Common Low-Density Lipoprotein Receptor p.G116S Variant Has a Large Effect on Plasma Low-Density Lipoprotein Cholesterol in Circumpolar Inuit Populations

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Background—Inuit are considered to be vulnerable to cardiovascular disease because their lifestyles are becoming more Westernized. During sequence analysis of Inuit individuals at extremes of lipid traits, we identified 2 nonsynonymous variants in low-density lipoprotein receptor (*LDLR*), namely p.G116S and p.R730W.

Methods and Results—Genotyping these variants in 3324 Inuit from Alaska, Canada, and Greenland showed they were common, with allele frequencies 10% to 15%. Only p.G116S was associated with dyslipidemia: the increase in LDL cholesterol was 0.54 mmol/L (20.9 mg/dL) per allele (P=5.6×10^{-49}), which was >3× larger than the largest effect sizes seen with other common variants in other populations. Carriers of p.G116S had a 3.02-fold increased risk of hypercholesterolemia (95% confidence interval, 2.34–3.90; P=1.7×10^{-17}), but did not have classical familial hypercholesterolemia. In vitro, p.G116S showed 60% reduced ligand-binding activity compared with wild-type receptor. In contrast, p.R730W was associated with neither LDL cholesterol level nor altered in vitro activity.

Conclusions—*LDLR* p.G116S is thus unique: a common dysfunctional variant in Inuit whose large effect on LDL cholesterol may have public health implications. **(*Circ Cardiovasc Genet*. 2015;8:100-105. DOI: 10.1161/CIRCGENETICS.114.000646.)**

Key Words: cardiovascular diseases ■ genetic variation ■ low-density lipoprotein cholesterol

Inuit were long believed to have lower cardiovascular disease (CVD) risk than nonindigenous populations.[1–3] However, re-evaluation of population studies indicates that ischemic heart disease rates are similar between Inuit and nonindigenous people.[4] Furthermore, ongoing Westernization in many Inuit communities has intensified their exposure to CVD risk factors such as smoking, calorie-dense processed foods, and a more comfortable but also sedentary lifestyle, all of which affect CVD risk and prevalence.[4–10] Among classical CVD risk factors, Inuit adults tend to have higher plasma concentrations of low-density lipoprotein (LDL) cholesterol (LDL-C) than nonindigenous populations.[11–15]

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The predominant monogenic cause of elevated LDL-C concentration in most global populations is familial hypercholesterolemia (FH; Online Mendelian Inheritance in Man, 143890).[16] Heterozygous FH prevalence may be as high as 1:200 in certain European populations, and it is a potent predisposition state for early CVD.[11–13] To date, DNA sequencing and biochemical studies have identified >1600 rare loss-of-function mutations in the gene encoding the LDL receptor (*LDLR*), which can increase LDL-C levels by ≥100%, and underlie >95% of cases of molecularly diagnosed FH.[16] But despite the relatively high levels of LDL-C observed in some Inuit, the role of *LDLR* gene variation has not been studied systematically.[13–15] We thus investigated the *LDLR* locus in Inuit and tested for association of variants therein with plasma lipids. Through Sanger sequencing and targeted genotyping, we found 2 new *LDLR* variants common to 5 Inuit subgroups from across North America and Greenland: (1) p.G116S was both dysfunctional in vitro and associated with a relatively large increase in plasma LDL-C levels, whereas (2) p.R730W had minimal dysfunction and impact on the lipid profile.

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Methods

For the purposes of this study, we referred to all participants as Inuit; however, we acknowledge that the circumpolar north is inhabited by a spectrum of diverse indigenous people.

Participants included Inuit >18 years of age (n=3324) residing in arctic communities across North America and Greenland. North American population-based samples were collected as part of regional health surveys that included (1) The Center for Alaska Native Health Research study of 2007, which covered 11 Southwest Alaska Yup'ik communities (n=1222),[17] (2) the Qanuippitaa Health Survey of 2004, which covered 14 coastal communities in Nunavik, Quebec (n=429),[6] (3) the Keewatin Health Assessment Study of 1990 to 1991, which surveyed the Keewatin (Kivalliq) region of Nunavut (n=210),[18] and (4) the Adult Inuit Health Survey of 2008, which surveyed the Inuvialuit region of the Northwest Territories (n=281).[19] Inuit living in West Greenland and Denmark (n=1182) were also included as part of our study cohort from a regional survey conducted in 1993 to 1994.[20]

The study was approved by the appropriate institutional research ethics boards including the Laval University Ethical Board and Comité Provincial de Santé Publique for use of Nunavik, Quebec samples; the University of Manitoba for use of Kivalliq samples; and McGill University for use of Inuvialuit samples. Yup'ik participants provided written informed consent using protocols approved by the University of Alaska Review Board, the National and Alaska Area Indian Health Service Institutional Review Boards, and the Yukon Kuskokwim Human Studies Committee. The Greenland population study was approved ethically by the Commission for Scientific Research in Greenland. Participants gave their written consent after being informed about the study both orally and in writing.

The *LDLR* promoter region and exons were Sanger sequenced within a discovery subset of 10 healthy Greenland Inuit with extreme plasma LDL-C concentrations >6.0 mmol/L (>95th percentile for non-Inuit adults). Two novel variants, p.G116S and p.R730W, were identified in *LDLR* exons 4 and 15, respectively (Figure I in the Data Supplement). Both variants were then genotyped in independent Inuit samples from 5 different regions (Table 1) with custom TaqMan single-nucleotide polymorphism (SNP) genotyping assays (Applied Biosystems; Foster City, CA). As a comparator, we genotyped a common polymorphism with a relatively large effect on LDL-C levels, namely the apoE gene (*APOE*) protein isoforms using TaqMan SNP genotyping assays for SNPs rs429358 and rs7412 (Applied Biosystems; Foster City, CA).[21] Genotypes were tested for association with blood lipid traits, including total cholesterol, LDL-C, high-density lipoprotein (HDL) cholesterol, non-HDL cholesterol and triglyceride, as well as apoB concentration, where available.

Detailed descriptions of methods are provided in the Methods in the Data Supplement.

Results

LDLR p.G116S and p.R730W Are Common and Exclusive to Inuit

To evaluate the genetic basis for elevated LDL-C in Inuit, we used candidate sequencing of the *LDLR* gene to screen 10 Inuit with plasma LDL-C concentrations >6.0 mmol/L (>95th percentile for non-Inuit adults). We found 2 heterozygous *LDLR* gene variants, namely p.G116S and p.R730W, in 3 and 4 Inuit with high LDL-C, respectively (Figure I in the Data Supplement). We then genotyped these variants in 3324 Inuit samples from Southwest Alaska, Northern Canada (Inuvialuit, Kivalliq, and Nunavik), and Greenland (Table 1). The p.G116S variant frequency ranged from 2% in Kivalliq to 13% in Greenland, with an overall frequency of 10% across all regions. The p.R730W variant frequency ranged from 11% in Greenland to 17% in Kivalliq, with an overall frequency of 14% across all regions. The variants were not in linkage disequilibrium (r^2=0.017; P=NS). Both variants were absent from other indigenous population samples and neither was observed in 4281 European samples and 2193 black samples from the National Heart, Lung, and Blood Institut Exome Sequencing Project database. However, p.G116S was reported previously in a single hypercholesterolemic subject of unspecified ethnic background ascertained in a lipid clinic in Denmark.[22]

