Paediatrics & Child Health, 2019, 111–115
doi: 10.1093/pch/pxy106
Original Article
Advance Access publication 6 August 2018

OXFORD

Original Article

The p.P479L variant in CPT1A is associated with infectious disease in a BC First Nation

Graham Sinclair PhD FCCMG[1], Sorcha Collins MSc[2], Laura Arbour MD MSc FRCPC FCCMG[2], Hilary Vallance MD FRCPC FCCMG[1]

[1]Department of Pathology and Laboratory Medicine, BC Children's Hospital, University of British Columbia, Vancouver, British Columbia; [2]Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia

Correspondence: Graham Sinclair, Department of Pathology and Laboratory Medicine Rm 2F24, BC Children's Hospital, University of British Columbia, Vancouver, British Columbia V6H 3N1. Telephone: 604-875-2345 ext. 7450, fax: 604-875-3434, e-mail gsinclair@cw.bc.ca

Abstract

Background: The hepatic carnitine palmitoyltransferase I (*CPT1A*) p.P479L variant is common in Aboriginal populations across coastal British Columbia, Alaska, the Canadian North, and Greenland. While the high frequency of this variant suggests positive selection, other studies have shown an association with sudden unexpected death in infancy and infection. We utilized administrative health data to evaluate hospitalizations for a single year cohort of children of First Nations descent genotyped for the variant and, matched for location of birth. Seven years of data were reviewed for 150 children split evenly between *CPT1A* genotypes (homozyous, heterozygous, and noncarrier of the p.P479L variant).

Results: Children homozygous for the p.P479L allele had a higher rate of hospital admissions at 2.6 per individual as compared to noncarriers at 0.86. Heterozygous children also showed a significant increase at 1.9 per person. Length of stay per admission was increased for both p.P479L homozygotes and heterozygotes. The odds ratio (OR) for at least one hospitalization for any reason was increased for p.P479L homozygotes relative to noncarriers (OR=10.2, confidence interval [CI] 3.5 to 30.0) as were admissions for dental caries (OR=3.4, CI 1.5 to 7.8), acute lower respiratory tract infections (OR=6.0, CI 1.6 to 22.4), and otitis media (OR=13.5, CI 1.7 to 109.4).

Conclusions: The CPT1A p.P479L variant is associated with an increased rate of hospitalization for those homozygous, primarily for infectious disease causes. Heterozygotes also showed a small but significant increase in hospitalization rates suggesting some dosage effect. Functional studies will be required to identify the underlying pathological mechanism.

Keywords: *Carnitine palmitoyltransferase; First Nation; Indigenous; Fatty acid oxidation; Infection.*

Carnitine palmitoyltransferase I (CPTI) is critical for importing long-chain fatty acids into the mitochondrion for beta-oxidation and is the rate limiting step in the utilization of fats as an energy source (1). CPTI activity is modulated through inhibition by malonyl-CoA, downregulating fatty acid oxidation and promoting fatty acid synthesis when the cell is in an energy replete state. Conversely, during periods of low glucose and/or high energy demand, malonyl-CoA levels drop and fatty acid oxidation is promoted for ketogenesis and ATP production.

A variant in the hepatic CPTI gene (*CPT1A*), widely described as the 'Arctic variant' (c.1436C>T, p.P479L), is common in coastal British Columbia First Nations, Native Alaskans, and across Inuit populations in the Canadian North and Greenland (2–5). While the estimated homozygosity rate in coastal BC First Nations is ~25%, in some Nunavut Inuit populations, more than

70% of community members are homozygous, and the T allele is essentially fixed in some populations in Greenland (3,4,6). This variant continues to present a paradox with respect to its clinical significance in these communities (7). A high allele frequency, evidence for positive genetic selection, and association with favourable measures of cardiovascular health all suggest that this variant is beneficial, perhaps as an adaptation to a traditional diet high in marine polyunsaturated fatty acids (5,8,9). Conversely, several studies in BC, Alaska, and Nunavut have shown an association of p.P479L homozygosity with increased rates of sudden unexpected death in infancy, possibly related to infectious disease, as well as reduced stature in adults (3,4,10–13).

