RESEARCH ARTICLE

Increased Missense Mutation Burden of Fatty Acid Metabolism Related Genes in Nunavik Inuit Population

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Abstract

Background

Nunavik Inuit (northern Quebec, Canada) reside along the arctic coastline where for generations their daily energy intake has mainly been derived from animal fat. Given this particular diet it has been hypothesized that natural selection would lead to population specific allele frequency differences and unique variants in genes related to fatty acid metabolism. A group of genes, namely CPT1A, CPT1B, CPT1C, CPT2, CRAT and CROT, encode for three carnitine acyltransferases that are important for the oxidation of fatty acids, a critical step in their metabolism.

Methods

Exome sequencing and SNP array genotyping were used to examine the genetic variations in the six genes encoding for the carnitine acyltransferases in 113 Nunavik Inuit individuals.

Results

Altogether ten missense variants were found in genes CPT1A, CPT1B, CPT1C, CPT2 and CRAT, including three novel variants and one Inuit specific variant CPT1A p.P479L (rs80356779). The latter has the highest frequency (0.955) compared to other Inuit populations. We found that by comparison to Asians or Europeans, the Nunavik Inuit have an increased mutation burden in CPT1A, CPT2 and CRAT; there is also a high level of population differentiation based on carnitine acyltransferase gene variations between Nunavik Inuit and Asians.
Conclusion
The increased number and frequency of deleterious variants in these fatty acid metabolism genes in Nunavik Inuit may be the result of genetic adaptation to their diet and/or the extremely cold climate. In addition, the identification of these variants may help to understand some of the specific health risks of Nunavik Inuit.

Introduction
Modern Inuit are descendants of the Dorset peoples from the second wave of migrations which took place over 1000 years ago [1]. Previous studies have established that these Inuit are genetically closest to the contemporary north-east Siberian populations [2], and belong to one of the three native ancestral groups that populated the Americas. The ancestors of today’s Nunavik (a region of northern Quebec) Inuit had migrated from Alaska to the east across the far north. With a population of barely 10,000 individuals, Nunavik Inuit form a very isolated population whose ancestral genetic profiles are likely to be well preserved until today. The Inuit enrolled in this study were recruited from 13 of the 14 inhabited villages, and they represent over 1% of the Nunavik Inuit population. As a part of their unique lifestyle, Nunavik Inuit derive approximately 75% of their daily energy intake from animal fat [3]. The traditional diet (high in fat and low in carbohydrates) of Nunavik Inuit suggests that they have high rates of gluconeogenesis, which is supported by their larger liver size compared to other populations [3].

The oxidative processing of fatty acids is critical for generating energy from a diet enriched with animal fat. Several enzymes are involved in the metabolism of fatty acids, including the carnitine acyltransferases [4]. The carnitine acyltransferase gene family is comprised of six members which encode for three types of enzymes: (1) carnitine palmitoyltransferases (CPTs) encoded by \textit{CPT1A}, \textit{CPT1B}, \textit{CPT1C} and \textit{CPT2}; (2) carnitine acetyltransferase (CrAT) encoded by \textit{CRAT}; and (3) carnitine octanoyltransferase (CrOT) encoded by \textit{CROT} [4]. The genomic features of these six genes are listed in Table 1. CPTs include two members, CPT-I and CPT-II. As a rate-limiting step in fatty acid oxidation, CPT-I converts long-chain fatty acyl molecules

| Encode                  | Genomic region of longest isoform (number of isoforms) | Number of exons | HGMD mutation numbers from of all isoforms | Loss of function variant* from EVS |
|-------------------------|--------------------------------------------------------|----------------|------------------------------------------|-----------------------------------|
| \textit{CPT1A}          | chr11:68522088–68609399 (2)                            | 20             | 29 (missense); 1 (splicing); 5 (indel)    | 3                                 |
| \textit{CPT1B}          | chr22:51007290–51017096 (4)                            | 21             | 2 (missense); 1 (splicing)               | 9                                 |
| \textit{CPT1C}          | chr19:50194365–50216988 (2)                            | 20             | N/A                                      | 4                                 |
| \textit{CPT2}           | chr1:53662101–53679869 (1)                             | 5              | 65 (missense); 4 (splicing); 20 (indel)   | 3                                 |
| \textit{CRAT}           | chr9:131857073–131873070 (1)                           | 15             | N/A                                      | 3                                 |
| \textit{CROT}           | chr7:86974951–86989425 (3)                             | 18             | N/A                                      | 12                                |

HGMD: Human Gene Mutation Database, including all published gene mutations responsible to human inherited disease.
EVS: NHLBI Exome sequencing project exome variant server, including approximately 4,294 European Americans and 2,200 African Americans.
*Including nonsense mutations, frameshift mutations, splicing donor and acceptor site mutations.