LDLR p.G116S Is Robustly Associated With Higher Plasma LDL-C Concentration

We stratified plasma lipoprotein profiles according to *LDLR* p.G116S or p.R730W genotype (Table 2). In each sample, p.G116S carriers had significantly higher total, non-HDL, and LDL-C concentrations compared with noncarriers (Tables IA and IIB in the Data Supplement). In the overall sample, p.G116S was associated with a ≈0.54 mmol/L (20.9 mg/dL) increase in LDL-C per copy (Table 3; P=5.6×10^{-49}); mean plasma apoB and non-HDL cholesterol concentrations were also proportionately higher per copy of p.G116S. In contrast, p.R730W was not significantly associated with LDL-C overall (P=0.13). In the combined Inuit samples, *LDLR* p.G116S genotype had an additive (codominant) effect on LDL-C concentration (Figure 1): mean LDL-C concentration was significantly higher in p.G116S heterozygotes than in p.G116 homozygotes (P=2.0×10^{-34}) and tended to be higher still in p.G116S homozygotes compared with heterozygotes (P=0.058). In contrast, the relationship between p.R730W and plasma LDL-C concentrations was not significant overall. Each copy of p.G116S was associated with increased risk of hypercholesterolemia, defined as a plasma LDL-C >5.0 mmol/L, which Canadian dyslipidemia guidelines[23] suggest as the cutpoint for prescription of lipid-lowering treatment (Figure 2; odds ratio, 3.02;

Table 1. Demographics and LDL Receptor Variant Frequencies for Select Circumpolar Populations

											MAF, %	
	n	Age, y	Female, %	BMI, kg/m^2	TC	LDL-C	HDL-C	Non–HDL-C	ApoB	TG	p.G116S	p.R730W
Greenland	1182	44±14	56	26±5	5.91±1.13	3.82±1.04	1.57±0.44	4.33±1.14	0.92±0.23	1.16±0.67	0.13	0.11
Kivalliq	210	37±16	54	27±4	5.00±1.03	3.09±0.92	1.45±0.41	3.55±1.01	0.98±0.26	1.03±0.57	0.02	0.17
Inuvialuit	281	45±16	67	30±7	5.05±0.99	2.91±0.89	1.37±0.42	3.68±1.03	0.91±0.25	1.74±1.27	0.05	0.13
Nunavik	429	37±14	56	27±6	4.99±0.99	2.79±0.86	1.63±0.43	3.33±1.02	0.96±0.24	1.23±0.72	0.09	0.13
SW Alaska	1222	38±16	53	28±6	5.20±1.15	3.20±0.98	1.64±0.44	3.61±1.08	n.d.	0.94±0.56	0.10	0.16
Combined	3324	40±16	56	27±6	5.40±1.16	3.30±1.05	1.58±0.44	3.83±1.15	0.93±0.24	1.13±0.74	0.10	0.14

All demographics are reported±SD. Lipid-related traits are all reported in mmol/L except apoB, which is in g/L. BMI indicates body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LDLR, LDL receptor; MAF, minor allele frequency; n.d., no data; TC, total cholesterol concentration; and TG, triglyceride concentration.

Table 2. Mean Lipid Traits Based on p.G116S and p.R730W Genotype in a Combined Inuit Cohort

	p.G116S genotype (A)			p.R730W genotype (T)		
Lipid Traits	GG	GA	AA	CC	CT	TT
TC	5.29±1.11	5.94±1.24	6.20±1.25*	5.43±1.17	5.37±0.17	5.47±1.07
LDL-C	3.22±0.98	3.88±1.14	4.21±1.14*	3.37±1.06	3.28±1.04	3.30±0.82
apoB	0.90±0.23	1.03±0.24	1.11±0.20*	0.93±0.23	0.91±0.24	0.97±0.27
HDL-C	1.57±0.44	1.59±0.43	1.58±0.49	1.56±0.44	1.61±0.46	1.68±0.45†
TG	1.13±0.75	1.09±0.62	0.97±0.38	1.12±0.76	1.11±0.67	1.11±0.68
Non–HDL-C	3.71±1.09	4.35±1.24	4.62±1.20*	3.86±1.15	3.76±0.16	3.80±1.10†

*$P<0.0001$ and †$P<0.05$ using ANOVA adjusted for age, sex, and body mass index. HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol concentration; and TG, triglyceride concentration.

95% confidence interval, 2.34–3.90; $P=1.7\times10^{-17}$). In contrast, p.R730W was not associated with increased risk of clinically actionable hypercholesterolemia.

LDLR p.G116S Has a Larger Effect Size on LDL-C Than the *APOE* E4 Isoform

We compared the effect size of p.G116S to that of the *APOE* E4 isoform, a well-established common variant associated with increased LDL-C.[24,25] In Inuit, *APOE* E4 allele frequencies ranged from 21% to 27% (Table II in the Data Supplement) and each copy of E4 increased LDL-C by 0.18 mmol/L (7.0 mg/dL; $P=9.0\times10^{-11}$). Furthermore, the top LDL-C–associated variants from genome-wide association studies had effect sizes per allele ranging from 0.05 to 0.18 mmol/L.[26] Thus, *LDLR* p.G116S in Inuit is unique, with >3-fold larger effect on LDL-C than any other common variant.

LDLR p.G116S Impairs LDLR Ligand-Binding Activity In Vitro

Finally, we investigated the function of both variants in vitro, using cell-based models transfected with plasmid constructs encoding wild-type, p.G116S, or p.R730W *LDLR* variants.

Table 3. Associations Between LDL Receptor Variants and Plasma LDL Cholesterol

Variant	Population	β, mmol/L	SE	*P* Value
p.G116S	Greenland	0.64	0.05	1.8×10^{-30}
	Kivalliq	1.02	0.27	1.7×10^{-4}
	Inuvialuit	0.52	0.16	0.0011
	Nunavik	0.40	0.09	3.7×10^{-5}
	SW Alaska	0.41	0.06	9.4×10^{-12}
	Combined	0.54	0.04	5.6×10^{-49}
p.R730W	Greenland	−0.11	0.06	0.077
	Kivalliq	0.003	0.09	0.97
	Inuvialuit	−0.13	0.11	0.24
	Nunavik	−0.05	0.08	0.52
	SW Alaska	−0.01	0.05	0.85
	Combined	−0.05	0.03	0.13

Effect sizes and *P* values are based on the minor alleles p.G116S or p.R730W. Greenland (n=1162), Kivalliq (n=204), Inuvialuit region (n=253), Nunavik (n=389), SW Alaska (n=1113), and combined (n=3121). LDL indicates low-density lipoprotein.

Overall, p.G116S tended to show increased mean mature LDLR expression by 31%, whereas p.R730W had reduced mean mature LDLR expression by 63% relative to the wild-type LDLR constructs. In vitro LDL-binding assays adjusted for total LDLR expression linked p.G116S with a significant 61% reduction in LDL-binding ability, whereas p.R730W had a nonsignificant 12% reduction in binding ability (Figure 3).

