was clearly linked to and associated with type 2 diabetes to a greater extent than any of the other markers tested. Also, there was no greater than 65% IBD allele sharing for any of the markers that showed suggestive linkage. In addition, the frequency of the most common allele for the four markers that showed a suggestive linkage and association was <0.50 in each case. Therefore, no common allele for any of these markers was present in the majority of the Oji-Cree subjects affected with type 2 diabetes in this study sample. Taken together, these observations suggest that there was no single genetic locus that was linked to, or allele that was associated with, type 2 diabetes in the Oji-Cree. The observation is consistent with the idea that the genetic susceptibility to type 2 diabetes might be complex in the Oji-Cree. Therefore, there might be two or more genetic loci that confer susceptibility to type 2 diabetes in the Oji-Cree.

There was a trend for a direct relationship between the heterozygosity in Oji-Cree and Caucasians. However, there was a wide disparity among markers with respect to the heterozygosities observed in Oji-Cree and those reported in Caucasians. In general, the observed marker heterozygosities observed in the Oji-Cree were lower than those reported in Caucasians. There were instances in which a marker was very informative in Caucasians, but was relatively uninformative in Oji-Cree. This emphasizes that marker heterozygosities can differ widely between ethnic groups. Since allele frequencies can affect linkage calculations, these findings support the importance of determining the allele frequencies within specific ethnic groups. It is likely that different sets of markers for genome-wide scanning will be required in different ethnic groups in order to optimize the informativeness for linkage calculations.

In conclusion, we have found suggestive linkages of, and associations between, type 2 diabetes and several chromosomal markers in this relatively small sample of Oji-Cree. There may be several genetic loci that confer susceptibility to type 2 diabetes in this study sample. We are following up on these preliminary leads by increasing the density of the markers within these linked and associated regions, and by increasing the number of subjects under study.

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