Published in final edited form as:
FEBS Lett. 2006 October 30; 580(25): 5953–5958.

A POLYMORPHISM IN NEW ZEALAND INBRED MOUSE STRAINS THAT INACTIVATES PHOSPHATIDYLCHOLINE TRANSFER PROTEIN

Huei-Ju Pan[1], **Diana S. Agate**[2], **Benjamin L. King**[3], **Michele K. Wu**[4], **Steven L. Roderick**[2], **Edward H. Leiter**[3], and **David E. Cohen**[4]

1 *Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan,*

2 *Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461,*

3 *The Jackson Laboratory, Bar Harbor, Maine 04609 and*

4 *Department of Medicine, Brigham and Women's Hospital, Harvard Medical School and Harvard-Massachusetts Institute of Technology, Division of Health Sciences and Technology, Boston, MA 02115*

Abstract

New Zealand Obese (NZO/HlLt) male mice develop polygenic diabetes and altered phosphatidylcholine metabolism. The gene encoding phosphatidylcholine transfer protein (PC-TP) is sited within the support interval for *Nidd3*, a recessive NZO-derived locus on Chromosome 11 identified by prior segregation analysis between NZO/HlLt and NON/Lt. Sequence analysis revealed that the NZO-derived PC-TP contained a non-synonymous point mutation that resulted in an Arg120His substitution, which was shared by the related NZB/BlNJ and NZW/LacJ mouse strains. Consistent with the structure-based predictions, functional studies demonstrated that Arg120His PC-TP was inactive, suggesting that this mutation contributes to the deficiencies in phosphatidylcholine metabolism observed in NZO mice.

Keywords

START domain; obesity; diabetes; protein structure; quantitative trait locus

Abbreviations

MLV, multilamellar vesicle; NON, Nonobese Nondiabetic; NZO, New Zealand Obese; PC, phopshatidylcholine; PC-TP, PC transfer protein; PEMT, phosphatidylethanolamine n-methyl transferase; PS, phosphatidylserine; QTL, quantitative trait locus; SUV, small unilamellar vesicle

Introduction

The New Zealand Obese (NZO) mouse strain is characterized by juvenile-onset obesity and maturity-onset hyperinsulinemia, hyperleptinemia, hyperglycemia, and hypertension. Approximately 50% of NZO males develop obesity-associated diabetes, or diabesity. Nonobese Nondiabetic (NON) is an unrelated mouse strain that spontaneously develops impaired glucose tolerance associated with defective insulin secretion by pancreatic beta cells.

Correspondence: David E. Cohen, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115. Phone: 001 (617) 525-7847; Fax: 001 (617) 264-6368; email: dcohen@partners.org.

In contrast to NZO, male NON mice do not progress from impaired glucose tolerance to hyperglycemia when maintained on standard diets. When NON and NZO are intercrossed, quantitative trait loci (QTL) derived from both strains produce (NZOxNON)F1 male mice of equivalent obesity to NZO parentals, but the frequency of males developing diabesity is increased to 90–100% [1,2].

In a effort to identify the genetic differences that underlie the tendency of NZO mice to develop diabesity, we recently showed that hepatic activities of two key enzymes of phosphatidylcholine (PC) metabolism, phosphatidylethanolamine n-methyl transferase (PEMT) and choline kinase were lower in (NZOxNON)F1 males than in either parental strain [3,4]. The *Pemt* gene encoding PEMT is sited on Chromosome 11 within the support interval for the more proximal of two linkage peaks designated as *Nidd3*, an NZO-derived QTL controlling serum insulin concentrations [1]. We tested the hypothesis that an additional NZO-derived gene contributing to the unexpected reduction in PC metabolism in the metabolic milieu of the F1 mice might reside in the support interval for the more distal linkage peak comprising *Nidd3*. Indeed, this region was found to contain *Pctp*, the gene encoding phosphatidylcholine transfer protein (PC-TP). PC-TP is a highly specific 25kDa PC binding protein that is enriched in cytosol of hepatocytes [5,6] and regulates lipid metabolism in the liver [7–9]. In the present report, we show that the NZO genome carries a functionally null *Pctp* allele, which was present only in two other closely related New Zealand mouse strains.

Materials and methods

Materials

Restriction endonucleases, as well as all other routine molecular reagents were purchased from either Roche Applied Science (Indianapolis, IN) or Invitrogen (Carlsbad, CA). Egg yolk PC, brain phosphatidylserine (PS) and bovine heart cardiolipin were from Avanti Polar Lipids (Alabaster, AL). L-α-1-palmitoyl-2-linoleoyl-[linoleoyl-1-^{14}C] PC (50 mCi/mmol) was from NEN Life Science Products (Boston, MA) and [1α,α(n)-^{3}H] and cholesteryl oleate (44 Ci/mmol) was from Amersham Biosciences (GE ealthcare Bio-Sciences Corp., Piscataway, NJ).

Mice

Mice were maintained on a chow diet containing 6% fat at The Jackson Laboratory (Bar Harbor, ME) in temperature and humidity controlled rooms with 14/10-hour light/dark cycles. At 8 weeks of age, mice were euthanized by CO_2 asphyxiation. Livers were harvested immediately and then snap frozen and stored at −80°C. Genomic DNA samples from individual mouse strains listed in Table 1 were obtained from the DNA Resource of The Jackson Laboratory. All protocols were approved by the institutions' Animal Care and Use Committees.

Quantitative real-time PCR

Hepatic mRNA expression of PC-TP was determined by quantitative real-time PCR. Total liver RNA was isolated for preparation of cDNA by standard techniques. *Pctp*-specific PCR primer sequences were 5′-ATAACCATCTACCGGCTGCT-3′ (forward) and 5′-CAAGGTTGCTTGGGAATGAG-3′ (reverse). Quantitative real-time PCR was performed on a LightCycler (Roche Applied Science) using SYBR green for DNA detection and cyclophilin RNA for normalization.

