useful. Risk calculation at a certain concentration of plasma homocysteine and assessment of significant response to treatment are the most important features.

In conclusion, the mean day-to-day change in fasting plasma homocysteine was 15.2% (1.3 μmol/L), and the maximum change was 62.2% (4.4 μmol/L). The mean CV was 13%, but there was heterogeneity of variance with CV values from 0% to 25%. The postprandial values did not differ systematically from the fasting values; therefore, it does not appear critical that the patient be in a fasting state when plasma homocysteine is measured. The orthostatic changes after 30 min were up to 29.9% (3.5 μmol/L), with a mean of 19% (2.1 μmol/L), and correlated only weakly to the albumin change. This might indicate a function of plasma homocysteine in the vaso-motor response, which needs further investigation.

This study was supported by The Medical Research Foundation of Northern Jutland County (Nordjylland Laegevidenskabelige Forskningsfond). We thank Gerda Mikkelsen and Susanne Øberg for excellent technical assistance.

**References**


Mannose-binding Lectin Gene Variation and Cardiovascular Disease in Canadian Inuit, Robert A. Hegele,1,7 Christopher P. Busch,1 T. Kue Young,2 Philip W. Connelly,3 and Henian Cao2 (1) Robarts Research Institute and Department of Medicine, University of Western Ontario, London, Ontario, Canada N6A 5K8; 2 Department of Community Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada R3E OW2; 3 Departments of Medicine and Biochemistry, St. Michael’s Hospital and University of Toronto, Toronto, Ontario, Canada M5B 1A6; * address correspondence to this author at: Blackburn Cardiovascular Genetics Laboratory, Robarts Research Institute, 406-100 Perth Dr., London, Ontario, Canada N6A 5K8; fax 519-663-3789, e-mail robert.hegele@rrri.on.ca

Canadian Inuit have an age-adjusted mortality from cardiovascular disease that is ~40% lower than the rest of Canada (1). This might result from the protective influence of certain environmental factors, such as the consumption of Arctic fish (1) or of certain genetic factors. For example, the thermolabile variant of the MTHFR gene, which encodes methylenetetrahydrofolate reductase, is one-sixth as prevalent in Inuit than in subjects of European origin (2). However, there are some inconsistent genetic findings in these people. For example, genetic variants that are associated with an increased risk of cardiovascular disease, such as the E4 allele of the APOE gene and the T235 allele of the AGT gene, are significantly more prevalent in Inuit than in whites (3). The resolution of such inconsistencies may come from the fact that several genes likely determine susceptibility to cardiovascular disease (4). It will thus be necessary to evaluate newer genetic determinants of cardiovascular disease risk in the Inuit.

One possible new genetic determinant for cardiovascular risk is the common coding sequence variation in the MBL gene, which encodes mannose-binding lectin (MBL) (5). MBL is an innate immune defense protein that binds mannose and other sugars on the surface of a variety of infectious agents, thereby facilitating phagocytosis and activation of the complement cascade (6,7). MBL likely modulates the severity of infection with Chlamydia pneumoniae (6,7), a pathogen linked by several lines of experimentation to the initiation and propagation of atherosclerosis (8). This might explain the association of genetic variation in MBL with severe atherosclerosis (5).

There are three common polymorphic sites in MBL. The Gly→Asp variant at MBL codon 54 (G54D) in exon 1, also called the “B allele”, has been associated with recurrent infections (6,7). In addition, MBL G54D destabilizes the sixth collagen repeat of MBL, and the B-type chains of MBL expressed in vitro fail to activate complement (6,7). Furthermore, subjects who are heterozygotes for the wild-type MBL A allele (G54) and the poorly functioning MBL B allele (D54) have a 20-fold reduction in MBL concentrations compared with G54 homozygotes because the MBL B allele product apparently acts as a dominant negative (6,7). The two other polymorphisms, namely G57E (also called the “C allele”) and R52C (also called the “D allele”)
have also been associated with reduced concentrations of MBL (6, 7). Because patients with severe atherosclerosis had a reduced frequency of the \textit{MBL} A allele and an increased frequency of the \textit{MBL} B, C, and D alleles compared with apparently healthy controls (5), we studied these alleles in Canadian Inuit from the Northwest Territories.