Biochemical evidence supports significant impairment in CPTI activity (~50% reduction) with the p.P479L variant (7,14). Most homozygotes have an atypical acylcarnitine profile with elevated free carnitine and reduced long chain acylcarnitines, consistent with reduced long-chain fatty acid flux into the mitochondrion for oxidation (4,11). This pattern is, however, moderated relative to classical complete deficiency in CPTI. Importantly, functional studies also showed that this variant has reduced inhibition from malonyl-CoA suggesting that it may be able to support higher levels of fatty acid oxidation in the fed state (14).

To date, clinical studies into the potential risks associated with p.P479L homozygosity have focused on its association with unexpected death in infancy and infectious disease in newborns, but little information is available on other clinical risks in childhood (13,15).

In our previous study of the association of the p.P479L variant with sudden unexpected death in infancy in BC First Nations, we performed *CPT1A* genotyping on a cohort of deidentified newborn screening samples for infants born in 2004 (4). Following consultation with some of the affected coastal BC First Nations, we proposed a linked administrative health study to evaluate clinical outcomes in a subset of the infants genotyped in the original study. Given that the previous work had been completed in a deidentified fashion, this presented a unique opportunity to evaluate the natural history of the variant over time in a cohort of individuals from the same First Nation, not identified clinically to have the p.P479L allele. This avoided concerns of bias due to population stratification, common to previous epidemiological studies of this variant. With consent from one coastal BC First Nation, we were able to link administrative health data over 7 years for a cohort of 150 children (50 homozygotes, 50 heterozygotes, and 50 noncarriers) from the same First Nation to evaluate rates of hospitalization and the primary diagnostic codes compared across all three *CPT1A* genotypes.

MATERIAL AND METHODS

Data sources

CPT1A p.P479L genotyping results were obtained from our previous study of all First Nations births in British Columbia in 2004 (4). The data set was subsequently limited to 150 First Nations children within a set of forward sortation areas (first 3 digits of the postal code). This data set included 50 p.P479L homozygotes, 50 heterozygotes, and 50 noncarriers of the variant randomly selected from the original cohort. All 150 individuals included in the study were born in 2004, had a mother's residence within one of 3 postal forward sortation areas encompassing the participating First Nation, and had a 'Status Indian' flag for the mother on the child's birth record. Linkage to administrative health data was performed by Population Data BC as an independent third party to maintain the blinding of the research team to individual identity. Using a specimen ID number linkable to demographic data in the BC Newborn Screening Program database, the personal health number (PHN) for each individual was extracted and provided to Population Data BC. That PHN was used to link administrative data from the BC Ministry of Health (hospital discharge abstracts) (16) and BC Vital Statistics (deaths) (17). The hospital discharge abstracts included all admissions to an acute care hospital in BC including day surgery, but excluding emergency room visits. Community encounters with primary care providers are not captured by this data set. Fields used for analysis included diagnosis code (ICD-10-CA), total length of stay, gestational age, and birth weight. Administrative data was inclusive of January 1, 2004 to December 31, 2011 providing 7 years of follow-up data.

Ethics

This study was designed as a deidentified, linked administrative health data protocol with written community support. The protocol was reviewed by the BC First Nations' Health Council and the Health Advisory Committee of the participating community, with written support for the deidentified population provided by the committee chair. The protocol was also approved, and a waiver of individual consent granted given the deidentified nature of the project, by the BC Newborn Screening Program Research Review Committee, data stewards at the British Columbia Ministry of Health and the BC Vital Statistics Agency, and the C&W UBC Research Ethics Board (H11-03494). Population Data BC, a nonprofit academic body associated with the University of British Columbia, acted as the third-party for all data linkage and facilitated the completion of data sharing agreements between the research team and provincial data stewards. All deidentified and linked data was maintained in the Population Data BC secure research environment with controlled access by the investigators.

