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REVIEW ARTICLE

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Clinical characteristics of primary carnitine deficiency: A structured review using a case-by-case approach

Loek L. Crefcoeur[1,2] | **Gepke Visser**[1,2] | **Sacha Ferdinandusse**[2] | **Frits A. Wijburg**[4] | **Mirjam Langeveld**[3] | **Barbara Sjouke**[3]

[1]Department of Metabolic Diseases, Wilhelmina Children's Hospital, University Medical Center Utrecht, Utrecht, The Netherlands

[2]Laboratory Genetic Metabolic Diseases, Amsterdam UMC location Academic Medical Center, University of Amsterdam, Amsterdam Gastroenterology and Metabolism, Amsterdam, The Netherlands

[3]Department of Endocrinology and Metabolism, Amsterdam UMC location Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

[4]Emma's Children's Hospital, Amsterdam UMC location Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Correspondence
Barbara Sjouke, Amsterdam UMC location Academic Medical Center, Department of Endocrinology and Metabolism, Meibergdreef 9, Room F5-169, PO box 22660, 1100 DD, Amsterdam, The Netherlands.
Email: b.sjouke@amsterdamumc.nl

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Abstract

A broad spectrum of signs and symptoms has been attributed to primary carnitine deficiency (PCD) since its first description in 1973. Advances in diagnostic procedures have improved diagnostic accuracy and the introduction of PCD in newborn screening (NBS) programs has led to the identification of an increasing number of PCD patients, including mothers of screened newborns, who may show a different phenotype compared to clinically diagnosed patients. To elucidate the spectrum of signs and symptoms in PCD patients, we performed a structured literature review. Using a case-by-case approach, clinical characteristics, diagnostic data, and mode of patient identification were recorded. Signs and symptoms were categorized by organ involvement. In total, 166 articles were included, reporting data on 757 individual patients. In almost 20% ($N = 136$) of the cases, the diagnosis was based solely on low carnitine concentration which we considered an uncertain diagnosis of PCD. The remaining 621 cases had a diagnosis based on genetic and/or functional (ie, carnitine transporter activity) test results. In these 621 cases, cardiac symptoms (predominantly cardiomyopathy) were the most prevalent (23.8%). Neurological (7.1%), hepatic (8.4%), and metabolic (9.2%) symptoms occurred mainly in early childhood. Adult onset of symptoms occurred in 16 of 194 adult patients, of whom 6 (3.1%) patients suffered a severe event without any preceding symptom (five cardiac events and one coma). In conclusion, symptoms in PCD predominantly develop in early childhood. Most newborns and mothers of newborns detected through NBS remain asymptomatic. However, though rarely, severe complications do occur in both groups.

KEYWORDS

carnitine, clinical characteristics, organic cation transporter novel 2, phenotyping, primary carnitine deficiency, screening

Primary data from this structured review are made publicly available through Figshare. https://doi.org/10.21942/uva.17722598

1 | INTRODUCTION

Primary carnitine deficiency (PCD) (OMIM #212140) is an inborn error of metabolism caused by pathogenic variants in the *SLC22A5* gene, which encodes the organic cation transporter novel 2 (OCTN2) protein.[1] This protein is responsible for the transport of carnitine across the plasma membrane into cells, as well as the reabsorption in renal tubuli.[2] Impaired function of OCTN2 leads to a significant decrease of intracellular carnitine concentrations, potentially hampering mitochondrial fatty acid oxidation. In older publications, patients have been reported to suffer from hepatic encephalopathy, cardiomyopathy, myopathy and arrhythmia, and/or sudden death.[3,4] However, with the introduction of PCD in newborn screening (NBS) programs, increasing numbers of individuals with biallelic *SLC22A5* variants are identified, most of whom are asymptomatic.[5-7]

The diagnostic criteria for PCD have changed over the past decades due to advances in diagnostic techniques (see Figure 1). Initially, patients were diagnosed solely based on low carnitine concentrations, either in muscle (termed "muscle carnitine deficiency") or blood/serum (termed "systemic carnitine deficiency"), distinguishing two forms of the disorder.[8,9] Currently, isolated muscle carnitine deficiency is no longer considered a form of PCD. In 1981, Gazzola et al.[10] described a method to measure carnitine transporter activity in cultured fibroblasts, proving that impaired transport of carnitine across the plasma membrane is the cause of PCD.[11,12] In 1998, Tamai et al.[2] successfully identified the transporter responsible for the transport of carnitine, the OCTN2. One year later, Nezu et al.[1] found the encoding gene (*SLC22A5*) and showed that variants in this gene lead to impaired carnitine transport across the plasma membrane. Subsequently, it has become common practice to diagnose PCD patients based on biallelic pathogenic variants in *SLC22A5*, and/or reduced carnitine transporter activity measured in fibroblasts.[3]