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into their corresponding acylcarnitines, which are then transported across the inner mitochondri-
drial membrane for beta-oxidation. CPT-II is responsible for the reversal of this process [5].
CrOT and CrAT catalyze the reversible transfer of fatty acyl groups between CoA and carni-
tine, and therefore determine the acetyl-CoA/CoA ratio [6].

CPT-I and II deficiencies are genetic disorders that are characterized by the reduced CPT
enzyme activity which can lead to severe consequences, such as liver failure and renal problems
[5, 7, 8]. Although these deficiencies are commonly caused by autosomal recessive mutations
in CPT1A and CPT2, heterozygous carriers of CPTs mutations can also be symptomatic [9, 10].

Considering their critical roles in lipid metabolism and energy generation, the six genes en-
coding carnitine acyltransferases may be subjected to evolutionary pressure, especially in popu-
lations such as the Inuit, which require an increased enzyme activity because of their special
diet and extreme living conditions. Recent studies have shown that the diet of Nunavik Inuit
led to changes in specific biomarkers. For instance, Nunavik Inuit have very high levels of n-3
polyunsaturated fatty acids (PUFA), especially in the elderly [11], as well as high levels of plas-
ma LDL cholesterol [12]. It is possible that these changes may be partially due to the variations
in the carnitine acyltransferase genes, which can affect their enzymatic activity. Because of
these biochemical characteristics, it is important to study the genes that are critical to fatty acid
metabolism. In addition to providing insight about the Nunavik Inuit adaptation, the identifi-
cation of variants in these genes may also help to understand some of their specific health risks.
To examine this hypothesis, we used exome sequencing and SNP array genotyping to investi-
gate genetic variants across these six genes in a cohort of Inuit from Nunavik.

Materials and Methods
Subjects
We recruited 113 Inuit individuals (62 males and 51 females, mean age 52 years) from 13 in-
habitied villages of Nunavik. The study was approved by Comité d’éthique de la recherche du
Centre hospitalier de l’Université de Montréal (ND 04.101) (Québec, Canada) and the Nunavik
Nutrition and Health Committee (Québec, Canada). Individual written consent was obtained
from each participant before entering this study.

Exome sequencing and coding variant identification from six carnitine
acyltransferase genes
The genomic DNA of 113 Nunavik Inuit individuals was extracted from peripheral blood lym-
phocytes using Gentra Systems PUREGENE DNA purification kit (Qiagen). A 50 μl DNA sam-
ple at a concentration of 100 ng/μl from each sample was captured by Agilent SureSelect
50mb/V4 capture kit. The library was subsequently sequenced at 100 bp pair-end using Illu-
mina Hiseq 2000, with 3 samples per lane to ensure an average coverage depth of 100-fold.

Raw fastq files were aligned to NCBI human reference GRCh37 using Burrows-Wheeler
Aligner (BWA) [13], with all PCR duplicates removed from the alignments. The aligned reads
were converted to binary format for further analysis using Sequence Alignment/Map (SAM)
tools [14]. Single nucleotide variant (SNV) and insertion/deletion (indel) calling were per-
fomed using Genome Analysis Toolkit (GATK) version 2.7 [15]. Variant annotation was per-
fomed using ANNOVAR program [16] with references to GRCh37/hg19, dbSNP version 132,
1000 Genomes project (1KGP) (2012 data release) [17], 69 Complete Genomics (2012 update)
and exome variant server (EVS) with approximately 6,500 exomes (NHLBI-ESP project, 2013
update) [18]. Finally, variant segregation analysis was performed using an in-house segregation
program, using more than 1,000 exomes of different ethnicities from our lab as controls.
In the variant analysis, intronic, intergenic and non-coding variants were excluded because of insufficient coverage. Quality filters were set at sequencing depth $\geq 20x$ and variant frequency $\geq 25\%$, with genotype quality $\geq 10$. Rare variants were defined as variants with minor allele frequency (MAF) $\leq 0.01$ in the aforementioned public databases. All potential missense and splicing site variants from the genes CPT1A, CPT1B, CPT1C, CPT2, CRAT and CROT were examined, and were further validated by manual inspection using the Integrative Genomics Viewer 1.4 (IGV) [19] and Sanger sequencing.