Western blot analysis

PC-TP Protein expression in liver homogenates was determined by Western blot analysis using three different polyclonal antibodies: 1) to the C-terminus of PC-TP (aa 200–214) [10], 2) to an internal peptide (aa 50–63) [11] and 3) to purified recombinant wild type PC-TP [11]. Blots

were stripped and reprobed with a MAP/ERK kinase 1 antibody (catalog number PRB-260C, Covance, Richmond, CA) to control for differences in protein loading. Detection was by enhanced chemiluminescence.

Nucleotide sequence analysis and predicted protein structures

The exons and 5′-flanking region of *Pctp* were amplified using PCR primers designed to the nucleotide sequence of the C57BL/6J strain (http://www.ensembl.org/Mus_musculus) and then subjected to nucleotide sequencing in both forward and reverse directions. Multiple nucleotide and amino acid sequence alignments were performed using CLUSTALW and visualized with Jalview [12,13]. The crystal structure of PC-TP in complex with di-linoleoyl-phosphatidylcholine (Protein Data Bank entry 1LN1) was modeled as the Arg120His mutant using O [14], which was also used to measure interatomic distances. The side chain dihedral angles near side chain rotamer values ($\chi 1 = 176°$) were modeled by inspection and produced no unacceptably close contacts to the remainder of the protein or ligand.

In vitro activity of wild type and mutated PC-TPs

A QuickChange Site-Directed Mutagenesis kit (Stratagene Cloning Systems, La Jolla, CA) was used to modify a synthetic human PC-TP cDNA that was cloned into pET11a (Novagen, EMD Biosciences, San Diego, CA) [11] so that it instead encoded Arg120His PC-TP. Mutagenesis was confirmed by nucleotide sequencing. Wild type and Arg120His in pET11a, as well as pET11a vector alone were transformed into *E. coli* BL21(DE3). Activities of native and recombinant expressed proteins were determined in cytosolic preparations from mouse livers and *E. coli*, respectively. Briefly, PC transfer activity was determined by measurement of intermembrane transfer of radiolabeled phospholipids [15]. Donor small unilamellar vesicles (SUVs) were prepared by bath sonication of PC, ^{14}C-PC, ^{3}H-cholesteryl oleate dispersed in 5 mM EDTA and 50 mM Tris at pH 7.2. Multilamellar vesicles (MLVs) consisting of PC and cardiolipin (95:5 mol%) in the same buffer were prepared by rotary shaking. Solutions containing PC-TP were incubated together in a 37°C shaking water bath with donor SUVs (50 μl), acceptor MLVs (100 μl) adjusted to a final volume of 500 μl with buffer. Control samples contained no added protein. Following 30 min of incubation, MLVs were pelleted by centrifugation and the [^{14}C]-PC and [^{3}H]-cholesteryl oleate contents in a 350 μl aliquot of SUVs that remained in suspension were determined by liquid scintillation counting. Because cholesteryl oleate is not transferred by PC-TP, ^{3}H concentrations were employed to correct for small losses of SUVs during centrifugation. In this manner, [^{14}C]-PC transferred from SUVs to MLVs was reflected by decreases in the ^{14}C/^{3}H ratio of SUVs. Specific activities were estimated by normalizing activities to cytosolic expression levels, which were quantified by densitometric analysis of Western blots [16].

Membrane binding of wild type and mutated PC-TPs

Binding of wild type and Arg120His PC-TP to SUVs was measured as previously described [11]. To produce wild type and mutated PC-TPs in the form of His-tag fusion proteins, we cloned cDNAs encoding wild type and Arg120His into pET19b (Novagen). His-tag proteins were purified from soluble bacterial proteins [11] and dialyzed against 10mM Tris-HCl, 20mM NaCl, 1mM DTT and 1mM NaN_3 at 4°C. Proteins were mixed together with SUVs (PC:PS molar ratio of 80:20) to a final volume 200μl. The final protein concentration (1.5 μM) was held constant, whereas the SUV phospholipid concentration was varied to achieve phospholipid/protein molar ratios of 0–240. Mixtures were incubated for 10 min at room temperature to allow binding of proteins to SUV, and then unbound proteins were collected by ultrafiltration through Microcon YM100 100kD molecular weight cut-off filter (Millipore, Bedford, MA) by centrifugation at 3,000 × g for 5min. Unbound proteins remaining within the filter were eluted by centrifugation (3,000×g for 2min) of an addition 300μl of 10mM MOPS

and were combined with initial ultrafiltrates. Ultrafiltrates containing unbound proteins were transferred to PVDF membranes (Immobilon-P, Millipore) using a Bio-Dot SF Cell slot-blot apparatus (Bio-Rad Laboratories, Hercules, CA) and probed with the antibody raised to the C-terminus of PC-TP. Detection was by enhanced chemiluminescence.