The clinical characterization of rare disorders relies on the combined data of reported cases. However, with advances in the diagnostic approach, revision of historical cases might uncover alternative underlying causes for the patients symptoms. A number of patients who were formerly diagnosed with PCD based on low serum carnitine levels turned out to have secondary carnitine deficiency due to medium-chain acyl-CoA dehydrogenase (MCAD) deficiency.[13-17] Moreover, the first reported PCD patient widely cited as a benchmark case showed elevated levels of C6, C7, and C8 dicarboxylic acids while on carnitine supplementation, which leads to a suspicion of MCAD deficiency.[9,18-20] These examples illustrate that historical case reports of a specific inborn error of metabolism need to be reappraised when determining the disorders clinical spectrum.

Introduction of PCD in NBS programs has enabled detection of newborns prior to the onset of symptoms.[21] However, NBS also detects many asymptomatic maternal cases, which might otherwise have remained unidentified.[22] It is uncertain what the health consequences for these individuals are making counseling on the need of follow-up and treatment particularly difficult.

We aim to update the clinical characterization of PCD, based on current diagnostic standards, through a structured review of previous case reports. The phenotypic spectrum of PCD is described, including separate analyses of patients diagnosed clinically and through screening. This overview aids counseling and treatment decisions in PCD and can facilitate the discussion on the benefit of NBS for this disorder.

2 | METHODS

2.1 | Data sources and search

PubMed and Embase were searched from inception to December 31, 2020. No restrictions were applied on publication date or article type. The search was constructed using terms and synonyms for carnitine deficiency or OCTN2 deficiency (see Supporting Information Table S1). Duplicates were manually removed.

2.2 | Study selection

Study selection was performed according to the PRISMA guidelines.[23] Title and abstracts were screened independently by LLC and BS with use of Covidence (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia. available at http://www.covidence.org). The articles that passed for full-text assessments were judged for inclusion and exclusion by predefined criteria as listed in Supporting Information Table S2. Articles were eligible for inclusion if a case of primary carnitine deficiency was reported, and original data of the clinical characteristics were available in the article. A case of PCD was defined as a serum, plasma, or blood carnitine level below the lower limit of the reference value at the study site reported in the article and/or confirmed low carnitine transporter activity in fibroblasts and/or the presence of biallelic variants in the *SLC22A5* gene. Articles were excluded if the following criteria applied: diagnosis of muscle carnitine deficiency, diagnosis solely based on low postmortem serum carnitine levels, incomplete diagnostics, low plasma carnitine