### SNP genotyping and common variant identification from six carnitine acyltransferase genes

SNP genotyping was performed on 113 Nunavik Inuit, using Illumina HumanOmnExpress-12 SNP array, which contains 730,525 SNPs. Raw data was processed and analyzed using Illumina GenomeStudio and quality control was performed by PLINK 1.07 [20]. SNPs within each gene region and 2,000 bp up/downstream flanking regions were extracted from all individuals. Variant concordance within these regions was calculated between data from exome sequencing and SNP array genotyping. Allele frequencies and Hardy–Weinberg equilibrium (HWE) of all selected SNP were calculated using PLINK. Calculation of linkage disequilibrium (LD) and haplotype analysis were performed using Haplovew 4.2 [21].

### Statistical analysis

Multidimensional scaling (MDS) and Admixture analyses [22] were performed on the Nunavik Inuit genotype data against HapMap genotype data from Asian (CHB and JPT), European (CEU) and African (YRI) populations to assess the mixture of Nunavik Inuit population.

Missense variations of the carnitine acyltransferase genes were retrieved from 286 Chinese (CHB) and Japanese (JPT) from the 1KGP database and European descendants from EVS, to serve as controls.

Mutation burden is defined as the average number of rare (MAF $\leq 0.01$) missense variant alleles per person. To examine whether there is an increased mutation burden in the selected genes in Nunavik Inuit compared to other populations, we performed a binomial test to compare rare allele frequencies between Nunavik Inuit and 1KGP Asians. The Adaptive Permutation test was also performed to compare the mutation burden in Nunavik Inuit with Asians. The Permutation test was performed five times based on the number of variant-containing genes, with Bonferroni correction for multiple comparisons applied and $p < 0.01$ was set as statistically significant.

We also calculated two F-statistics ($F_{ST}$ and $F_{IS}$) scores based on variants identified in the carnitine acyltransferase genes. The $F_{ST}$ value provides an indication about the population genetic difference that is due to the genetic drift between subpopulations. The $F_{IS}$ value measures the proportion of total inbreeding within a population that is due to non-random mating within subpopulations [23]. To further compare the impact of these variants in Nunavik Inuit to other populations, we generated a scatterplot using the R package "ggplot2" of all functional variants identified in CPT1A, CPT1B, CPT1C, CPT2 and CRAT in Nunavik Inuit, 1KGP Asians and EVS Europeans. Mann-Whitney U test was performed to compare the PolyPhen-2 scores of all rare missense variants found in Nunavik Inuit and 1KGP Asian populations, with $p < 0.05$ as significant.

### Results

#### Data quality control

Using the data from the HumanOmnExpress SNP array, four individuals with sex mismatch were identified by PLINK and removed from the subsequent analysis. After MDS testing, the
genomes of nine additional individuals were found to have a mixture of both Inuit and European genomes and were therefore also removed from subsequent analysis. This mixture is probably the results of intermarriages between Nunavik Inuit and French-Canadians. Therefore a total of 100 Inuit samples were included in the genetic/statistical analysis (Fig 1).

Based on GATK’s DepthOfCoverage Walker [15] program, the average coverage depth of the coding regions of CPT1A, CPT1B, CPT1C, CPT2, CRAT and CROT were 102X, 134X, 107X, 102X, 101X and 80X, respectively. The percentage of targeted region with coverage depth of more than 20X for each gene were 92.7%, 97.3%, 99.6%, 94.1%, 98.6% and 90.2%, respectively (S1 Fig).

Of the 6 carnitine acyltransferase genes, coding variants were identified only in CPT1A, CPT1B, CPT1C, CPT2 and CRAT in Nunavik Inuit. Since no coding variants were found in CROT, all subsequent analyses and discussions were focused on the other 5 genes. Across these genes, 16 variants were identified by both the exome sequencing and SNP array, 4 of which were exonic variants. The concordance rate of the exonic variants between the two platforms was 100%, while for the intronic variants it was 77% (S1 Table). VCF (Variant Call Format) files of the exome sequencing data and PLINK genotype files of the SNP array covering the carnitine acyltransferase gene regions can be found in S1 Archive.