The *LDLR* p.G116S variant in exon 4 resides within the ligand-binding domain.[27] Of missense or nonsense mutations in *LDLR* that cause monogenic FH, ≈20% reside within exon 4, which is considered to be a mutational hot-spot.[28] The

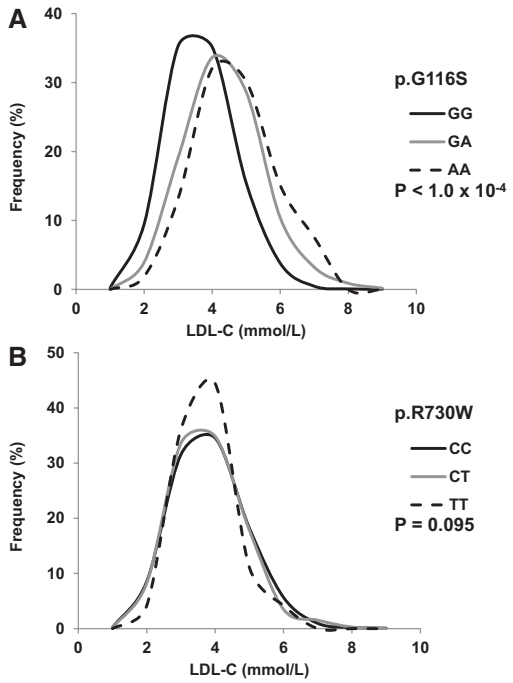

Figure 1. Low-density lipoprotein (LDL) receptor (*LDLR*) variant associations with LDL cholesterol (LDL-C) in a combined Inuit cohort. Inuit participants were separated based on *LDLR* p.G116S or p.R730W genotype and LDL cholesterol concentration. **A**, Frequency distribution of Inuit participants based on p.G116S carrier status and LDL-C concentration. Mean LDL cholesterol concentrations between noncarriers (GG; n=2585), heterozygotes (GA; n=559), and homozygotes (AA; n=53) were compared using 1-way ANOVA with the *P* value as indicated. **B**, Frequency distributions were similarly constructed for p.R730W noncarriers (CC; n=2408), heterozygotes (CT; n=717), and homozygotes (TT; n=72) and were also compared using 1-way ANOVA.

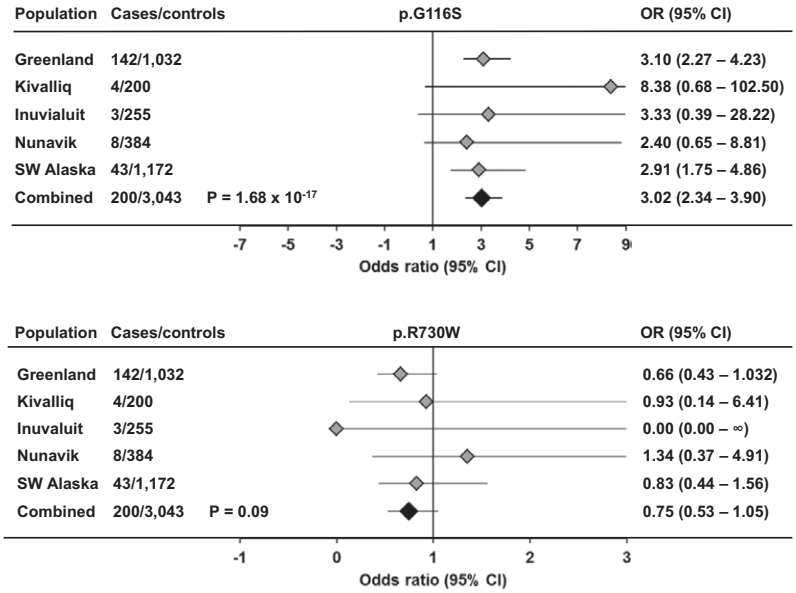

Figure 2. Association between Inuit low-density lipoprotein (LDL) receptor (*LDLR*) variants and elevated LDL cholesterol (LDL-C) concentration. Forest plots indicate odds ratios (ORs) and 95% confidence intervals (95% CIs) from association testing between both *LDLR* p.G116S or p.R730W variant genotypes and severely elevated LDL-C status (LDL-C >5.0 mmol/L).

pathogenic relevance of p.G116 in receptor function was supported by identification of the p.G116C variant in a Polish patient with hypercholesterolemia.[29] In contrast, p.R730W is within in exon 15, which encodes an attachment site for O-linked carbohydrate chains; this domain has no clear functional role.[27] Less than 1% of disease-causing *LDLR* mutations reside within exon 15.[28] Sequence conservation analysis suggested stronger evolutionary conservation at p.G116 compared with p.R730 (Figure II in the Data Supplement), whereas multiple algorithms predicted a more deleterious effect for p.G116S than p.R730 on LDLR function (Table III in the Data Supplement). The role of p.R730 in LDLR function remained unclear as a different mutation at p.R730, namely p.R730Q, was found in a sample from a Dutch FH cohort but was predicted to be benign and was reported as likely not disease causing.[30]

Discussion

LDLR p.G116S thus seems to be an example of the hypothesized but to date elusive entity in lipoprotein genetics, namely a common genetic variant whose LDL-C raising effect, is intermediate between the modest effects attributable to genome-wide association study alleles and the large effects of rare *LDLR* mutations causing monogenic disease (FH). Although bioinformatic predictions further supported a functional consequence of p.G116S, the functional studies ultimately corroborate the observed phenotypic effect of p.G116S. The ≈ 60% reduced ligand-binding ability of cells expressing the p.G116S is intermediate between that of wild-type LDLR and of rare FH-causing mutations, which show ≤100% reductions of ligand-binding ability.[27]

Our discovery of the association between p.G116S and LDL-C concentration is of particular interest from a public health perspective, as Inuit communities may currently be at the tipping point of environment-related increased risk of CVD and metabolic disorders. In other populations, every 1-mmol/L increase in LDL-C corresponds to a ≈20% increase in CVD and ≈15% increase in all-cause mortality.[31] Thus, the ≈0.5-mmol/L increase in LDL-C per p.G116S allele could potentially lead to ≈10% and ≈7.5% increased risk of CVD and all-cause mortality, respectively. Our analyses indicated that p.G116S carriers were at a ≈3-fold increased risk of high LDL-C (>5 mmol/L), which suggested that p.G116S carriers were also more likely to be candidates for pharmacological intervention than noncarriers (odds ratios, 3.02; 95% confidence interval, 2.34–3.90; P=1.7×10^{-17}). Unfortunately, data on CVD end points were not systematically collected in the surveys that comprised this study, so the possible impact of p.G116S on metabolic and CVD risk among the Inuit cannot be directly inferred at this time. A link between this genetic variant and CVD risk would need to be formally evaluated, for instance, using Mendelian randomization or another appropriate prospective study design. Furthermore, it would be of interest to detect possible interactions between lifestyle factors, other risk factors, and the phenotypic impact of *LDLR* p.G116S. Although baseline between-population differences in lipid profiles might be consistent with environmental effects (Table 1), we have not systematically collected comprehensive diet and lifestyle data; although we would like to do this in the future, such an analysis is beyond the scope of the present report.