Analysis

A two-tailed, unpaired T-test was used to compare mean hospital admissions per individual and average days of stay per admission between genotype groups. Data from the initial newborn admission was omitted for all individuals. Primary reasons for hospital admission were extracted from the ICD-10-CA codes

in the hospital discharge abstracts. All admissions for any reason were evaluated, but comparisons were limited to primary admission groups with greater than 10 total admission counts across all genotypes. An individual was counted if they had one or more admissions for that primary admission reason (i.e., an individual with multiple admissions for the same reason was counted only once). Individual codes were grouped as follows: Dental Caries (K02), Otitis Media (H65-H66), Acute Lower Respiratory Tract infection (J12-J22), and acute Upper Respiratory Tract infection (J00- J06). OR and 95% confidence intervals were calculated for each code group with p.P479L homozygotes and heterozygotes compared to noncarriers separately.

RESULTS

Population characteristics

There were no significant differences in birth weight or gestational age at birth between the three genotype groups, although there was a slight under-representation of females in the noncarrier group (Table 1). The proportion of premature births did not differ between genotypes, although the overall rate of 10.7% was higher than that reported for the province as a whole in 2004 (9.2%) (18). Such an increased rate of prematurity has, however, been previously reported for First Nations births in BC (19,20). Matching of genotype and administrative data records was unsuccessful for one noncarrier and the final data set for analysis was 149 individuals.

Primary outcome

The a priori primary outcome for this study was the number of hospital admissions (excluding the initial newborn birthing admission) per individual over the 7 years data set. As shown in Table 1, p.479L homozygotes had a statistically higher rate of admission at 2.6 hospitalizations per individual over the first 7 years of life as compared to noncarriers at 0.86 (P<0.0001, two-tailed T-test). Interestingly, p.P479L heterozygotes also showed a significant increase in admissions at 1.9 per individual (P=0.009, two-tailed T-test) as compared to noncarriers. A similar trend is evident from the average days of stay per admission with an increased length of stay for both p.P479L homozygotes and heterozygotes (Table 1). There were no deaths recorded for any individual in any genotype group.

Secondary outcomes

Given the small sample sizes available, only limited evaluation of the reasons for hospitalization is possible. Evaluations were limited to primary admission groupings with greater than 10 total events to ensure a favourable case to variable ratio (>5:1). Overall, the OR for at least one hospitalization for any reason was increased for p.P479L homozygotes relative to noncarriers (OR=10.2, CI 3.4 to 30.0). Although all admission reasons

Table 1. Hospital admissions and primary admission reasons for 7 years of administrative data

	Homozygotes	Heterozygotes	Noncarrier	OR (Hom vs. NC) (95% CI)	OR (Het vs. NC) (95% CI)
Total Individuals	50	50	49		
Female (%)	24 (48)	23 (46)	21 (43)		
Mean birth weight ± SD (g)	3,371 ± 637	3,266 ± 767	3,412 ± 597		
Premature (<37 weeks)	6	5	5		
Non-newborn Hospital Admissions (per person)					
Mean (Median)	2.6 (2)	1.9 (1)	0.8 (0)		
Range	(0–12)	(0–13)	(0–6)		
T-Test (vs. Noncarrier)[a]	P<0.001	P=0.009			
Days of Stay (per admission)					
Mean (Median)	3.6 (3)	2.9 (2)	1.8 (1)		
Range	1–14	1–14	1–7		
T-test (vs. Noncarrier)[a]	P=0.001	P=0.001			
Individuals with at least one hospital admission					
Any reason	45	33	23	10.2 (3.4–30.0)	2.2 (1.0–4.9)
Dental Carries	30	25	15	3.4 (1.5–7.8)	2.3 (1.0–5.2)
Acute Lower Resp. Inf.	14	9	3	6.0 (1.6–22.4)	3.4 (0.9–13.3)
Otitis Media	11	1	1	13.5 (1.5–7.8)	1.0 (0.06–16.1)

CI Confidence interval; NC Noncarrier; OR Odds ratio.