**Missense variants of the carnitine acyltransferase genes in different populations**

In the 100 individuals included in the analysis, 10 missense and 3 synonymous variants were found in 5 carnitine acyltransferase genes. Three missense variants, CPT1C p.T265M, CPT2 p. R477W, CRAT p.S78F and one synonymous variant CPT1A p.V616V, were unique to the Nunavik Inuit population (Table 2 and S2 Table). In addition, an Inuit specific variant CPT1A p.P479L was also observed. These five variants and a rare synonymous CRAT p.A575A variant were validated by Sanger sequencing (S2 Fig). The frequencies of the 10 missense variants were compared between the Nunavik Inuit, Asian, European and African populations. *In silico*
prediction using PolyPhen-2 [24] and MutationTaster [25] suggested that the 4 Inuit specific missense variants are potentially deleterious (Table 3). Even with the exclusion of the Inuit high frequency variant CPT1A p.P479L (rs80356779), the Polyphen-2 scores of all the rare variants found in Nunavik Inuit and 1KGP Asian population were still significantly higher (Mann-Whitney U test, \( P = 0.02 \)) for Nunavik Inuit (Table 4 and S3 Fig).

## Mutation burden test and F-statistics of variants in the carnitine acyltransferase genes in Nunavik Inuit

In order to characterize the genetic profile of the carnitine acyltransferase genes in Nunavik Inuit, the mutation burden for each gene was calculated in the Inuit and compared to the mutation burden in 286 Asians (CHB and JPT) from 1KGP. Nunavik Inuit had significantly more mutated alleles per individual in the CPT1A, CPT2 and CRAT genes than Asians (Binomial test, \( p < 0.01 \)). Permutation test showed similar results with marginally significant \( p \) values in CPT2 and CRAT (not significant after Bonferroni correction) (Table 5).

### Table 2. Coding variants of carnitine acyltransferase genes discovered in Nunavik Inuit.

| Gene | Variant | Variation type | Annotation |
|------|---------|----------------|------------|
| CPT1A | p.V616V | synonymous | novel |
| CPT1A | p.P479L | missense | rs80356779 |
| CPT1A | p.F417F | synonymous | rs2228502 |
| CPT1B | p.E531K | missense | rs470117 |
| CPT1B | p.S427C | missense | rs8142477 |
| CPT1B | p.I66V | missense | rs3213445 |
| CPT1C | p.T265M | missense | novel |
| CPT2 | p.F352C | missense | rs2229291 |
| CPT2 | p.V368I | missense | rs1799821 |
| CPT2 | p.R477W | missense | novel |
| CRAT | p.A603P | missense | rs17459086 |
| CRAT | p.A575A | synonymous | rs375414636 |
| CRAT | p.S78F | missense_splicing | novel |

CHB, JPT, CEU, YRI are from 1KGP.

*Based on the derived allele from the reference genome.

### Table 3. Variant frequencies and deleterious score predictions of carnitine acyltransferase genes.

| Gene | Protein coding variants | SNP        | MutationTaster | PolyPhen v2 | Frequency* in Nunavik Inuit (100) | Frequency in CHB-JPT (286) | Frequency in CEU (178) | Frequency in YRI (250) |
|------|-------------------------|------------|----------------|-------------|-----------------------------------|-----------------------------|------------------------|------------------------|
| CPT1A | p.P479L | rs80356779 | 0.997899 | 1 | 95.5% | 0 | 0 | 0 |
| CPT1B | p.E531K | rs470117 | 0.606166 | 0.303 | 30.5% | 48.3% | 46.5% | 8.8% |
| CPT1B | p.S427C | rs8142477 | 0 | 0 | 67.5% | 51.7% | 93.3% | 25.8% |
| CPT1B | p.I66V | rs3213445 | 0.251908 | 0 | 17.5% | 35.8% | 4.9% | 10.2% |
| CPT1C | p.T265M | rs80356779 | 0.999717 | 0.998 | 0.5% | 0 | 0 | 0 |
| CPT2 | p.F352C | rs2229291 | 0.999596 | 0.999 | 28.0% | 20.0% | 0 | 1.3% |
| CPT2 | p.V368I | rs1799821 | 0.06145 | 0.001 | 52.5% | 77.5% | 54.9% | 25.2% |
| CPT2 | p.R477W | rs2229291 | 0.997459 | 1 | 3.0% | 0 | 0 | 0 |
| CRAT | p.A603P | rs17459086 | 0.999409 | 0.013 | 9.0% | 7.0% | 1.7% | 0.8% |
| CRAT | p.S78F | rs375414636 | 0.999657 | 0.906 | 2.5% | 0 | 0 | 0 |

CHB, JPT, CEU, YRI are from 1KGP.