Results

Figure 1 presents a schematic diagram of the previously-reported NZO-derived QTL for *Nidd3* on Chromosome 11 [1] that drew our attention to the possibility that the NZO *Pctp* allele, because of its chromosomal position, might contribute to the impaired PC metabolism. Indeed, as shown in Figure 2, multiple nucleotide sequence polymorphisms distinguished the NZO *Pctp* allele from the other strains chosen for comparative sequencing (NON/Lt, NOD/Lt, and C57BL/6J). Within the gene promoter region 86 bp upstream from the transcription initiation site, an A-to-G substitution within the CAAT-box of the putative promoter [17] distinguishes NZO from the other 3 unrelated strains sequenced. Between the CAAT box and transcription initiation site in NZO mice, we also observed that the 12 bp spanning between bp −80 to −69 were deleted from the promoter region of *Pctp*. Two polymorphisms were observed within the NZO coding region of *Pctp*. A synonymous C-to-T nucleotide substitution in exon 3 (338 bp downstream from the transcription initiation site at bp 330 of the open reading frame) did not alter the identity of Ser110. By contrast, a non-synonymous G-to-A substitution was identified in exon 4 (367 bp downstream from the transcription initiation site at bp 359 of the open reading frame). This replaced Arg120 with His (i.e. Arg120His) in NZO mice. In the 3′-UTR, a series of 7 G bases beginning at bp 935 downstream of the transcription initiation site in C57BL/6J mice was replaced with 9 G bases in NZO and 11 G bases in NON. Beginning at bp 1492, the 15 bp sequence in C57BL/6J was replaced by 17 G bases in NZO mice and 14 G bases in NON mice. To gain additional insights into the temporal origins of this substitution among mouse strains, we amplified and sequenced exon 4 of *Pctp* from a variety of mouse strains including the two closely-related NZB and NZW strains (Table 1). The Arg120His substitution was present only in the *Pctp* alleles of the NZO-related NZB and NZW strains, but not in a variety of other strains.

The finding of NZO polymorphisms in the gene promoter and 3′-UTR of *Pctp* suggested the possibility that mRNA expression levels might be altered. However, quantitative real-time PCR revealed that mRNA levels of *Pctp* in livers of NZO mice were the same as in the NON and the F1 hybrid (NZOXNON)F1 mouse strains. By Western blot analysis using each of three different anti-PC-TP antibodies, PC-TP protein concentrations did not differ in livers of NZO and NON mice (data not shown). Expression levels were also the same in (NZOxNON)F1 mice.

Figure 3 illustrates the environment surrounding residue 120 in the three dimensional structure of PC-TP [18]. The crystal structure of PC-TP in its native form [16] reveals that Arg120 interacts directly with the side chains of Asp70 and Asp122. Asp70 is a member of helix α2 (residues 64–74), which donates two additional residues to the PC binding site: Leu68, which interacts with the sn-2 acyl chain of the bound PC molecule, and Tyr72, which also interacts with the sn-2 chain and additionally interacts with the phosphoryl group of PC via its side chain hydroxyl. Although mutation of Arg to His at position 120 conserves the positive charge, the His side chain is shorter than the native Arg residue and would not be expected to interact with Asp70 in the same manner although it could interact with Asp122 (imidazole NE2 atom to Asp 122 side chain OD1 atom distance of 2.50 Å). This altered interaction of residue 120 with α2 would be predicted to affect the conformation or position of this helix and mediate altered interactions of Leu68 and Tyr72 with the ligand, and perhaps alter the activity of the protein as a result. Based upon this structure-based prediction, we evaluated the influence of the Arg120His mutation on the activity of native and recombinant PC-TP. We did not detect a

difference in the PC transfer activity of cytosolic proteins (up to 200 μg added in the *in vitro* assay) from livers of NZO and NON mice. However, as shown in Figure 4A, robust PC transfer activity was observed for recombinant wild type protein, which increased in this assay in proportion to the amount that was added, whereas minimal activity was observed for the recombinant Arg120His mutant protein, which did not differ from the vector control.

Because genetic outcross between NZO and NON produces an F1 male that is comparably obese, but develops diabesity at a higher frequency than observed in NZO parental males [19], we questioned whether the increased diabesity penetrance might entail a further impairment of PC homeostasis via a dominant-negative inactivation of the NON wild type (Arg120) protein by the mutant NZO His120 PC-TP protein. Using the *in vitro* assay, we admixed wild type and mutant recombinant PC-TPs in varied molar ratios (Figure 4B). We observed an essentially linear increase in the activity of the wild type PC-TP as its molar percentage in the mixture increased, arguing against a dominant-negative effect of the mutant protein.

Figure 4C compares membrane binding of wild type and Arg120His PC-TPs, as measured by the concentration of free protein that passed through a filter that retained SUVs. As the phospholipids/protein ratio was increased, reduced band intensity was indicative of PC-TP binding to SUVs. These data demonstrate that the Arg120His mutation did not reduce the capacity of PC-TP bind membranes.

Discussion

The present study shows that the NZO/HlLtJ strain and its two most closely related strains, NZB/BlNJ and NZW/LacJ all share the same inactivating Arg120His mutation in their *Pctp* allele. PC-TP is a member of the steroidogenic acute regulatory (StAR)-related transfer (START) domain-containing superfamily of proteins [20,21] and has also been designated StarD2 [22]. The gene encoding PC-TP is highly conserved among mammals [6,17,23,24]. It is expressed in a number of human [24] and mouse [23] tissues, but highest levels are generally found in the liver. Experiments in tissue culture [25,26] and in mice with homozygous disruption of *Pctp* [7–9] suggest a role for PC-TP in hepatobiliary elimination of plasma cholesterol.

Although we observed nucleotide sequence polymorphisms in both the promoter region and the 3′UTR of the NZO *Pctp* gene, these did not influence hepatic mRNA expression levels. Similarly there were no differences in PC-TP protein in livers of NZO or NON mice, indicating that the Arg120His substitution did not alter protein stability. However, a notable observation in this study was that the Arg120His amino acid substitution inactivated recombinant expressed PC-TP in an assay of intermembrane PC exchange. Although we did not observe a difference in PC transfer activity between cytosolic protein preparations from livers of NZO and NON mice, this result most likely reflects the relative insensitivity of the *in vitro* assay when PC-TP comprises a small fraction of the total protein in the assay, taken together with the contributions of other soluble proteins in liver that exhibit PC transfer activity (e.g. sterol carrier protein 2, StarD10 and phosphatidylinositol transfer protein) [27]. It is also possible that another lipid transfer protein may have compensated for the loss of PC-TP activity in livers of NZO mice.