[a]Two-tailed unpaired T-test.

were reviewed, the only diagnoses to meet the 10 event threshold were those related to an infectious origin. OR for at least one hospital admission due to dental caries (OR=3.4, CI 1.4–7.8), acute lower respiratory tract infections (OR=6.0, CI 1.6 to 22.4), and otitis media (OR=13.5, CI 1.5 to 109.4) were all elevated for p.P479L homozygotes as compared to noncarriers (Table 1). A similar trend was observed for p.P479L heterozygotes but the results were not statistically significant. Of note, there were few or no admissions for hypoglycemia or seizures.

DISCUSSION

The CPTI p.P479L variant presents substantial uncertainty for families and clinicians providing care in communities with high allele frequencies given the unclear clinical risks. While previous studies have shown a small but statistically significant association with sudden death in infancy, we report here an additional association between homozygosity for the variant and increased rates of hospitalization in childhood for a number of infectious disease-related causes. There is mounting evidence suggesting that this variant is both conditionally beneficial and conditionally detrimental, likely dependent upon a variety of environmental and/or genetic modifiers yet to be determined.

A number of genetic studies have confirmed a high frequency for the variant in coastal northern Indigenous populations (2–5). The variant has not been identified in any other ethnic groups outside of these regions (21). There is good evidence from previous epidemiological studies to support positive genetic selection for the p.P479L variant, arguing for a fitness benefit (6,12,21–23). These arguments have centered on an adaptation towards a diet high in fat, particularly enriched with marine polyunsaturated (omega-3) fatty acids (5,6,8,22). It is compelling to speculate that the observed reduced inhibition by malonyl-CoA could lead to persistent fatty acid oxidation and ketogenesis even in the event of opportunistic carbohydrate consumption (4). Alternatively, it has been suggested that the variant may alter the substrate specificity of the CPTI enzyme, increasing flux for unsaturated fatty acids, reflecting the dietary intake (5,8,15). Evidence from Alaska and Greenland studies have confirmed favourable alterations in plasma lipid profiles and waist circumference in association with the p.P479L variant, particularly for those with high marine fat intake (5,8,12).

These potential benefits are important but don't negate growing evidence for clinical risks associated with the variant. Studies on sudden unexpected death in infancy in BC, Alaska, and Nunavut have provided evidence supporting an association between p.P479L homozygosity and infant mortality (3,10,11,24). While this suggests an increased proportional risk for homozygotes, the absolute risk remains small in comparison to the frequency of the genotype in the affected communities. It was hypothesized that the sudden death risk was likely the result of hypoketotic hypoglycemia due to an inability of homozygotes to increase fatty acid flux in response to fasting. In addition, work by Collins et al. (13) and Gesner et al. (15) has provided evidence suggesting that infectious disease was a contributor to the increased deaths in homozygous infants.

The work presented here provides further evidence to support an association between infectious disease and the p.P479L variant. This study utilized administrative health data from a single First Nation with individuals not identified clinically to have the p.P479L allele. This avoided bias due to population stratification, common to previous epidemiological studies of this variant but limited our ability to evaluate potential confounders such as infection risks in the home, environmental exposures, sleep practices, and socioeconomic variables. Based on 7 years of hospital admission data and a cohort of First Nations children matched for age and location of birth, p.P479L homozygotes had, on average, three times as many hospital admissions per person as those who did not carry the variant and remained in hospital twice as long per admission. This suggests both an increase in significant adverse health events and an increase in the severity of those events. Although low total case numbers limit the parsing of these events based on primary reasons for admission, a trend of increased admissions related to infectious disease is seen. It is important to note that our study was not targeted specifically to infectious disease; all admission reasons were evaluated but the only diagnoses with sufficient frequency for analysis were related to infectious causes. Increased odds for lower respiratory tract infection and otitis media mirror the data reported by groups investigating sudden deaths in infancy (13,15). The finding of increased odds of admission for dental caries, also an infectious disease, is unique to this study but previous studies were limited to infant mortality. Of note, admissions for hypoglycemia were not observed and other clinical markers of an energy deficit (seizures, failure to thrive) had an insufficient numbers for analysis. However, primary health care encounters for hypoglycemia and related causes have not been captured in this data set and may be under-represented.