*Based on the derived allele from the reference genome.
FST and FIS scores were calculated for 100 Nunavik Inuit and 83 HapMap Asians (CHB and JPT). The CPT1A p. P479L variant had the highest FST value (0.92), and the mean FST of the 4 Inuit specific variants was also statistically significant (0.84, p < 0.01). However, the mean FIS of all variants did not significantly deviate from zero after 1,000 randomizations (-0.0092) (Table 6).

Fig 2 depicted the frequencies and deleterious scores of the functional variants of the carnitine acyltransferase gene in different populations. The allele frequencies of all missense variants in European descendants (EVS database), Asians (1KGP) and the Nunavik Inuit were plotted against the PolyPhen-2 score of each variant. Variants with PolyPhen-2 score >0.8 (predicted to be highly deleterious) were presented at higher frequencies in the Nunavik Inuit compared to Asians and European descendants (Fig 2).

Common missense variants in the carnitine acyltransferase genes

HWE testing did not show a significant deviation for any of the exonic variants identified in the 5 genes. Haplotype analysis found that 3 common Nunavik Inuit CPT1B missense variants (rs470117, rs8142477 and rs3213445) were in an 8 kb LD block. The frequency of this haplotype in the Inuit (0.66) was significantly higher than in Asians (0.46) (Binomial test,

Table 4. Deleterious scores of all rare missense variants in carnitine acyltransferase genes found in Nunavik Inuit and 1KGP Asians.

| Gene | Variant | PolyPhen v2 | Derived allele frequency (%) |
|------|---------|-------------|-----------------------------|
|      |         |             | Nunavik Inuit (100)         |
| CPT1A| p.P479L | 1           | 95.5                        |
| CPT1C| p.T265M | 0.998       | 0.5                         |
| CPT2 | p.R477W | 1           | 3                           |
| CRAT | p.S78F  | 0.906       | 2.5                         |
|      |         |             | 1KGP Asians (286)           |
| CPT1A| p.I491T | 0           | 0.35                        |
| CPT1B| p.C659W | 1           | 0.35                        |
| CPT1C| p.R514Q | 1           | 0.35                        |
| CPT1C| p.Q97H  | 0.08        | 0.35                        |
| CPT2 | p.S122F | 0.018       | 0.6                         |
| CRAT | p.V411M | 0.803       | 0.35                        |

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Table 5. Mutation burden scores of identified rare mutations (MAF<0.01) of carnitine acyltransferase genes in Nunavik Inuit and 1KGP Asians.

| Gene | Variants | # variant alleles/Inuit (100) | # variant alleles/Asian (286) | Inuit/Asian ration | Base-pair sequenced | P 1 | P 2 |
|------|----------|------------------------------|-----------------------------|-------------------|-------------------|-----|-----|
| CPT1A| p.P479L*, p.I491T | 1.91                         | 0.007                       | 272.80            | 2319              | 2.2e-16 | 0.000006 |
| CPT1B| p.C659W  | 0                            | 0.007                       | 0.00              | 2316              | 1   | 0.75 |
| CPT1C| p.T265M*, p.Q97H, p.R514Q | 0.01                     | 0.014                       | 0.71              | 2409              | 1   | 1   |
| CPT2 | p.R477W*, p.S122F | 0.06                        | 0.017                       | 3.53              | 1974              | 0.003 | 0.03689 |
| CRAT | p.S78F*, p.V411M | 0.05                        | 0.007                       | 7.14              | 1815              | 0.00076 | 0.01305 |

1: Binomial test, Bonferroni corrected, significant p<0.01.

2: Empirical p-value of permutation test, 10^6 permutations.

*Variants only identified in Inuit.