PC-TP binds PC non-covalently in a tight 1:1 stoichiometric complex [5,16]. Its activity as a lipid transfer protein has been well characterized using carefully standardized *in vitro* assays [5]. To accomplish PC exchange, PC-TP must bind to a donor membrane, release a PC molecule within its lipid-binding pocket and then bind to a new one. Structural studies of PC-TP [11, 16] indicate that PC exchange at the membrane requires a major conformational change in the protein. Once exchange occurs, PC-TP must then desorb from the donor and bind to an acceptor

membrane, where a similar exchange of PC occurs. A failure of any of these steps could have disrupted the activity of recombinant PC-TP that was measured in the *in vitro* transfer activity used in this study [28]. Arg in position 120 of PC-TP is conserved among humans, cows, rats and mice [6], supporting the likelihood that its conversion to His might lead to significant alterations in protein structure and function. Based upon the predicted structure of Arg120His PC-TP, it appears likely that the inability to transfer PC between membranes results from altered binding of PC within the lipid-binding pocket of the protein. This possibility is supported by the observation that the Arg120His substitution did not influence membrane binding by PC-TP, which is mediated at least in part by the C-terminal α-helix of the protein [11]. Although the physiological significance of inactivating PC-TP is not known, these results are suggestive that the spontaneous mutation of PC-TP adds to the additional defects in PC biosynthesis elicited by reduced activities of PEMT and choline kinase in the liver of NZO males [3].

The finding that only NZO, NZB, and NZW mice share the Arg120His mutation indicates that the mutation had become fixed in the outbred progenitors maintained at the University of Otago, New Zealand, prior to initiation of NZ inbred strain development in 1948. The NZO and NZB strain had common agouti progenitors and were separated at the third generation of sib mating, at which point the latter strain was selected for black coat color while selection for agouti coat and later, obesity, was continued in the NZO line [29]. Although deriving from the same outbred stock, the NZW strain was independently selected for albino coat color from different progenitors than used to form the NZO and NZB lines [30].

In summary, nucleotide sequence analysis of *Pctp* revealed differences between the NZO and NON alleles in both non-coding and coding regions. One of the polymorphisms in the coding region of the NZO allele resulted in an Arg120His substitution, a change that abrogated the *in vitro* activity of PC-TP without inhibiting membrane binding. The finding of the same Arg120His substitution in NZB and NZW *Pctp* shows this mutation is not of recent origin. Admixture experiments *in vitro* with cytosolic proteins from transfected *E. coli* producing wildtype or mutant PC-TP failed to show a dominant negative effect of the null allele product on wildtype activity. Although the physiological significance of inactivating PC-TP is not known, the demonstration of a functionally null *Pctp* allele in the NZO genome, coupled with reduced activities of the PC biosynthetic PEMT and choline kinase enzymes [3] in the liver of NZO males, further indicates significant derangements in lipid metabolism in this mouse model of polygenic obesity and diabesity.

Acknowledgements

This study was supported by NIH Grants DK56853 (EHL) and DK56626 and DK48873 (DEC). H-J P was supported by a Mentor-based Fellowship from the American Diabetes Association. Institutional shared services at The Jackson Laboratory were supported by National Cancer Institute Center Support Grant CA34196.

References

1. Leiter EH, Reifsnyder PC, Flurkey K, Partke HJ, Junger E, Herberg L. NIDDM genes in mice: deleterious synergism by both parental genomes contributes to diabetogenic thresholds. Diabetes 1998;47:1287–95. [PubMed: 9703330]
2. Watkins SM, Reifsnyder PR, Pan HJ, German JB, Leiter EH. Lipid metabolome-wide effects of the PPARgamma agonist rosiglitazone. J Lipid Res 2002;43:1809–17. [PubMed: 12401879]
3. Pan HJ, Reifsnyder P, Vance DE, Xiao Q, Leiter EH. Pharmacogenetic analysis of rosiglitazone-induced hepatosteatosis in new mouse models of type 2 diabetes. Diabetes 2005;54:1854–62. [PubMed: 15919809]
4. Pan HJ, Lin Y, Chen YE, Vance DE, Leiter EH. Adverse hepatic and cardiac responses to rosiglitazone in a new mouse model of type 2 diabetes: relation to dysregulated phosphatidylcholine metabolism. Vascul Pharmacol 2006;45:65–71. [PubMed: 16750656]