As with all association studies, a potential risk of population stratification artifacts exists. However, there are several reasons why we think that this is not a major issue here. First, we adjusted for geographic location in the association and correlation analyses for the combined Inuit cohort; the association of G116S with LDL-C was highly significant with this adjustment variable included. Second, although the minor allele of G116S varies by geographic region, the directionality of the association by genotype is the same and is individually significant, in each of the 5 subpopulations for LDL-C and the related traits of total cholesterol, non–HDL cholesterol, and apoB (see Tables IA and IB in the Data Supplement). Third, we have functionally evaluated the variants in vitro in 2 different cell lines and show a significant loss of binding function for the variant that is significantly associated with LDL-C levels, but no functional

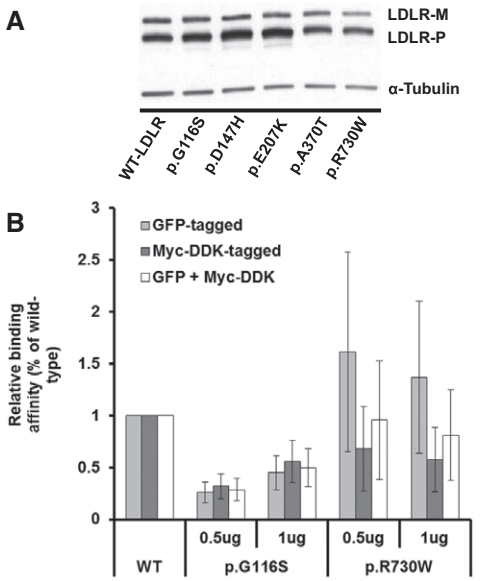

Figure 3. Comparison of low-density lipoprotein (LDL) receptor (LDLR) expression and ligand-binding ability between wild-type (WT) and Inuit-identified variants. **A**, COS7 cells were transfected with expression vectors encoding Myc-DDK–tagged LDLR and LDLR variants mutated to recreate the Inuit-identified polymorphisms or established LDLR variants previously reported. Total cell lysates were analyzed and transfections for this model were performed twice. LDLR-M refers to the mature protein, and LDLR-P to the precursor protein. **B**, LDLR binding activity was measured in HepG2 cells and normalized based on expression data from the corresponding mature LDLR isoforms. Binding activity experiments were performed in triplicate at 2 doses of LDL and were averaged. Average binding activity estimates were then normalized based on the expression data determined from the Myc-DDK–tagged, green fluorescent protein (GFP)-tagged, or combined GFP–tagged and Myc-DDK–tagged expression models. Error bars represent normalized SE. All binding read-outs for p.G116S transfectants are significantly different from WT (all $P<0.05$), whereas none of the read-outs for p.R730W transfectants are significantly different from WT (all $P>0.05$).

impact of the variant that is not associated with LDL-C levels. The findings for dysfunction of G116S are similar in quality, although smaller in magnitude, than those that we have seen for our patients with clinical FH with mutations in the *LDLR* gene. Finally, principal component analysis performed using genome-wide markers from the exome array on 3 of the 5 Inuit subpopulations shows a distinctive clustering, with no overlap at all with white or black clusters (data not shown). However, some small stratification artifacts are still possible.

Although we studied *LDLR* variation, we did not screen the additional FH genes *APOB* and *PCSK9*, so we cannot rule out similar additional effects on this quantitative trait. Furthermore, genome-wide association studies in other global populations have recently implicated >30 genes that modulate LDL-C concentration; cumulatively these might have a larger impact than the 0.54-mmol/L per allele effect of p.G116S.[26] A comprehensive genetic screen for LDL-C–related variants using multilocus high-density genotyping strategies or microarrays, while of potential interest in these samples, is far beyond the scope of the studies reported here. Also, the reason that these distinct variants arose in circumpolar people in the first place cannot be determined or even reasonably speculated on at this time. The effect size on LDL-C is not consistent with any known or obvious survival advantage nor does there seem to be any potential for negative selection because CVD onset typically follows decades after the onset of the reproductive years. Finally, although the p.R730W variant seemed to have minimal impact on LDL-C at the population level, a possible impact on other pathways or networks cannot be ruled out from the studies performed here.

Thus, our screen for FH-related variation in the Inuit uncovered a unique genetic variant among global populations: *LDLR* p.G116S is a common, dysfunctional variant that is strongly associated with a large LDL-C–raising effect, although not causing classical FH. It seems to embody the type of variant that has been long sought-after in the post–genome-wide association study era and warrants consideration in evaluating clinical and public health implications as part of the fabric of CVD risk in the circumpolar north.

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Disclosures

None.

References

1. Bjerregaard P, Dyerberg J. Mortality from ischaemic heart disease and cerebrovascular disease in Greenland. *Int J Epidemiol*. 1988;17:514–519.
2. Middaugh JP. Cardiovascular deaths among Alaskan Natives, 1980-86. *Am J Public Health*. 1990;80:282–285.
3. Young TK, Moffatt ME, O'Neil JD. Cardiovascular diseases in a Canadian Arctic population. *Am J Public Health*. 1993;83:881–887.
4. Bjerregaard P, Young TK, Hegele RA. Low incidence of cardiovascular disease among the Inuit–what is the evidence? *Atherosclerosis*. 2003;166:351–357.
5. Bjerregaard P, Mulvad G, Pedersen HS. Cardiovascular risk factors in Inuit of Greenland. *Int J Epidemiol*. 1997;26:1182–1190.
6. Chateau-Degat ML, Dewailly E, Louchini R, Counil E, Noël M, Ferland A, et al. Cardiovascular burden and related risk factors among Nunavik (Quebec) Inuit: insights from baseline findings in the circumpolar Inuit health in transition cohort study. *Can J Cardiol*. 2010;26:190–196.
7. Ebbesson SO, Adler AI, Risica PM, Ebbesson LO, Yeh JL, Go OT, et al. Cardiovascular disease and risk factors in three Alaskan Eskimo populations: the Alaska-Siberia project. *Int J Circumpolar Health*. 2005;64:365–386.
8. Howard BV, Comuzzie A, Devereux RB, Ebbesson SO, Fabsitz RR, Howard WJ, et al. Cardiovascular disease prevalence and its relation to risk factors in Alaska Eskimos. *Nutr Metab Cardiovasc Dis*. 2010;20:350–358. doi:10.1016/j.numecd.2009.04.010.
9. Jernigan VB, Duran B, Ahn D, Winkleby M. Changing patterns in health behaviors and risk factors related to cardiovascular disease among American Indians and Alaska Natives. *Am J Public Health*. 2010;100:677–683. doi:10.2105/AJPH.2009.164285.
10. Kellett S, Poirier P, Dewailly E, Sampasa H, Chateau-Degat ML. Is severe obesity a cardiovascular health concern in the Inuit population? *Am J Hum Biol*. 2012;24:441–445. doi:10.1002/ajhb.22241.