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In CPT2, two common missense variants rs2229291 (p.F352C) and rs1799821 (p.V368I) are in a 1 kb LD block (Fig 3). These variants along with the Nunavik Inuit specific variant p. R477W form four major haplotypes in the studied cohort: T-G-C (45%), T-A-C (24%), G-A-C (28%) and T-G-T (3%), respectively. While in Europeans and Africans, only the T-G-C and T-A-C haplotypes were found. In Nunavik Inuit the G-A-C haplotype frequency is 28% compared to 10% in Asians [26], and absent in Europeans (Binomial test, p = 3.481e-07).

Discussion

Carnitine acyltransferase gene variants in the Nunavik Inuit

Our results indicate that Nunavik Inuit are genetically distinct from European, African and Asian populations, with the closest relationship to Asians. In 100 Nunavik Inuit we found three novel missense variants and a known Inuit specific missense variant in 4 carnitine acyltransferase genes. The rare missense mutations are significantly more deleterious and their frequencies are significantly higher in Nunavik Inuit, compared to other populations. Interestingly, we didn’t find CROT missense variants in either Nunavik Inuit or Asians.

Mutation burden testing shows that mutations in the CPT1A, CPT2 and CRAT genes are significantly more frequent in Nunavik Inuit compared to Asians. The mean FST value (0.18) of all variants indicates a great genetic differentiation between Nunavik Inuit and Asians ($F_{ST}>0.15$); it is also noteworthy that the 4 Inuit specific variants are indicating an even higher degree of differentiation for this population ($F_{ST}>0.25$ (Table 6). However the $F_{IS}$ value shows no statistical significance, which may due to the limited number of variants available. Nevertheless, the negative mean $F_{IS}$ value suggests that there could be an excess of heterozygotes within the populations.

Table 6. The $F_{ST}$ and $F_{IS}$ value of 13 variants in the population containing Nunavik Inuit and HapMap Asians.

| Variant | SNP           | $F_{ST}$  | $F_{IS}$  |
|---------|---------------|-----------|-----------|
| CPT1A: p.V616V |               | 0.0196    | -0.04     |
| CPT1A: p.P479L | rs80356779   | 0.9171*   | -0.0452   |
| CPT1A: p.F417F | rs2228502    | 0.0506    | -0.1067   |
| CPT1B: p.E531K | rs470117     | 0.0414    | -0.0273   |
| CPT1B: p.S427C | rs8142477    | 0.034     | 0.0382    |
| CPT1B: p.I66V | rs3213445    | 0.0453    | 0.0114    |
| CPT2: p.F352C | rs2229291    | 0.0298    | -0.0925   |
| CPT2: p.V368I | rs1799821    | 0.0546    | 0.0349    |
| CPT2: p.R477W |               | 0.0146    | -0.0297   |
| CRAT: p.A603P | rs17459086   | 0.0045    | -0.0227   |
| CRAT: p.A575A | rs375414636  | 0.0097    | -0.0196   |
| CRAT: p.S78F |               | 0.0122    | -0.0246   |
| CPT1C: p.T265M |               | 0.0024    | -0.0048   |
| Mean (all variants) | | 0.1833    | -0.0092   |
| Mean (missense variants) | | 0.1907    | -0.0053   |
| Mean (Inuit specific variants) | | 0.8401*   | -0.0393   |

$F_{ST}$: The fixation index, which is the proportion of total genetic variance contained in a subpopulation relative to the total genetic variance. The value of $F_{ST}$ ranges from 0 to 1, the higher value implies higher degrees of population differentiation.

$F_{IS}$: The inbreeding coefficient, which is the proportion of genetic variance in the subpopulation contained in an individual.

*Statistical significance, 1000 randomizations

p = 7.501e-05). In CPT2, two common missense variants rs2229291 (p.F352C) and rs1799821 (p.V368I) are in a 1 kb LD block (Fig 3). These variants along with the Nunavik Inuit specific variant p. R477W form four major haplotypes in the studied cohort: T-G-C (45%), T-A-C (24%), G-A-C (28%) and T-G-T (3%), respectively. While in Europeans and Africans, only the T-G-C and T-A-C haplotypes were found. In Nunavik Inuit the G-A-C haplotype frequency is 28% compared to 10% in Asians [26], and absent in Europeans (Binomial test, p = 3.481e-07).
Nunavik Inuit population specific variants