5. Wirtz KW. Phospholipid Transfer Proteins. Annu Rev Biochem 1991;60:73–99. [PubMed: 1883207]
6. van Helvoort A, et al. Mice without phosphatidylcholine transfer protein have no defects in the secretion of phosphatidylcholine into bile or into lung airspaces. Proc Natl Acad Sci USA 1999;96:11501–6. [PubMed: 10500206]
7. Wu MK, Cohen DE. Phosphatidylcholine transfer protein regulates size and hepatic uptake of high-density lipoproteins. Am J Physiol 2005;289:G1067–G1074.
8. Wu MK, Cohen DE. Altered hepatic cholesterol metabolism compensates for disruption of phosphatidylcholine transfer protein in mice. Am J Physiol 2005;289:G456–G461.
9. Wu MK, Hyogo H, Yadav S, Novikoff PM, Cohen DE. Impaired response of biliary lipid secretion to a lithogenic diet in phosphatidylcholine transfer protein-deficient mice. J Lipid Res 2005;46:422–31. [PubMed: 15576839]
10. Shoda J, et al. Etiologic significance of defects in cholesterol, phospholipid, and bile acid metabolism in the liver of patients with intrahepatic calculi. Hepatology 2001;33:1194–205. [PubMed: 11343249]
11. Feng L, Chan WW, Roderick SL, Cohen DE. High-level expression and mutagenesis of recombinant human phosphatidylcholine transfer protein using a synthetic gene: evidence for a C-terminal membrane binding domain. Biochemistry 2000;39:15399–409. [PubMed: 11112525]
12. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673–80. [PubMed: 7984417]
13. Cuff JA, Clamp ME, Siddiqui AS, Finlay M, Barton GJ. JPred: a consensus secondary structure prediction server. Bioinformatics 1998;14:892–3. [PubMed: 9927721]
14. Jones TA, Zou JY, Cowan SW, Kjeldgaard M. Improved methods for building protein models in electron density maps and the location of errors in these models. Acta Crystallogr A 1991;47:110–9. [PubMed: 2025413]
15. Feng L, Cohen DE. Baculovirus-mediated expression of recombinant at phosphatidylcholine transfer protein. J Lipid Res 1998;39:1862–9. [PubMed: 9741699]
16. Roderick SL, Chan WW, Agate DS, Olsen LR, Vetting MW, Rajashankar KR, Cohen DE. Structure of human phosphatidylcholine transfer protein in complex with its ligand. Nature Struct Biol 2002;9:507–11. [PubMed: 12055623]
17. Wu MK, Boylan MO, Cohen DE. Cloning and gene structure of rat phosphatidylcholine transfer protein, *Pctp*. Gene 1999;235:111–20. [PubMed: 10415339]
18. DeLano, WL. The PyMOL User's Manual. DeLano Scientific; San Carlos, CA: 2002.
19. Reifsnyder PC, Leiter EH. Deconstructing and reconstructing obesity-induced diabetes (diabesity) in mice. Diabetes 2002;51:825–32. [PubMed: 11872687]
20. Ponting CP, Aravind L. START: a lipid-binding domain in StAR, HD-ZIP and signalling proteins. Trends Biochem Sci 1999;24:130–2. [PubMed: 10322415]
21. Tsujishita Y, Hurley JH. Structure and lipid transport mechanism of a StAR-related domain. Nat Struct Biol 2000;7:408–14. [PubMed: 10802740]
22. Soccio RE, Breslow JL. StAR-related lipid transfer (START) proteins: mediators of intracellular lipid metabolism. J Biol Chem 2003;278:22183–6. [PubMed: 12724317]
23. Geijtenbeek TBH, Smith AJ, Borst P, Wirtz KWA. cDNA cloning and tissue specific expression of the phosphatidylcholine transfer protein gene. Biochem J 1996;316:49–55. [PubMed: 8645232]
24. Cohen DE, Green RM, Wu MK, Beier DR. Cloning, tissue-specific expression, gene structure and chromosomal localization of human phosphatidylcholine transfer protein. Biochim Biophys Acta 1999;1447:265–70. [PubMed: 10542325]
25. Baez JM, Barbour SE, Cohen DE. Phosphatidylcholine transfer protein promotes apolipoprotein A-I-mediated lipid efflux in Chinese hamster ovary cells. J Biol Chem 2002;277:6198–206. [PubMed: 11751880]
26. Baez JM, Tabas I, Cohen DE. Decreased lipid efflux and increased susceptibility to cholesterol-induced apoptosis in macrophages lacking phosphatidylcholine transfer protein. Biochem J 2005;388:57–63. [PubMed: 15628972]
27. Wirtz KW. FEBS Lett. 2006in press.

28. DiCorleto PE, Zilversmit DB. Protein-catalyzed exchange of phosphatidylcholine between sonicated liposomes and multilamellar vesicles. Biochemistry 1977;16:2145–50. [PubMed: 861201]

29. Bielschowsky M, Goodall CM. Origin of inbred NZ mouse strains. Cancer Res 1970;30:834–6. [PubMed: 5425297]

30. Festing, MFW. Origins and characteristics of inbred strains of mice. In: Lyon, MF.; Rastan, S.; Brown, SDM., editors. Genetic Variants and Strains of the Laboratory Mouse. Oxford University Press; Oxford: 1996. p. 1537-76.