11. Haase A, Goldberg AC. Identification of people with heterozygous familial hypercholesterolemia. *Curr Opin Lipidol*. 2012;23:282–289. doi:10.1097/MOL.0b013e3283556c33.
12. Jørgensen ME, Bjerregaard P, Kjaergaard JJ, Borch-Johnsen K. High prevalence of markers of coronary heart disease among Greenland Inuit. *Atherosclerosis*. 2008;196:772–778. doi:10.1016/j.atherosclerosis.2007.01.008.
13. Redwood DG, Lanier AP, Johnston JM, Asay ED, Slattery ML. Chronic disease risk factors among Alaska Native and American Indian people, Alaska, 2004-2006. *Prev Chronic Dis*. 2010;7:A85.
14. Bjerregaard P, Jørgensen ME, Borch-Johnsen K; Greenland Population Study. Serum lipids of Greenland Inuit in relation to Inuit genetic heritage, westernisation and migration. *Atherosclerosis*. 2004;174:391–398. doi:10.1016/j.atherosclerosis.2004.02.010.
15. Ebbesson SO, Schraer C, Nobmann ED, Ebbesson LO. Lipoprotein profiles in Alaskan Siberian Yupik Eskimos. *Arctic Med Res*. 1996;55:165–173.
16. Goldstein JL, Brown MS. The LDL receptor. *Arterioscler Thromb Vasc Biol*. 2009;29:431–438. doi:10.1161/ATVBAHA.108.179564.
17. Mohatt GV, Plaetke R, Klejka J, Luick B, Lardon C, Bersamin A, et al; Center for Alaska Native Health Research. The Center for Alaska Native Health Research Study: a community-based participatory research study of obesity and chronic disease-related protective and risk factors. *Int J Circumpolar Health*. 2007;66:8–18.
18. Moffatt ME, Young TK, O'Neil JD, Eidelheit S, Fish I, Mollins J. The Keewatin Health Assessment Study, NWT, Canada. *Arctic Med Res*. 1993;52:18–21.
19. Saudny H, Leggee D, Egeland G. Design and methods of the Adult Inuit Health Survey 2007–2008. *Int J Circumpolar Health*. 2012;71.
20. Bjerregaard P, Curtis T, Borch-Johnsen K, Mulvad G, Becker U, Andersen S, et al. Inuit health in Greenland: a population survey of life style and disease in Greenland and among Inuit living in Denmark. *Int J Circumpolar Health*. 2003;62(Suppl 1):3–79.
21. Fullerton SM, Clark AG, Weiss KM, Nickerson DA, Taylor SL, Stengård JH, et al. Apolipoprotein E variation at the sequence haplotype level: implications for the origin and maintenance of a major human polymorphism. *Am J Hum Genet*. 2000;67:881–900. doi:10.1086/303070.
22. Damgaard D, Larsen ML, Nissen PH, Jensen JM, Jensen HK, Soerensen VR, et al. The relationship of molecular genetic to clinical diagnosis of familial hypercholesterolemia in a Danish population. *Atherosclerosis*. 2005;180:155–160. doi:10.1016/j.atherosclerosis.2004.12.001.
23. Anderson TJ, Grégoire J, Hegele RA, Couture P, Mancini GB, McPherson R, et al. 2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. *Can J Cardiol*. 2013;29:151–167. doi:10.1016/j.cjca.2012.11.032.
24. Khan TA, Shah T, Prieto D, Zhang W, Price J, Fowkes GR, et al. Apolipoprotein E genotype, cardiovascular biomarkers and risk of stroke: systematic review and meta-analysis of 14,015 stroke cases and pooled analysis of primary biomarker data from up to 60,883 individuals. *Int J Epidemiol*. 2013;42:475–492. doi:10.1093/ije/dyt034.
25. Ward H, Mitrou PN, Bowman R, Luben R, Wareham NJ, Khaw KT, et al. APOE genotype, lipids, and coronary heart disease risk: a prospective population study. *Arch Intern Med*. 2009;169:1424–1429. doi:10.1001/archinternmed.2009.234.
26. Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. *Nature*. 2010;466:707–713. doi:10.1038/nature09270.
27. Hobbs HH, Russell DW, Brown MS, Goldstein JL. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu Rev Genet*. 1990;24:133–170. doi:10.1146/annurev.ge.24.120190.001025.
28. Stenson PD, Ball EV, Howells K, Phillips AD, Mort M, Cooper DN. The Human Gene Mutation Database: providing a comprehensive central mutation database for molecular diagnostics and personalized genomics. *Hum Genomics*. 2009;4:69–72.
29. Chmara M, Wasag B, Zuk M, Kubalska J, Wegrzyn A, Bednarska-Makaruk M, et al. Molecular characterization of Polish patients with familial hypercholesterolemia: novel and recurrent LDLR mutations. *J Appl Genet*. 2010;51:95–106. doi:10.1007/BF03195716.
30. Fouchier SW, Kastelein JJ, Defesche JC. Update of the molecular basis of familial hypercholesterolemia in The Netherlands. *Hum Mutat*. 2005;26:550–556. doi:10.1002/humu.20256.
31. Cholesterol Treatment Trialists' (CTT) Collaborators, Mihaylova B, Emberson J, Blackwell L, Keech A, Simes J, et al. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet*. 2012;380:581–590.

CLINICAL PERSPECTIVE

The low-density lipoprotein (LDL) receptor (*LDLR*) gene has taught us much about lipoprotein metabolism and atherosclerosis risk. Severe loss-of-function mutations in *LDLR* occur in ≈1:250 people and can double plasma LDL cholesterol, causing familial hypercholesterolemia, an autosomal codominant condition that underlies ≈4% of early coronary heart disease cases. In contrast, common SNPs in *LDLR* have minor allele frequencies of ≈0.2, with LDL cholesterol raising effects of ≈0.12 mmol/L (5 mg/dL), which contributes to polygenic coronary heart disease risk in the general population. We report an *LDLR* variant, namely p.G116S, that is intermediate between these 2 extremes. It is common among Inuit people (Eskimos) living in Alaska, Northern Canada, and Greenland, with an overall frequency of ≈10%. It raises LDL cholesterol substantially by 0.54 mmol/L (21 mg/dL) per allele. In vitro studies show compromised ability of the variant receptor to bind LDL cholesterol. Although p.G116S heterozygotes are 3× more likely than noncarriers to have LDL cholesterol >5 mmol/L (194 mg/dL), they do not express classical heterozygous familial hypercholesterolemia. Similarly, p.G116S homozygotes have LDL cholesterol levels far below those observed in classical homozygous familial hypercholesterolemia. The p.G116S variant is thus unique: it is relatively common among Inuit, is associated with moderate loss-of-function, and raises LDL cholesterol by a moderately large amount. Carriers have a phenotype that is less severe than classical familial hypercholesterolemia; nonetheless, p.G116S is predicted to increase coronary heart disease risk among the Inuit, who are considered to be a vulnerable population in transition.

SUPPLEMENTAL MATERIAL

Methods

Lipid measurements

Methods for lipid measurement in the individual health surveys were previously reported.[1-4] Generally, participants fasted for either 12 hours or overnight prior to blood sample collection. LDL cholesterol was estimated using the Friedewald equation in all samples, except for those from Alaska, in which LDL cholesterol was directly measured.