**CRAT and CPT2.** The CRAT p.S78F mutation is located at the splicing site and is predicted to affect splicing, potentially leading to a loss of function of CrAT and carnitine acetyltransferase deficiency. Moreover, in a muscle-specific CrAT knockout mouse model, CrAT acts as a modulator of whole-body glucose homeostasis and metabolic flexibility [27]. The Inuit appear to tolerate drastic changes in metabolic homeostasis, hence the loss of CrAT function may be beneficial to them. Symptomatic CPT-II deficiency is usually caused by
homozygous or compound heterozygous mutations in CPT2. The CPT2 p.R477W mutation found in the Nunavik Inuit is likely to lead to the loss of function as it is located in a highly conserved region where mutations known to cause CPT-II deficiency occur [28, 29]. It is possible that heterozygote loss of function mutations in CPT2 may have a beneficial effect on the enzyme activity in Nunavik Inuit.

The high frequency CPT1A p.P479L variant. In general, missense mutations are rare in CPT1A; yet in some Inuit populations, the p.P479L loss of function mutation has a high frequency, ranging from 44% to 83% from Alaska to Greenland [30, 31]. In our study of the Nunavik Inuit, the p.P479L mutation has an allele frequency of 95.5%, the highest reported to date. The frequency of this variant seems to increase from the west to the east along the arctic tree line and from inland to the shore (S4 Fig), correlating with the migration timeline and with the higher consumption of animal fat of residents near the shore. The CPT1A p.P479L mutation is significantly more frequent among Nunavik Inuit compared to 243 Kivalliq Inuit (western Nunavut) (χ² = 15.629, p = 0.0004) [31]. Interestingly, the CPT1A p.P479L mutation is absent from all other worldwide populations, including East Asians (Table 3). This variant was initially believed to cause CPT-I deficiency, since the first discovery was made in a Canadian First Nation man with myopathy [32], a typical subtype of CPT1A deficiency. Therefore, this variant has been included in the Alaska newborn screening protocol [33]. In addition, in Canadian Inuit and First Nation families with severe CPT-I deficiency, the only missense mutation found in the CPT1A gene was the homozygous p.P479L variant [34]. It was further demonstrated that the presence of the p.P479L variant in both CPT1A alleles resulted in reduced CPT-I activity in cultured fibroblasts and affected malonyl-CoA interaction with CPT1A [10]. However, other studies of this variant in Yupik Eskimos and Greenland Inuit yielded different results. In these studies, the L479 allele was reported to be associated with infant mortality [35], with impaired fasting tolerance [36], reduced adiposity [37] and with higher levels of HDL-cholesterol and apoA-I cardioprotective factors [38]. Nevertheless, it is important to note that Yupik Eskimos and Canadian Inuit are genetically different [39]. The reduced CPT1A activity associated with the homozygous state of p.P479L variant in Inuit may indicate two scenarios: A) lower activity of the p.P479L variant is beneficial for a state of permanent ketoadaptation in Inuit [34] or B) another Inuit specific variant in the regulatory region may serve as a rescue factor to the enzyme activity.

Although different disorders and traits associated with this variant were reported in different studies, the presence of this variant in very high frequency in Nunavik Inuit suggests that it is likely to be beneficial in this population. For example, it may help the arctic residents to adapt to the extremely cold environment and/or their ketogenic diet. This assumption is supported by the mean age difference found in previous studies and in the current study. Previously, it was reported that the p.P479L variant frequency is higher among Inuit newborns and children [31, 35]. However, the current study shows that among older populations (mean age of 52 years at enrollment) the L479 allele remains predominant. Furthermore, infant mortality rates are higher in Nunavik than in Nunavut (25 vs 14.6 per 1,000 live births) and Quebec (5 per 1,000 live births), but lower in Kivalliq (32.3 per 1,000 live births) [40]. These conflicting data suggest that further investigation is needed to determine whether there is an association between the p.P479L variant and infant death. Since the p.P479L variant is absent from Chinese and Japanese populations, it probably occurred more recently, after the migration across the Bering Strait of their Asian ancestors. Genotyping the p.P479L variant in Siberian and Mongolian populations will be interesting to trace the origin and the occurrence of this variant.
CPT1B and CPT2 haplotypes in the Nunavik Inuit population

The mutation burden and tolerance tests did not show any excess of rare missense CPT1B variant in Nunavik Inuit. Nevertheless, the relative frequencies of different CPT1B locus rs470117/rs8142477/rs3213445 containing haplotypes found in the Nunavik Inuit are different from those seen in Asian populations. Interestingly, this haplotype is in the same LD block as the SNP rs5770917, which was reported to be associated with narcolepsy in the Japanese population [41, 42]. Given that the Inuit live in the far north with the midnight sun and the polar night, variants in this locus may therefore have some roles in sleep, possibly benefiting the Inuit while predisposing to narcolepsy in Japan.