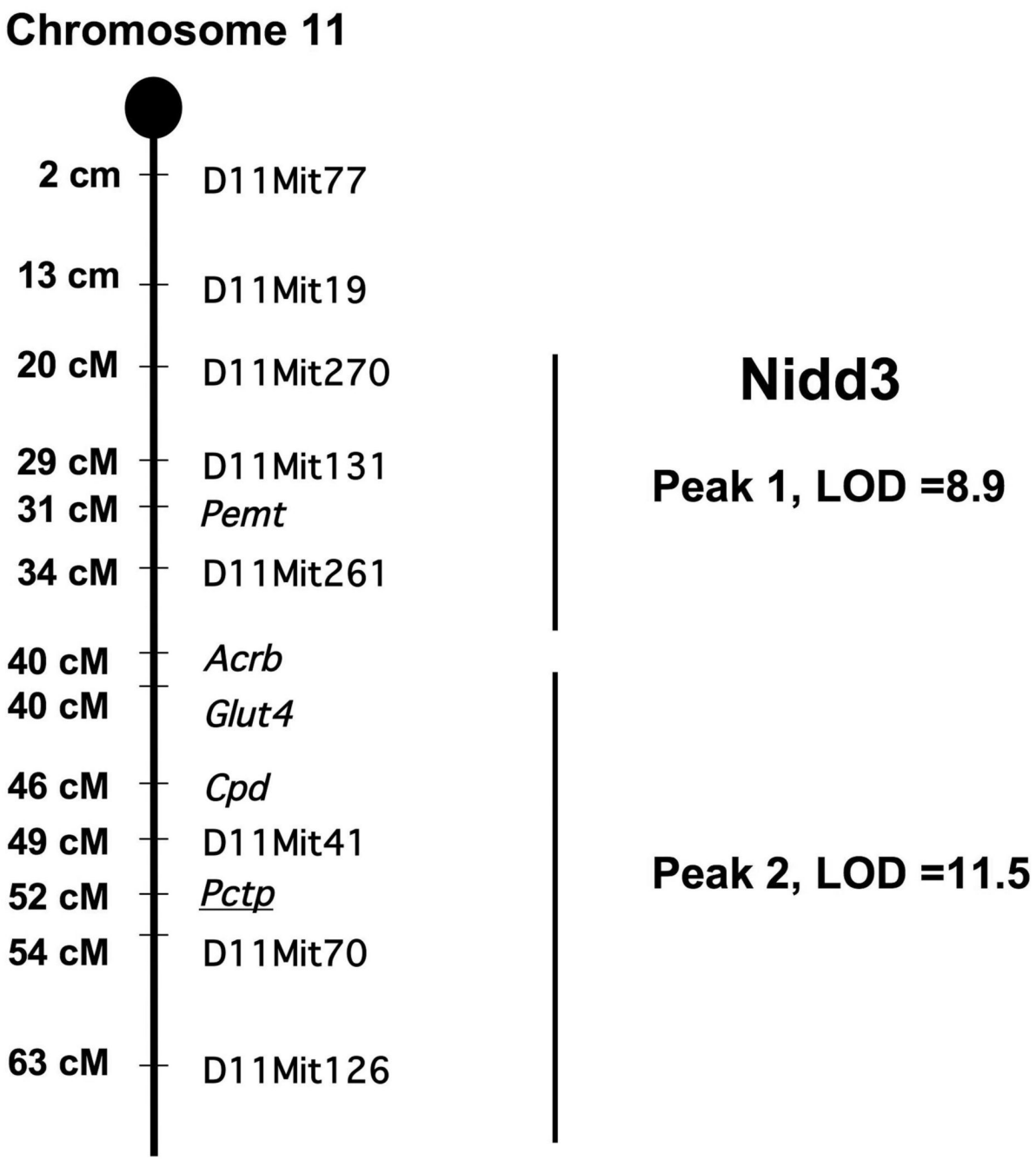

Figure 1. Localization of *Pctp* within the *Nidd3* locus

The *Pctp* gene maps within a previously published QTL peak for *Nidd3* affecting both serum insulin and body weight [1]. The diabetogenic contribution was from NZO.