Bioinformatic analysis

LDLR variant effects on receptor function were assessed using multiple *in silico* prediction algorithms including PMUT,[5] PolyPhen,[6] MutPred,[7] and SIFT.[8] Algorithm scores were included where available. Evolutionary conservation of amino acid residues at *LDLR* p.G116 and p.R730 was assessed using the BLAST alignment tool, which aligns homologous regions from a range of species.[9] Previously reported FH mutations in *LDLR* were referenced from the Human Gene Mutation Database[10] and the Western Database of Lipid Variants.[11] Large-scale genome sequencing databases from the 1000 Genomes Project (1,092 sequenced genomes from a multi-ethnic cohort)[12] and National Heart, Lung, and Blood Institute Grand Opportunity (NHLBI GO) Exome Sequencing Project (ESP)[13] Exome Variant Server (EVS; variants from exome sequencing from 2,203 African-American and 4,300 European-American samples, details at http://evs.gs.washington.edu/EVS/) were used to determine the frequencies of *LDLR* p.G116S and p.R730W in non-Inuit populations.

Site-directed mutagenesis

Wild-type LDLR cDNA / pCMV6 Entry Myc –DDK or GFP tagged plasmid was obtained from Origene (Cedarlane, Burlington, ON). Site-directed mutagenesis was performed to generate LDLR mutants, using Stratagene QuikChange II XL Site-Directed Mutagenesis kit (Agilent, Mississauga, ON). For functional studies, generated mutant clones included LDLR-p.G116S and LDLR-p.R730W; as positive controls, we also generated the previously reported FH-causing LDLR-p.D147H and LDLR-p.E207 variants.[14]

Cell culture and transfection

COS-7 cells or CHO cells were cultured in Dulbecco's Modification of Eagle's Medium (DMEM), or F-12K culture medium containing 1X penicillin and streptomycin, or 1X antibiotic-antimycotic, and supplemented with 10% fetal bovine serum (FBS), at 37ºC in 5% CO_2. Cells were seeded at density of 6 X 10^5 per 60 mm dish at the day before transfection. 1ug wild-type or mutant plasmid DNA was transfected per dish using Effectene Transfection Reagent and Enhancer (Qiagen, Mississauga, ON). After 48 hours, cells were washed twice with PBS, lysed in preheated at 95ºC for 5 min in sodium dodecyl sulfate (SDS) buffer (50mM Tris•Cl [pH 6.8], 2% SDS, 10% glycerol). After cell lysates were heated, they were stored at –80ºC.

Western blot analysis

Protein concentrations from whole cell lysates were determined using the DC Protein Assay Kit (Bio-Rad, Mississauga, ON). Lysates were supplemented with 50mM Tris•Cl (pH 6.8), 100mM dithiothreitol (DTT), 2% SDS, 0.1% bromophenol blue, 10% glycerol, and heated to 95^o C for 5 min, loaded on 4 -12% gradient SDS-polyacrylamide (PAGE) gels (Invitrogen, Burlington, ON)

and transferred to polyvinylidene difluoride (PVDF) membranes (Invitrogen) in a transfer module (Bio-Rad). Hybridization to anti-green fluorescent protein (GFP) monoclonal antibody (1:4000) or proprietary 4C5 anti DDK monoclonal antibody (1:1500) (Cedarlane) anti-α- tubulin (1:25,000) (Sigma, Mississauga, ON) was performed and signals were visualized using SuperSignal West Pico Chemiluminescent Substrate Kit (Pierce, Rockford, IL). The amount of GFP or Myc-DDK-tagged protein was measured and normalized to α-tubulin via densitometry.

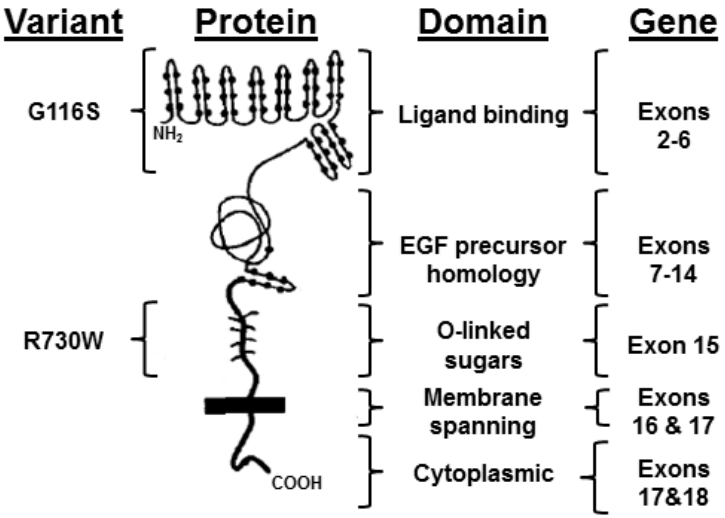
DiI-LDL uptake

HepG2 cells were plated at a density of 30,000 cells/well in 96-well poly-D-lysine-coated plate (Corning, Tewkesbury, MA) in DMEM with 10% FBS, and transient transfected with LDLR-WT and mutant clones for 24 hours and then incubated in culture medium supplemented with 10% lipoprotein deficient serum (LPDS, Biomedical Technologies, Stoughton MA) and 1 mg/ml 25-hydroxycholesterol (Sigma, Markham, ON) for 24 hours. DiI-LDL (Biomedical Technologies) was supplied at concentration of 0, 5, 10 ng/ul (0, 0.5, and 1 μg/well) with 10% LPDS and 25-hydroxycholesterol in each well, and incubated at 37 °C for 5 hours. After incubation, cells were washed twice with Tris-buffered saline containing 2 mg/ml bovine serum albumin (BSA, Sigma), and three times with TBS only. Cells were lysed with RIPA buffer (Cell Signaling, Beverly, MA), lysates were transferred to a 96-well black plate (Perkin Elmer, Walham, MA) and fluorescence was measured using SpectraMax (Molecular Devices, Downington, PA) at excitation wavelength of 514 nm and emission wavelength of 550 nm. The levels of DiI LDL for LDLR-p.E207K transfected cells were used as lack of function baseline background for DiI LDL uptake of WT and mutant plasmid transfected cells. The levels of DiI-

LDL uptake for LDLR-WT were used as reference standard for LDLR-p.G116S and LDLR-p.R730W.