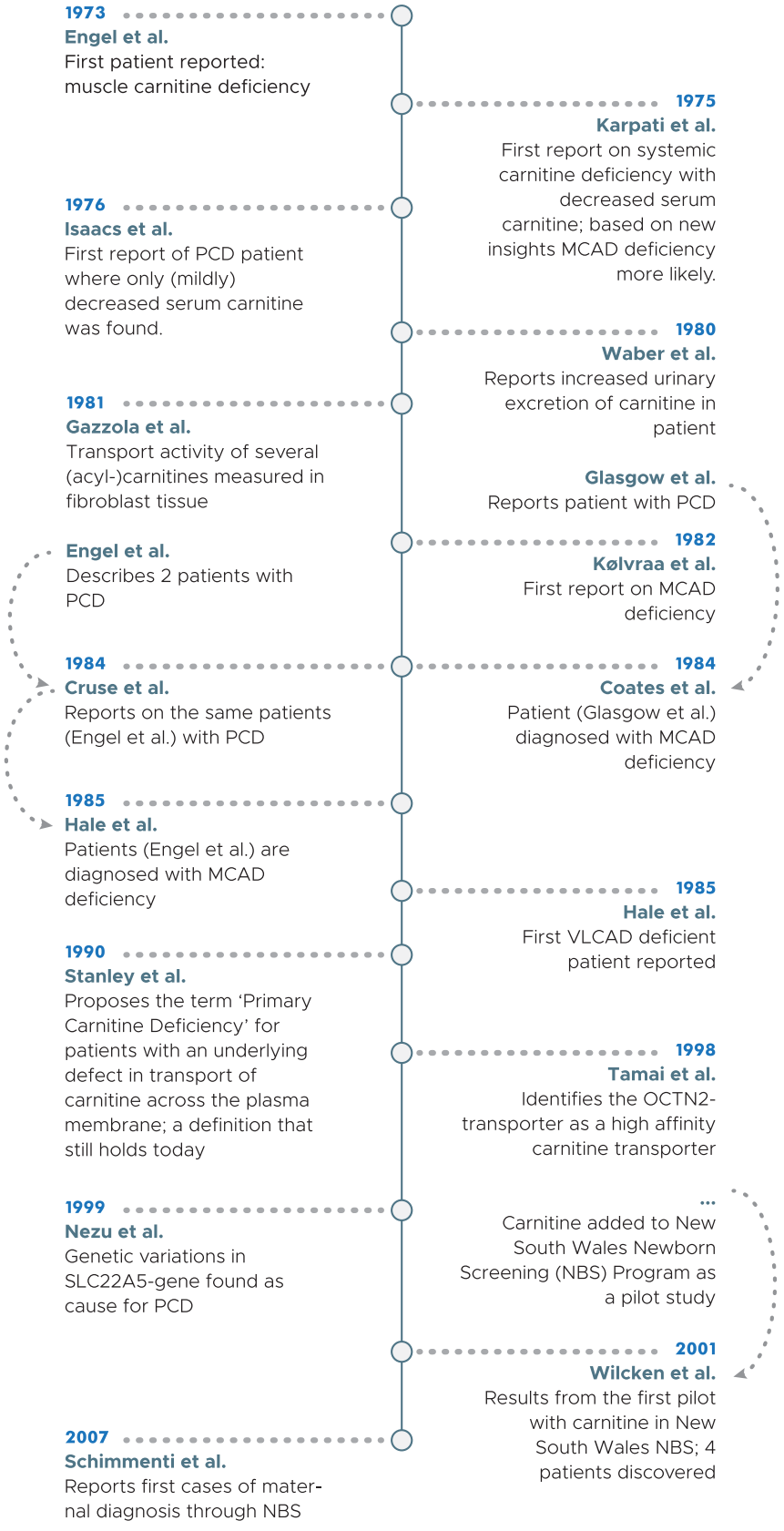

FIGURE 1 Timeline of key moments that impacted the (differential) diagnosis of primary carnitine deficiency

levels more likely caused by a disorder other than PCD, the article did not report data of (an) individual case(s), the article did not concern PCD and finally, and the article was written in a language other than English, Dutch, German, French, or Danish. If full text was unavailable and the abstract contained a case description, the abstract was included in the study. If the abstract did not contain enough data, we requested the full text from

corresponding authors for articles published from 1990 onward, and since from then, the diagnostic definition of PCD is in line with the currently used definition (see Figure 1). If there was disagreement on whether or not to include an article, consensus was reached through discussion between LLC and BS. A full list of included articles is available in Supporting Information Data S1.

2.3 | Data extraction

The following data were extracted for individual cases from the included articles: age at diagnosis, diagnostic test results (plasma carnitine concentration, carnitine transport activity in fibroblasts, and *SLC22A5* variants), clinical symptoms, age at start of symptoms, additional tests performed for symptoms (eg, imaging, laboratory tests), carnitine supplementation dose, and effect of carnitine supplementation on symptoms. If the patient had died, all information on the cause of death and the results of postmortem tests were extracted. If multiple articles described the same case (ie, when referenced in the article or when multiple identifying data points and [part of] the research group overlapped), information from all articles was used but the case was included only once. In case of missing data, this was recorded.

2.4 | General analysis of clinical characteristics

The entire study population was divided into several subgroups for analysis. First, patients were grouped based on the reliability of the diagnosis of PCD, separating patients that were diagnosed solely based on low plasma/serum/blood carnitine concentration (unconfirmed PCD) from patients whose diagnosis was confirmed by either decreased OCTN2 transporter activity and/or biallelic variants in the *SLC22A5* gene (confirmed PCD). Second, patients were grouped based on the mode of detection of the disorder: clinical presentation, screening of high-risk individuals (family screening or a nationwide adult screening campaign in the Faroe Islands[24]), NBS, or maternal diagnosis after NBS of the child. Patient characteristics and symptom prevalence were compared between these groups.