CPT2 missense mutations are rare in the general population, suggesting that the variations in the CPT2 gene seen in Nunavik Inuit may be functional, possibly related to the energy metabolism requirements unique to this population. The haplotype containing p.F352C and p.V386I variants was previously named as a thermolabile CPT-II variant [43] with decreased CPT-II activity. It was further reported as a risk factor for infection-induced acute encephalopathy and for continuous high-grade childhood fever, which leads to a systemic and metabolic energy crisis in Japanese and Chinese populations [26, 43, 44]. The CPT2 p.F352C variant was also reported in three individuals from one Inuit family with CPT-II deficiency [34]. However, there was no clinical description of acute encephalopathy in this family. Since this variant is temperature sensitive, it may act differently or even lead to higher enzyme activities in cold temperatures, which may explain their increase of the thermolabile haplotype frequency in the Inuit. Of note, encephalopathy was not reported in the Nunavik Inuit cohort, suggesting that the same genetic variation may be associated with different phenotypes in different populations.

In this study, we observed an increased frequency of rare divergent variants in the carnitine acyltransferases family of genes in Nunavik Inuit in Quebec, as compared to other populations. There are few missense mutations in these genes, suggesting that they do not tolerate variation. As Asians are thought to have common ancestors with the Inuit [45], the excess of variants in these genes in Nunavik Inuit also suggests an effect of selective pressure. It is possible that these variants are related to their high fat diet, as the carnitine acyltransferase genes are essential for fat metabolism.

Since data on metabolic measurements was not available in our cohort, we could not determine whether the high frequency of variations in the carnitine acyltransferase genes in Nunavik Inuit affects their enzyme levels and activities. Our cohort was comprised of healthy Nunavik Inuit individuals and individuals with a family history of brain aneurysms; none of them showed any of the severe symptoms caused by CPTs or CrAT deficiencies. We hypothesize that these variants, while being part of the Inuit ‘healthy genomes’, could be harmful in other populations. Further studies in other Inuit populations, with the inclusion of measurements of these enzymes’ levels and activity are necessary to confirm our results and conclusions.

Supporting Information
S1 Archive. Data of carnitine acyltransferase genes from exome sequencing and SNP array. (7Z)
S1 Fig. The average coverage of gene CPT1A, CPT1B, CPT1C, CPT2, CRAT and CROT from the exome sequencing data of 113 Nunavik Inuit. (TIF)
S2 Fig. Chromatograms of Sanger sequencing validation of 4 Inuit specific missense variants and 2 rare synonymous variants identified in Nunavik Inuit. CPT1A p.P479L variant was only shown in a selected of samples.

(TIF)

S3 Fig. Rare missense variants from Nunavik Inuit and 1KGP Asians with their gradation profile of Polyphen-2 scores.

(TIF)

S4 Fig. The CPT1A p.P479L variant frequencies in different Inuit settlements in Canada and the migration patterns of Inuit. Original map from "Canada's Relationship with Inuit: A History of Policy and Program Development".

(TIF)

S1 Table. Variant concordance of CPT1A, CPT1B, CPT1C, CPT2 and CRAT from SNP array and exome sequencing. ¹Variant only in individuals with mixed ethnicity. ²Variant with MAF = 1 in Inuit, Asians and Europeans.

(DOCX)

S2 Table. All exonic variants of CPT1A, CPT1B, CPT1C, CPT2 and CART in 100 Nunavik Inuit.

(DOCX)

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Author Contributions

Conceived and designed the experiments: GAR PAD LX. Performed the experiments: SZ LX AA CVB. Analyzed the data: SZ PX ADL DS. Contributed reagents/materials/analysis tools: MTG ADL DS EH OD. Wrote the paper: SZ LX PAD GAR.

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