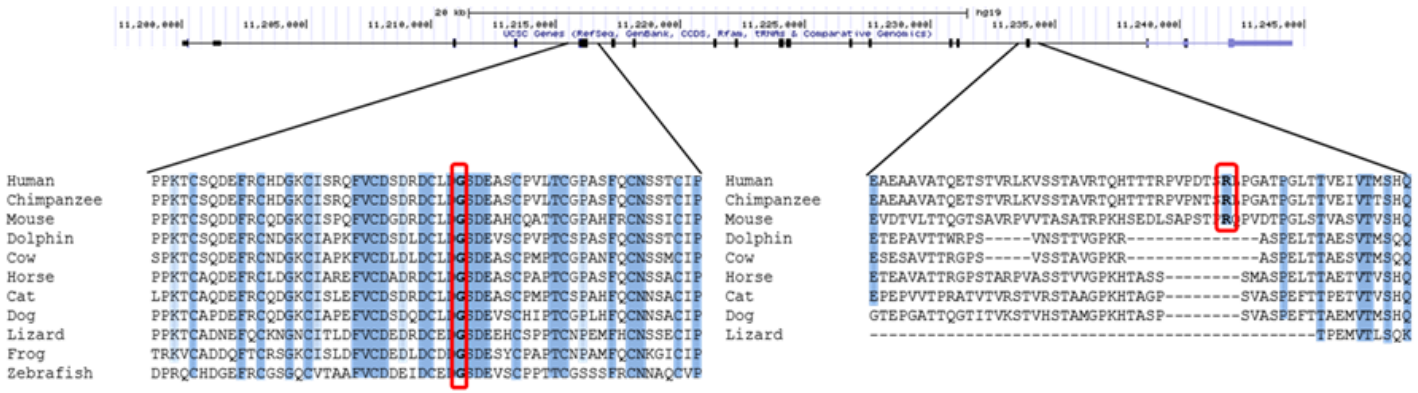
Statistical analyses

Population demographics were assessed in relation to *LDLR* variant genotypes with two-tailed unpaired t-tests or ANOVA for continuous variables using SAS v.9.2 (SAS Institute, Cary, NC). Nominal statistical significance was set at $P<0.05$. Multivariate linear regression adjusted for age, gender, and body mass index (BMI) was then used to test for association between *LDLR* p.G116S or p.R730W and blood lipid traits. Multivariate linear regression in the combined Inuit cohort was adjusted based on geographic location. Linear regression analyses used either *LDLR* genotypes or *APOE* isoform status. Multivariate logistic regression adjusted for age, gender, and BMI was used to test for association between *LDLR* genotypes and elevated LDL cholesterol status (LDL-C >5.0 mmol/L). The LDL-C cutoff of >5.0 mmol/L was based on the current Canadian Cardiovascular Society recommendations for severe dyslipidemia requiring pharmacologic lipid-lowering treatment.[15] Logistic regression was additionally adjusted based on geographic location for the combined Inuit cohort. Pairwise linkage disequilibrium and haplotype phase were assessed using PLINK (http://pngu.mgh.harvard.edu/purcell/plink/).[16] Differences in LDLR expression and activity in cell culture between wild-type and mutant LDLR were compared using two-tailed unpaired t-tests.



Supplemental Figure 1. Structural organization of the human LDL receptor protein and the relative positions of the p.G116S and p.R730W variants.

The 839-amino acid mature protein is shown here with corresponding exon and domain annotations. Modified from Hobbs *et al.*[17]



Supplemental Figure 2. Amino acid conservation in the vicinity of p.G116S and p.R730W.

Multiple amino acid residue sequence alignments from divergent species show conservation at amino acids 116 and 730 in *LDLR* (outlined in red). Amino acid residues conserved between homologs are highlighted in blue and indicate local conservation. LDLR gene representation was made using the UCSC Genome Browser.

Supplemental Table 1A: Plasma lipid traits in Inuit based on *LDLR* p.G116S or p.R730W genotype.

	total cholesterol (mmol/L)			LDL-C (mmol/L)			apolipoprotein B (g/L)		
p.G116S genotype	**GG**	**GA**	**AA**	**GG**	**GA**	**AA**	**GG**	**GA**	**AA**
Greenland	5.74±1.09	6.40±1.07	6.70±1.32**	3.63±0.99	4.34±0.98	4.70±1.12**	0.89±0.22	1.02±0.24	1.10±0.20**
Kivalliq	4.93±0.97	6.02±0.97*	n.d.	3.02±0.87	4.11±0.90*	n.d.	0.95±0.24	1.36±0.12**	n.d.
Inuvialuit	5.00±0.98	5.58±1.07	5.05±0.11*	2.88±0.86	3.43±1.03	3.27±0.20*	0.90±0.25	1.03±0.25	0.95±0.04*
Nunavik	4.90±0.94	5.38±1.13	6.27±0.58**	2.72±0.82	3.11±0.97	3.90±0.77**	0.94±0.24	1.02±0.24	1.24±0.19*
SW Alaska	5.16±1.10	5.62±1.32	5.57±0.96**	3.12±0.91	3.58±1.17	3.61±0.96**	n.d.	n.d.	n.d.
Combined	5.29±1.11	5.94±1.24	6.20±1.25**	3.22±0.98	3.88±1.14	4.21±1.14**	0.90±0.23	1.03±0.24	1.11±0.20**

	total cholesterol (mmol/L)			LDL-C (mmol/L)			apolipoprotein B (g/L)		
R730W genotype	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Greenland	5.91±1.12	5.90±1.17	6.1±1.09	3.83±1.03	3.78±1.08	3.73±0.72	0.92±0.22	0.91±0.24	0.95±0.31
Kivalliq	4.98±1.00	4.87±1.00	5.18±0.91	3.08±0.90	2.96±0.92	3.19±0.76	0.97±0.25	0.95±0.26	0.98±0.22
Inuvialuit	5.10±1.01	4.86±0.97	5.15±0.81	2.97±0.90	2.78±0.86	2.98±0.40	0.92±0.26	0.87±0.24	0.91±0.18
Nunavik	4.94±0.96	4.93±0.90	5.52±0.93	2.80±0.84	2.71±0.85	3.14±1.04	0.95±0.23	0.93±0.24	1.04±0.28
SW Alaska	5.23±1.16	5.29±1.14	5.18±1.08	3.21±1.00	3.22±0.94	3.19±0.85	n.d.	n.d.	n.d.
Combined	5.43±1.17	5.37±0.17	5.47±1.07	3.37±1.06	3.28±1.04	3.20±0.82*	0.93±0.23	0.91±0.24	0.97±0.27

* indicates P<0.05 and **indicates P<0.0001 using ANOVA adjusted for age, sex and BMI. Abbreviations as in Table 1.

Supplemental Table 1B: Plasma lipid traits in Inuit based on *LDLR* p.G116S or p.R730W genotype.

	HDL-C (mmol/L)			Non-HDL-C (mmol/L)			Triglyceride (mmol/L)		
p.G116S genotype	**GG**	**GA**	**AA**	**GG**	**GA**	**AA**	**GG**	**GA**	**AA**
Greenland	1.58±0.45	1.54±0.40	1.58±0.48	4.16±1.09	4.84±1.12	5.13±1.18**	1.18±0.68	1.16±0.66	0.96±0.30
Keewatin	1.44±0.41	1.51±0.47	n.d.	3.48±0.94	4.51±0.89*	n.d.	1.01±0.55	0.87±0.39	n.d.
Inuvialuit	1.35±0.41	1.39±0.40	1.17±0.15	3.65±1.03	4.20±1.03	3.88±0.04*	1.74±1.32	1.86±0.81	1.34±0.54
Nunavik	1.61±0.42	1.71±0.47	1.92±0.77	3.25±0.99	3.68±1.05	4.35±0.90*	1.24±0.75	1.25±0.56	0.99±0.32
SW Alaska	1.64±0.44	1.64±0.45	1.56±0.46	3.52±1.03	3.98±1.26	4.01±1.01**	0.96±0.58	0.90±0.47	0.94±0.47
Combined	1.57±0.44	1.59±0.43	1.58±0.49	3.71±1.09	4.35±1.24	4.62±1.20**	1.13±0.75	1.09±0.62	0.97±0.38