2.5 | Categorization of signs and symptoms

To systematically categorize reported signs and symptoms, two groups were defined: (1) sign/symptom reported by authors (description of a specific sign or symptom only without information on specific test results, for example, laboratory tests, cardiac ultrasound, etc) and (2) sign/symptom confirmed (data on additional test results on which the description of the sign or symptom was based were available, see Supporting Information Table S3). If confirmatory tests and the reported sign/symptom contradicted each other, the conclusion of additional testing was used, overruling the sign/symptom reported by the authors. For instance, if authors reported dilated cardiomyopathy but the cardiac ultrasound showed normal ejection fractions, this was classified as cardiac involvement NOS, since ultrasound contradicts the presence of dilated cardiomyopathy.

All symptoms were registered verbatim in the database and translated to standardized terms using the human phenotype ontology (HPO version 1.7.0; http://www.human-phenotype-ontology.org). These standardized terms were consequently clustered based on organ involvement, resulting in the following groups: (1) cardiac function disorder (cardiomyopathy, cardiac involvement not otherwise specified [NOS]), (2) electrophysiological abnormalities (sustained ventricular tachycardia, ventricular fibrillation, cardiac arrest, sudden cardiac death, conduction disorder NOS, and arrhythmia NOS). For cardiac events (ventricular fibrillation [VF], sustained ventricular tachycardia [sus-VT], cardiac arrest, and sudden cardiac death), if multiple cardiac events occurred in one patient, only one was used for categorization, to prevent double entries in the dataset. For this, the most specifically documented cardiac event was used. For instance, a patient reported with cardiac arrest and VF, would be categorized as VF. (3) Neurological symptoms (coma, encephalopathy, seizure, and neurological involvement NOS), (4) hepatic symptoms (steatosis, hepatic failure, impaired liver function, elevated liver enzymes, and hepatomegaly), (5) muscle symptoms (myopathy, rhabdomyolysis, atrophy, myalgia, weakness, and muscle involvement NOS), (6) metabolic symptoms (hypoglycemia, hyperammonemia, and metabolic acidosis), (7) other symptoms (anemia, motor delay, general developmental delay, dysmorphia, and other/nonspecific), and (8) death. For the full list of standardized terms that were applied and a summary of how these were designated in characteristic-groups, see Supporting Information Table S4.

2.6 | Genetic data

Extracted variants were annotated using the comprehensive overview of molecular changes in *SLC22A5* by Frigeni et al.,[25] the OCTN2 database provided by ARUP Scientific Resource for Research and Education, and Alamut Visual

V2.11 (Interactive Biosoftware).[26] For analysis of genotype-phenotype associations, variants were grouped into (1) missense variants and in-frame deletions/insertions and (2) nonsense, frameshift, and splice site variants.

2.7 | Statistics

Statistical analysis was performed using R (version 3.6.2, R Core Team, Vienna, Austria). For comparison between categorical data, the chi-square test was used. Mann–Whitney U tests were used for comparison between two groups with continuous data. Bonferroni correction was applied to correct for multiple testing. Significance was assumed for a *P*-value <0.05.

3 | RESULTS

With the search, 1532 articles were identified for title and abstract screening of which 166 met the inclusion criteria for this review, yielding 757 unique cases. Of these, 53 cases were described in two or more articles. The results of data selection are presented in a PRISMA flow diagram (Figure 2). One hundred thirty-six patients had unconfirmed PCD (diagnosis based on serum, plasma, or blood carnitine concentration only), while the remaining 621 patients had confirmed PCD. Of those, 26 were diagnosed by decreased carnitine transporter activity, 534 by the detection of biallelic variants in the *SLC22A5* gene and 61 by results from both tests. An overview of patient characteristics of unconfirmed and confirmed PCD patients is provided in Table 1. The median year of publication differed significantly between unconfirmed and confirmed PCD (2010 [interquartile range (IQR) 1990–2020] and 2014 [IQR 2010–2020] respectively, $P < 0.001$). Neurological, muscle, hepatic, and metabolic symptoms were all reported more often in unconfirmed PCD (15.4, 33.8, 16.2, 19.9%, respectively) compared to the confirmed population (7.1, 10.3, 8.4, 9.2%, respectively; *P* values: 0.023, <0.001, 0.071, 0.005, respectively; only for hepatic symptoms was no significant difference observed). In the unconfirmed PCD group, 42.6% of individuals were asymptomatic, vs 55.1% in the confirmed PCD group ($P = 0.090$). Finally, mortality