It has been suggested that this apparent association with infectious disease may reflect either an increase in severity of response to common pathogens due to an inability to upregulate fatty acid oxidation during intercurrent illness, or an increased susceptibility to those pathogens due to direct impairment of the immune system (15). A number of studies have outlined the reliance of memory T-cells on fatty acid oxidation for long-term viability, which would support the hypothesis of an increased risk for repeated infections in p.P479L homozygotes. While activated effector T-cells are highly anabolic and rely primarily on glycolysis for energy production, memory T-cells display a more catabolic metabolism with a switch to fatty acid oxidation to support long-term quiescence (25). It is argued

that this metabolic switch helps to maintain excess respiratory capacity allowing for rapid expansion of memory T-cells with subsequent pathogen challenge. An inability to upregulate fatty acid oxidation in p.P479L homozygous T-cells could limit the survival of memory T-cells, increasing susceptibility to repeated infections. Prospective cohort studies to evaluate response to infection and external stressors, along with functional studies to evaluate the metabolic profiles of p.P479L homozygous T-cells will be required to investigate this hypothesis.

CONCLUSIONS

Our results support the growing literature on the potential effect of the CPTI p.P479L variant on infection risk in infants and children. Given small sample numbers, all of these findings must be interpreted with caution; however, there does appear to be a clear trend towards increased hospital admissions and longer days of stay related to infectious disease in the p.P479L homozygotes. Unexpectedly, an intermediate phenotype is suggested in the p.P479L heterozygotes. Although there is evidence for a historical selective advantage to the CPT1A p.P479L variant, the association of the variant with increased rates of hospitalization, sudden death, and infectious disease suggests a clinical risk that warrants further investigation.

Acknowledgements

We would like to acknowledge support from the Health Advisory Committee of the participating First Nation and funding support from the BC Children's Hospital Foundation (Telethon Competition). All inferences, opinions, and conclusions drawn in this report are those of the authors, and do not reflect the opinions or policies of the Data Stewards.

This work was completed at BC Children's Hospital and was approved by the C&W UBC Research Ethics Board (H11-03494). Funding provided by the BC Children's Hospital Foundation Telethon Competition. The authors have no conflicts of interest to disclose.