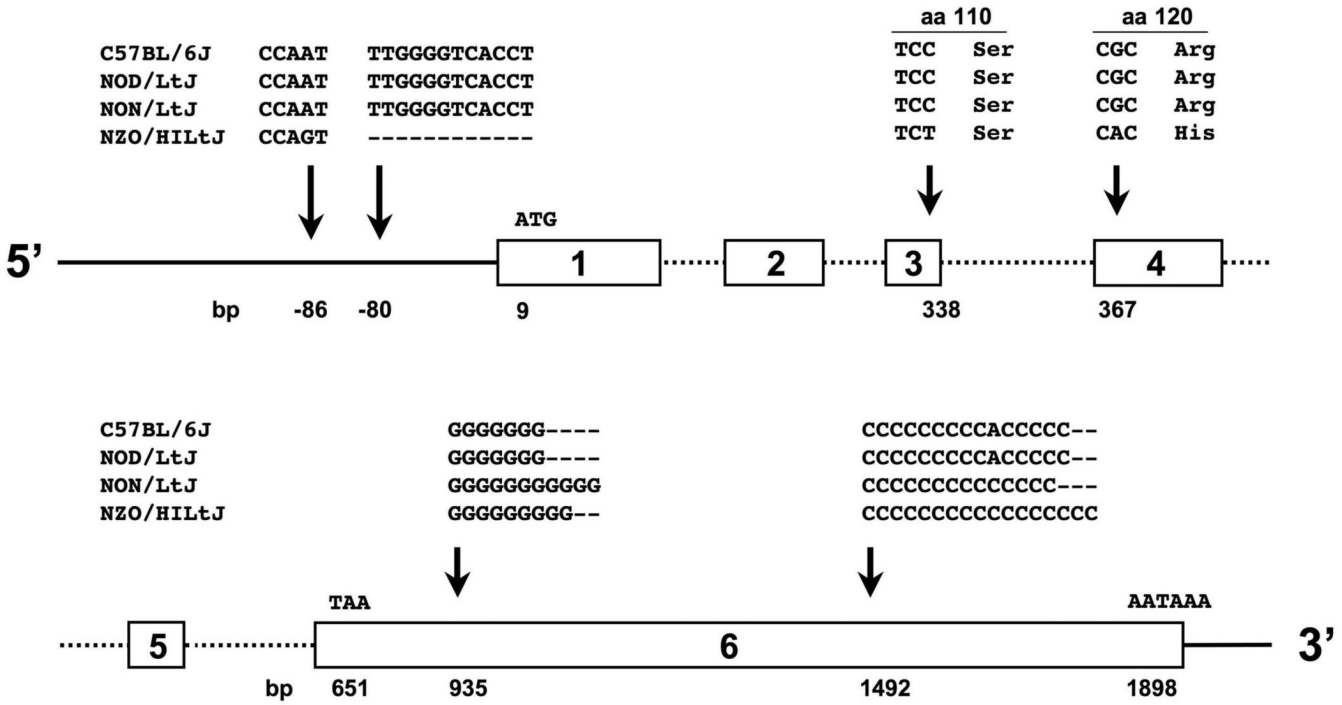

Figure 2. Sequence variations of *Pctp* in NZO compared with other mouse strains

In this schematic diagram of *Pctp*, the boxes represent exons. The solid lines indicate the 5′ promoter region and 3′ flanking DNA. The dashed lines indicate introns, which are not drawn to scale. Numeric values indicate bp positions upstream (negative) or downstream (positive) with respect to the transcription initiation site **(**http://www.ensembl.org/Mus_musculus**)**. The positions of the ATG start codon, the TAA stop codon and AATAAA polyadenylation sequence are depicted as reference points. Arrows indicate location and identity of sequence variations identified in NZO *Pctp*: There was an A to G substitution at bp −86 within in the CCAAT-box of the promoter region and a deletion (represented by dashes) of 12 bp from −80 to −69. A C-to-T substitution at bp 338 within exon 3 was synonymous, leaving Ser at aa 110 unaltered. However, a G-to-A substitution at bp 367 in exon 4, was non-synonymous, resulting the substitution of Arg at aa 120 with His. There were two additional sequence differences outside the coding region in exon 6, each of which consisted of two bp insertions in NZO mice, as well as a 4 bp insertion and a 1 bp deletion in the NON strain.

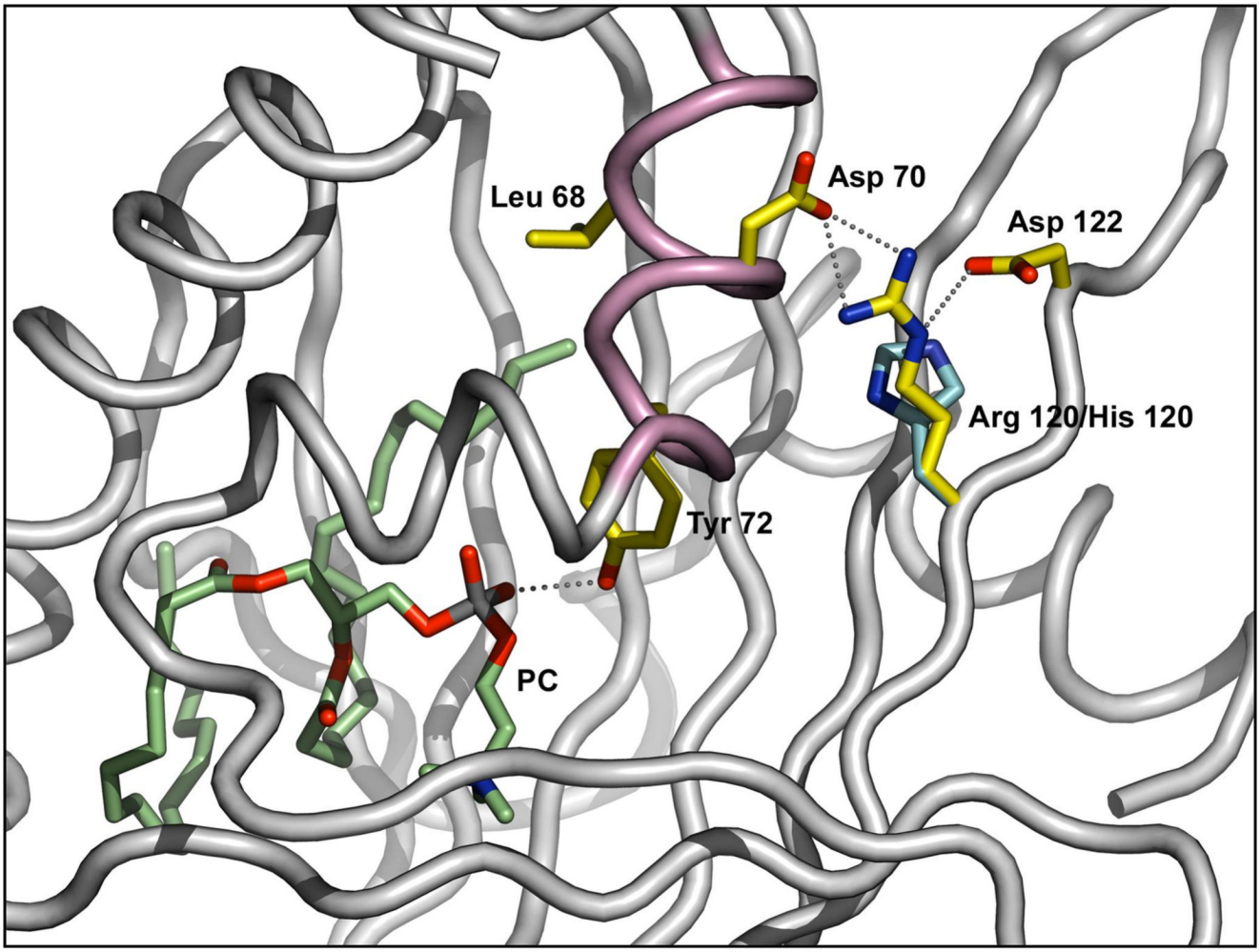

Figure 3. Predicted influence of the Arg120His substitution on PC-TP structure

The three-dimensional structure of PC-TP (gray) in complex with phosphatidylcholine (green). The interactions of Arg120 with Asp70 and Asp122 are depicted by dotted lines, as are the interactions of the phenolic hydroxyl group of Tyr72 with the ligand. A modeled position of His120 (blue) is also depicted. Residues Leu68, Asp70 and Tyr72 are donated from helix α2 (residues 64 – 74; pink). This helix may mediate the effects of the Arg120His mutation to the lipid binding pocket.