The Northwest Territories are located above the 60th parallel of latitude and comprise one-third of the landmass of Canada. In 1986, the population of Northwest Territories was 52,000. Of these, 35% were Inuit (or Eskimos), 15% were Dene (or Athapaskan Indians), and 50% were predominantly migrants of European origin from other parts of Canada. The present study involved residents of eight communities from the Nunavut region, mainly from the western shore of Hudson Bay (2, 3, 9).

Five hundred sixteen randomly selected individuals, ages 18 to 80 years, participated; of these, 281 reported themselves as being Inuit, 112 reported themselves as being of mixed ethnic background, 92 reported themselves as being of European background (white), and 50 reported themselves as being of an ethnic background other than Inuit, mixed, or white. At the time of the study, these communities continued to adhere to a more traditional lifestyle, including the consumption of Arctic fish at least three times per week. Of great interest was the very high prevalence of cigarette smoking, approaching 80% of adult subjects (9). The white subjects were included as a contrast sample to estimate allele frequencies from a regional control white population.

The project was approved by the Institutional Review Boards of the Universities of Manitoba and Toronto. Blood samples were obtained with informed consent. The first exclusion criterion was a self-reported ethnic background that was neither Inuit nor white. This left 373 subjects. The second exclusion criterion was an inadequate blood sample for genetic determinations. This left 192 subjects, of whom 148 were Inuit and 44 were white. Established procedures were used to genotype \textit{MBL} (10).

Among the 148 unrelated healthy Inuit, 51.4% were women, the mean (± SD) age was 38.2 ± 15.0 years, and the mean body mass index was 26.8 ± 4.5 kg/m². Among the 44 unrelated healthy whites, 38.6% were women, the mean age was 37.9 ± 11.4 years, and the mean body mass index was 26.1 ± 4.1 kg/m². The quantitative variables did not differ significantly between the samples from the two ethnic groups.

The genotype frequencies are shown in Table 1. Genotype frequencies in both races did not differ significantly from the expectations of the Hardy-Weinberg equation. We found that the \textit{MBL} A/A homozygotes were significantly more frequent in the Inuit than in whites (83% vs 57%; \textit{P}<0.0001). This difference was attributable to fewer \textit{MBL} A/B and A/D heterozygotes in the Inuit compared with the whites (11).

The allele frequencies are also shown in Table 1. The \textit{MBL} B, C, and D alleles together accounted for 9.5% of 296 \textit{MBL} alleles among the Inuit sample, which was significantly less than the frequency of 22.7% of these alleles among the 88 alleles in the regional control white sample (\textit{P}<0.0001). The frequency of the B allele was 7.8% in the Inuit, which was significantly lower than the frequency of 17.0% in whites (\textit{P}<0.025). The D allele frequency was also significantly lower in the Inuit compared with whites (\textit{P}<0.05).

Allele frequencies in the Inuit were also compared with those published previously for reference European controls (5). The \textit{MBL} B, C, and D alleles together accounted for 9.5% of 296 \textit{MBL} alleles among the Inuit sample, which was significantly less than the frequency of 21.0% of 200 alleles in the European controls (\textit{P}<0.0001) (5). We also observed that the \textit{MBL} A/A homozygotes were significantly more frequent in the Inuit than in the published apparently healthy European controls (83% vs 61%; \textit{P}<0.0001).

Thus, Canadian Inuit have significantly different frequencies of \textit{MBL} alleles compared with both regional reference whites and published European controls (5). In particular, there is a significantly higher proportion of Inuit who are homozygous for the “resistant” \textit{MBL} A allele compared with whites. In addition, Inuit have a significantly lower prevalence of the “susceptible” \textit{MBL} B, C, and D alleles. These differences in allele frequencies produced significant differences in genotype frequencies between the ethnic groups. More than 80% of Inuit had the resistant \textit{MBL} A/A homozygous genotype compared with ~60% of whites from both the reference sample residing in Nunavut and the published European control subjects (both \textit{P}<0.0001) (5).