References

1. Bartlett K, Eaton S. Mitochondrial beta-oxidation. Eur J Biochem 2004;271(3):462–9.
2. Gessner BD, Gillingham MB, Johnson MA, et al. Prevalence and distribution of the c.1436c→t sequence variant of carnitine palmitoyltransferase 1A among Alaska native infants. J Pediatr 2011;158(1):124–9.
3. Collins SA, Sinclair G, McIntosh S, et al. Carnitine palmitoyltransferase 1A (CPT1A) P479L prevalence in live newborns in Yukon, northwest territories, and Nunavut. Mol Genet Metab 2010;101(2–3):200–4.
4. Sinclair GB, Collins S, Popescu O, McFadden D, Arbour L, Vallance HD. Carnitine palmitoyltransferase I and sudden unexpected infant death in British Columbia First Nations. Pediatrics 2012;130(5):e1162–9.
5. Rajakumar C, Ban MR, Cao H, Young TK, Bjerregaard P, Hegele RA. Carnitine palmitoyltransferase IA polymorphism p479L is common in Greenland Inuit and is associated with elevated plasma apolipoprotein A-I. J Lipid Res 2009;50(6):1223–8.
6. Fumagalli M, Moltke I, Grarup N, et al. Greenlandic Inuit show genetic signatures of diet and climate adaptation. Science 2015;349(6254):1343–7.
7. Greenberg CR, Dilling LA, Thompson GR, et al. The paradox of the carnitine palmitoyltransferase type ia P479L variant in Canadian aboriginal populations. Mol Genet Metab 2009;96(4):201–7.
8. Lemas DJ, Wiener HW, O'Brien DM, et al. Genetic polymorphisms in carnitine palmitoyltransferase 1A gene are associated with variation in body composition and fasting lipid traits in Yup'ik Eskimos. J Lipid Res 2012;53(1):175–84.
9. Das M, Sha J, Hidalgo B, et al. Association of DNA methylation at CPT1A locus with metabolic syndrome in the genetics of lipid lowering drugs and diet network (GOLDN) study. Plos One 2016;11(1):e0145789.
10. Gessner BD, Wood T, Johnson MA, Richards CS, Koeller DM. Evidence for an association between infant mortality and homozygosity for the arctic variant of carnitine palmitoyltransferase 1A. Genet Med 2016;18(9):933–9.
11. Gessner BD, Gillingham MB, Birch S, Wood T, Koeller DM. Evidence for an association between infant mortality and a carnitine palmitoyltransferase 1A genetic variant. Pediatrics 2010;126(5):945–51.
12. Andersen MK, Jørsboe E, Sandholt CH, et al. Identification of novel genetic determinants of erythrocyte membrane fatty acid composition among Greenlanders. Plos Genet 2016;12(6):e1006119.
13. Collins SA, Surmala P, Osborne G, et al. Causes and risk factors for infant mortality in Nunavut, Canada 1999 – 2011. BMC Pediatr 2012;12(1):1.
14. Brown NF, Mullur RS, Subramanian I, et al. Molecular characterization of L-CPT I deficiency in six patients: Insights into function of the native enzyme. J Lipid Res 2001;42(7):1134–42.
15. Gessner BD, Gillingham MB, Wood T, Koeller DM. Association of a genetic variant of carnitine palmitoyltransferase 1A with infections in Alaska native children. J Pediatr 2013;163(6):1716–21.
16. Canadian Institute for Health Information [creator]. Discharge Abstract Database (Hospital Separations). V2. Population Data BC [publisher]. Data Extract. MOH, 2013. <http://www.popdatabc.ca/data> (Accessed July 29, 2014).
17. BC Vital Statistics Agency [creator]. Vital Statistics Deaths. V2. Population Data BC [publisher]. Data Extract BC Vital Statistics Agency, 2013. <http://www.popdatabc/data> (Accessed July 29, 2014).
18. British Columbia Perinatal Health Program. Preterm Birth in British Columbia. Vancouver, BC. March 2018.
19. Smylie J. Our Babies, Our Future: Aboriginal Birth Outcomes in British Colombia. Prince George, BC: Natl Colloborating Cent Aborig Heal, 2011.
20. Luo ZC, Kierans WJ, Wilkins R, Liston RM, Uh SH, Kramer MS. Infant mortality among first nations versus non-first nations in British Columbia: Temporal trends in rural versus urban areas, 1981-2000. Int J Epidemiol 2004;33(6):1252–9.
21. Clemente FJ, Cardona A, Inchley CE, et al. A selective sweep on a deleterious mutation in cpt1a in arctic populations. Am J Hum Genet 2014;95(5):584–9.
22. Zhou S, Xiong L, Xie P, et al. Increased missense mutation burden of fatty acid metabolism related genes in Nunavik Inuit population. PLoS One 2015;10(5):1–15.
23. Cardona A, Pagani L, Antao T, et al. Genome-wide analysis of cold adaptation in indigenous Siberian populations. Plos One 2014;9(5):e98076.
24. Patsoukis N, Bardhan K, Chatterjee P, et al. PD-1 alters T-cell metabolic reprogramming by inhibiting glycolysis and promoting lipolysis and fatty acid oxidation. Nat Commun 2015;6:6692. <http://www.nature.com/ncomms/2015/150326/ncomms7692/full/ncomms7692.html> (Accessed March 11, 2016).
25. Powell JD, Pollizzi K. Fueling memories. Immunity 2012;36(1):3–5.