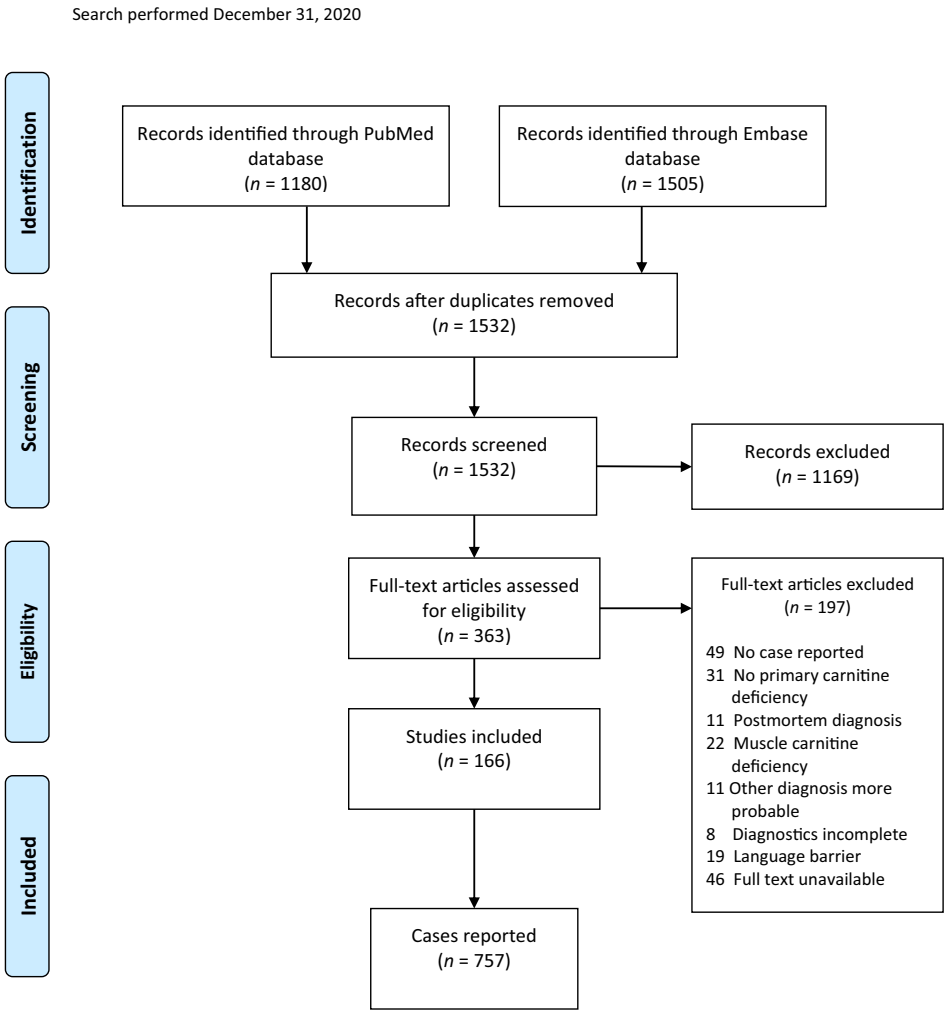

FIGURE 2 PRISMA flow diagram of literature selection and inclusion

TABLE 1 Patient characteristics of patients with a possible and a certain diagnosis of PCD