The significant differences in \textit{MBL} allele and genotype frequencies suggest that Canadian Inuit could be less
susceptible to certain infections than whites. For example, interindividual differences in susceptibility to infection with *C. pneumoniae* have been suggested to be determined by genetic variation in MBL (5, 8). Several lines of experimental evidence have implicated infection with *C. pneumoniae* as a factor that contributes to atherosclerosis (8, 12). If the MBL allele frequencies in the Inuit actually conferred resistance to *C. pneumoniae* infection, this might explain in part their apparent resistance to cardiovascular disease. Alternatively, the associations may have been related to linkage disequilibrium with other structural differences in MBL or at a linked locus, which would have provided the mechanistic basis for the associations.

The high prevalence of the MBL A allele and the low prevalence of the MBL B, C, and D alleles among the Inuit could have been the result of either a distant founder effect or selection. For example, the Inuit probably had been relatively disease free before contact with Europeans because the Bering Strait “cold-screen” eliminated many pathogens (13). However, after contact with Europeans, the introduction and spread of certain infectious diseases might have led to selection of the MBL B, C, and D alleles out of the Inuit population. A possible genetic association between MBL variation and the susceptibility to infections among the Inuit needs to be explored. Findings in Greenland Inuit are also consistent with these observations (14, 15).

Although the basis for the distinctive genetic architectural features of Canadian Inuit may never be determined, these people have significant differences in MBL allele frequencies compared with whites. Given the increasing interest in infectious causes for atherosclerosis and cardiovascular disease endpoints, it is reasonable to consider the possibility that interindividual differences in genetic susceptibility to infections may contribute to differences in the expression of cardiovascular endpoints. Our results, specifically the high frequencies of the resistant MBL A allele and A/A genotype and the low frequencies of the other MBL alleles in the Inuit, are thus consistent with the well-documented low prevalence of cardiovascular disease in these people. However, the findings are indirect and the relationship between these polymorphisms and atherosclerosis can only be validated in disease association studies.

This work was supported by grants from the MRC (Canada, Grant no. MT13430), the Heart and Stroke Foundation of Ontario (Grant no. 3628), and the Blackburn Group. We acknowledge the cooperation and assistance of the members of the Health Canada research team. R.A. Hegele is a Career Investigator (CI-2979) of the Heart and Stroke Foundation of Ontario. T.K. Young is a Senior Scientist of the MRC (Canada).

**References**


**Simple and Rapid Detection of BRCA1 and BRCA2 Mutations by Multiplex Mutagenically Separated PCR, Pak Cheung R. Chan,† Betty Y.L. Wong,2 Hilmi Ozcelik,1,3 and David E.C. Cole1,2,4† (Departments of 1 Laboratory Medicine and Pathobiology and 4 Medicine and Pediatrics (Genetics), University of Toronto, Toronto, Ontario, Canada M5G 1L5; 2 Genetic Repository, Toronto General Hospital, Toronto, Ontario, Canada M5G 2C4; 3 Department of Pathology and Laboratory Medicine and Centre for Cancer Genetics, Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada M5G 1X5; 4 Laboratory Medicine and Pathobiology, Rm. 402, Banting Institute, 100 College St., University of Toronto, Toronto, Ontario, Canada M5G 1L5; fax 416-978-5650, e-mail davidec.cole@utoronto.ca)

BRCA1 and BRCA2 are tumor suppressor genes that are inactivated during neoplastic development (1, 2). Germ-line mutations of the two genes are transmitted in the autosomal dominant fashion and predispose carriers to the development of ovarian and/or breast cancers (3, 4). Mutations in BRCA1 are present in approximately one-half of the early-onset breast cancer families and 80% of the early-onset breast and ovarian cancer families (5), whereas BRCA2 mutations are believed to account for a comparable percentage of inherited breast cancer cases (6). Women with germ-line mutations in BRCA1 have a lifetime risk of 85% and up to 50% for breast and ovarian cancers, respectively.