	HDL-C (mmol/L)			Non-HDL-C (mmol/L)			Triglyceride (mmol/L)		
p.R730W genotype	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Greenland	1.57±0.44	1.56±0.45	1.65±0.46	4.33±1.13	4.34±1.20	4.45±1.17	1.14±0.64	1.24±0.75	1.35±0.95
Keewatin	1.43±0.41	1.46±0.37	1.65±0.53	3.57±0.97	3.41±1.02	3.53±0.80	1.04±0.54	0.98±0.59	0.74±0.28
Inuvialuit	1.36±0.41	1.40±0.46	1.42±0.35	3.73±1.05	3.48±0.99	3.67±0.76	1.80±1.38	1.54±0.78	1.70±0.72
Nunavik	1.61±0.43	1.67±0.42	1.83±0.38	3.31±0.98	3.23±0.99	3.69±1.17	1.56±0.63	1.21±0.72	1.20±0.44
SW Alaska	1.62±0.43	1.68±0.47	1.70±0.42	3.62±1.10	3.61±1.05	3.48±1.06	0.95±0.58	0.95±0.52	1.00±0.54
Combined	1.56±0.44	1.61±0.46	1.68±0.45*	3.86±1.15	3.76±0.16	3.80±1.10*	1.12±0.76	1.11±0.67	1.11±0.68

* indicates P<0.05 and **indicates P<0.0001 using ANOVA adjusted for age, sex and BMI. Abbreviations as in Table 1.

Supplemental Table 2. *APOE* E4 effect on LDL cholesterol in Inuit.

Population	E4 frequency	β (mmol/L)	SE	P-value
Greenland	0.22	0.22	0.049	8.2×10^{-6}
Kivalliq	0.21	0.06	0.10	0.56
Inuvialuit	0.23	0.006	0.10	0.95
Nunavik	0.27	0.09	0.06	0.17
SW Alaska	0.24	0.25	0.045	3.6×10^{-8}
Combined	0.23	0.18	0.027	9.0×10^{-11}

APOE E4 effect sizes were calculated in comparison to E3 carriers using linear regression adjusted for age, sex and BMI in Kivalliq (N=200), Greenland (N=1,096), Inuvialuit region (N=212), Nunavik (N=383), SW Alaska (N=1,203) and a combined cohort (N=3,094). *APOE* E4 frequencies were calculated from larger populations including *APOE* E2, E3 and E4 carriers. *APOE* isoforms were inferred using TaqMan SNP genotyping assays for SNPs rs429358 and rs7412 (Applied Biosystems; Foster City, CA).[18]

Supplemental Table 3. *In silico* analyses of *LDLR* p.G116S and p.R730W and LDLR function.

Position	Variant	Nucleotide substitution	*in silico* algorithm	Score	Prediction
Chr19:11,215,991	p.G116S Exon 4	c.409G>**A**	PMUT	n.d.	Neutral
			SIFT	0.01	Damaging
			PolyPhen	0.999	Probably damaging
			MutPred	n.d.	0.81 probability of deleterious mutation
Chr19:11,233,960	p.R730W Exon 15	c. 2251C>**T**	PMUT	n.d.	Pathological
			SIFT	0.04	Damaging
			PolyPhen	0.951	Possibly damaging
			MutPred	n.d.	0.51 probability of deleterious mutation

Amino acid positions for mutations refer to the mature protein. Abbreviations as in Table 1.

Supplemental References

1. Mohatt GV, Plaetke R, Klejka J, Luick B, Lardon C, Bersamin A, et al. The Center for Alaska Native Health Research Study: a community-based participatory research study of obesity and chronic disease-related protective and risk factors. I*nt J Circumpolar Health.* 2007;66:8-18.

2. Chateau-Degat ML, Dewailly E, Louchini R, Counil E, Noel M, Ferland A, et al. Cardiovascular burden and related risk factors among Nunavik (Quebec) Inuit: insights from baseline findings in the circumpolar Inuit health in transition cohort study. *Can J Cardiol.* 2010;26:190-196.

3. Moffatt ME, Young TK, O'Neil JD, Eidelheit S, Fish I, Mollins J. The Keewatin Health Assessment Study, NWT, Canada. *Arctic Med Res.* 1993;52:18-21.

4. Bjerregaard P, Mulvad G, Pedersen HS. Cardiovascular risk factors in Inuit of Greenland. *Int J Epidemiol.* 1997;26:1182-1190.

5. Ferrer-Costa C, Gelpi JL, Zamakola L, Parraga I, de la Cruz X, Orozco M. PMUT: a web-based tool for the annotation of pathological mutations on proteins. *Bioinformatics.* 2005;21:3176-3178.

6. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. *Nat Methods.* 2010;7:248-249.

7. Li B, Krishnan VG, Mort ME, Xin F, Kamati KK, Cooper DN, et al. Automated inference of molecular mechanisms of disease from amino acid substitutions. *Bioinformatics.* 2009;25:2744-2750.

8. Ng PC, Henikoff S. Predicting deleterious amino acid substitutions. *Genome Res.* 2001;11:863-874.

9. Kent WJ. BLAT--the BLAST-like alignment tool. *Genome Res.* 2002;12:656-664.

10. Stenson PD, Ball EV, Howells K, Phillips AD, Mort M, Cooper DN. The Human Gene Mutation Database: providing a comprehensive central mutation database for molecular diagnostics and personalized genomics. *Hum Genomics.* 2009;4:69-72.

11. Fu J, Kwok S, Sinai L, Abdel-Razek O, Babula J, Chen D, et al. Western Database of Lipid Variants (WDLV): a catalogue of genetic variants in monogenic dyslipidemias. *Can J Cardiol.* 2013;29:934-939.

12. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature.* 2012;491:56-65.

13. Tennessen JA, Bigham AW, O'Connor TD, Fu W, Kenny EE, Gravel S, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. *Science.* 2012;337:64-69.

14. Hobbs HH, Brown MS, Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. *Hum Mutat.* 1992;1:445-466.

15. Anderson TJ, Gregoire J, Hegele RA, Couture P, Mancini GB, McPherson R, et al. 2012 update of the Canadian Cardiovascular Society guidelines for the diagnosis and treatment of dyslipidemia for the prevention of cardiovascular disease in the adult. *Can J Cardiol.* 2013;29:151-167.

16. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81:559-575.

17. Hobbs HH, Russell DW, Brown MS, Goldstein JL. The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein. *Annu Rev Genet.* 1990;24:133-170.

18. Fullerton SM, Clark AG, Weiss KM, Nickerson DA, Taylor SL, Stengard JH, et al. Apolipoprotein E variation at the sequence haplotype level: implications for the origin and maintenance of a major human polymorphism. *Am J Hum Genet.* 2000;67:881-900.

Circulation
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Common Low-Density Lipoprotein Receptor p.G116S Variant Has a Large Effect on Plasma Low-Density Lipoprotein Cholesterol in Circumpolar Inuit Populations

Joseph B. Dubé, Jian Wang, Henian Cao, Adam D. McIntyre, Christopher T. Johansen, Scarlett E. Hopkins, Randa Stringer, Siyavash Hosseinzadeh, Brooke A. Kennedy, Matthew R. Ban, T. Kue Young, Philip W. Connelly, Eric Dewailly, Peter Bjerregaard, Bert B. Boyer and Robert A. Hegele

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