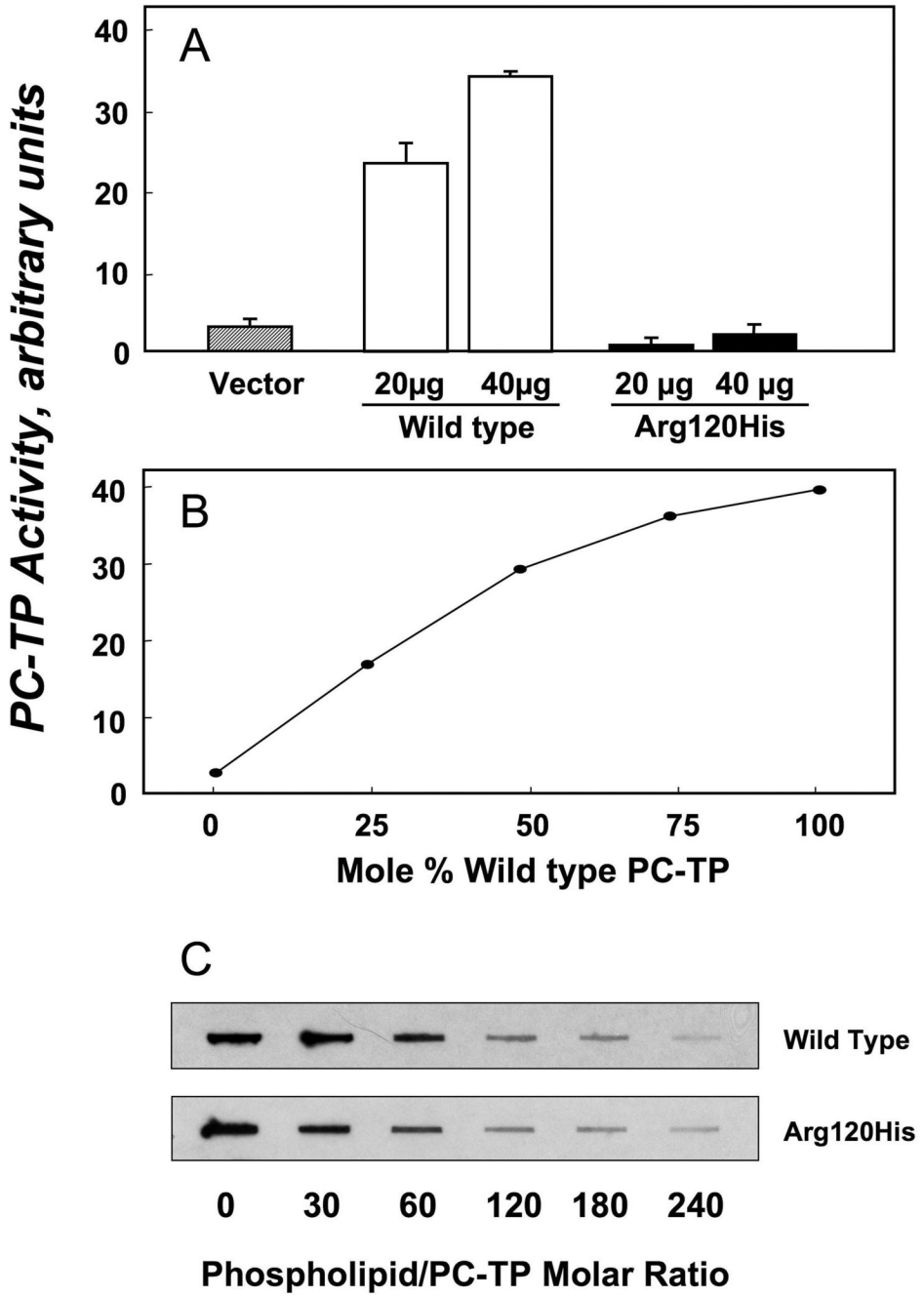

Figure 4. The Arg120His substitution inactivates PC-TP *in vitro* without altering membrane binding

(A) PC-TP activities were compared among vector, vector containing wild type or Arg120His PC-TP. The indicated amounts of PC-TP in *E. coli* cytosol were used to measure PC transfer activity *in vitro*. The assay of vector control consisted of the corresponding amount of cytosolic protein. Data are represented as mean ± SD. (B) Samples of cytosol containing wild type and mutant PC-TP were admixed (50µg total PC-TP) to achieve varied molar ratios of wild type and Arg120His PC-TP. The nearly linear increase in activity as a function of increasing proportion of wild type PC-TP indicated that Arg120His did not inactivate the wild type protein *in vitro.* (C) Membrane binding of His-tag wild type and Arg120His PC-TPs was determined

according to the free fraction of protein as a function of increasing phospholipids/protein ratios. The data are representative of duplicate determinations in two experiments.

Table 1

Comparison of Arg120His variant among 12 mouse strains

Mouse strain	Nucleotide variant on exon 4*	Amino acid variant at residue 120
NZO/HILtJ	A	His
NZW/LacJ	A	His
NZB/BlNJ	A	His
NON/LtJ	G	Arg
EL/SuzSeyFrkJ	G	Arg
DDY/JclSidSeyFrkJ	G	Arg
NOD/LtJ	G	Arg
ALR/LtJ	G	Arg
ALS/LtJ	G	Arg
DBA/2J	G	Arg
C57BL/6J	G	Arg
TallyHo/Jng	G	Arg

* Located 367 bp downstream from the transcription initiation site