		Possible diagnosis of PCD (diagnosis based on low plasma/serum/blood carnitine concentration only) $N = 136$		Certain diagnosis of PCD (diagnosis based on decreased activity and/or bialleic variants in the *SLC22A5* gene) $N = 621$		
		Median/*N*	**[Min–Max]/(%)**	**Median/*N***	**[Min–Max]/(%)**	***p* value**
Year published		2010	[1976–2020]	2014	[1979–2021]	**<0.001**
Sex (male)		45	(34.4%)	234	(42.4%)	
Age at diagnosis (years)		3.75	[0.0–45.0]	1.00	[0.0–79.0]	
Infancy		48	(35.6%)	286	(46.7%)	
Childhood		42	(31.1%)	133	(21.7%)	**0.025**
Adulthood		45	(33.3%)	194	(31.6%)	
Age at first symptom (years)		1.00	[0.0–40.0]	1.00	[0.00–39.0]	
Infancy		29	(42.6%)	63	(33.0%)	
Childhood		27	(39.7%)	112	(58.6%)	**0.003**
Adulthood		12	(17.6%)	16	(8.4%)	
Carnitine[a]						
Free carnitine	(*N* samples)	82		470		
	(Ratio)	0.31	[0.01–095]	0.34	[0.00–1.00]	
Total carnitine	(*N* samples)	50		68		
	(Ratio)	0.49	[0.00–1.37]	0.17	[0.00–3.58]	
Unspecified carnitine	(*N* samples)	9		4		
	(Ratio)	0.59	[0.25–0.89]	0.13	[0.00–0.72]	
Transporter activity (% of controls)[b]		-		5.0	[0.0–22.0]	
Cardiac symptoms		34	(25.0%)	148	(23.8%)	1.000
Neurological symptoms		21	(15.4%)	44	(7.1%)	**0.023**
Muscle symptoms		46	(33.8%)	64	(10.3%)	**<0.001**
Hepatic symptoms		22	(16.2%)	52	(8.4%)	0.071
Metabolic symptoms		27	(19.9%)	57	(9.2%)	**0.005**
Only nonspecific symptoms		3	(2.2%)	72	(11.6%)	**0.012**
Asymptomatic		58	(42.6%)	342	(55.1%)	0.090
Deceased		10	(7.4%)	25	(4.0%)	0.057

Note: Data presented as: *N* (%) or median [Min–Max]. Significant *p* values are in bold. For sex, age at diagnosis, age period at diagnosis, age at first symptom and age period at first symptom, the number of missing data were, respectively: possible diagnosis: 5, 12, 1, 20, 12; certain diagnosis: 69, 54, 8, 139, 88.
Abbreviation: PCD, primary carnitine deficiency.

[a]If possible, ratios were calculated for serum/plasma/blood carnitine concentrations as follows: $\frac{\text{Carntine concentration}}{\text{Lower reference value at study site}}$. Provided data are: N available samples, ratio [Min–Max].

[b]If possible, results were converted to % of controls as follows: $\left(\frac{\text{Carnitine transporter activity}}{\text{Mean activity of controls}}\right) \cdot 100\%$. This conversion was possible for 67/87 samples.

was higher in the unconfirmed PCD group (7.4 vs 4.0%; $P = 0.057$), though this observation was not significant.

Analyses in the following paragraphs were performed in the confirmed PCD group only.

3.1 | Clinical characteristics based on mode of detection

Of the 621 patients, 161 were identified after presenting with symptoms (clinical diagnosis), 102 maternal cases were identified through abnormal results in the NBS of their child (maternal cases), 249 cases were identified through NBS and finally, and 109 patients were detected through high-risk screening.

Clinically diagnosed patients ($N = 161$) were mostly diagnosed in infancy/childhood ($N = 145$; 90.1%). Only five of 161 patients (3.1%) experienced their first symptom after childhood. The median age at first symptom was 1 year for the clinically diagnosed patients [IQR 0.5–5 years]. In contrast, none of the maternal cases had experienced symptoms in childhood. Of the symptomatic

maternal cases (17/102; 16.7%), nine experienced exclusively pregnancy-related/nonspecific symptoms (see Table 2) and eight patients developed the following symptoms: VF and hypotonia ($N = 1$; 1.0%), sus-VT ($N = 1$; 1.0%), cardiomyopathy with cardiac dilation ($N = 1$; 1.0%), hepatic steatosis with hypoglycemia ($N = 1$; 1.0%), muscle weakness ($N = 3$; 3.9%), and fasting intolerance ($N = 1$; 1.0%). The signs and symptoms in NBS patients (25/249; 10.0%) were, respectively: nonspecific ($N = 11$), cardiac involvement ($N = 4$; atrial/

TABLE 2 Clinical characteristics of PCD patients based on mode of detection

		Symptomatic presentation		Risk screening		Maternal NBS		NBS	
		$N = 161$		$N = 109$		$N = 102$		$N = 249$	
Age at diagnosis (years)		3.0	[0.0–57.0]	29.0	[0.0–79.0]	29.0	[20.0-43.0]	0.0	[0.0-0.7]
Infancy		32	(19.9%)	5	(4.6%)	0	(0.0%)	249	(100%)
Childhood		113	(70.2%)	20	(18.3%)	0	(0.0%)	0	(0.0%)
Adulthood		13	(8.1%)	79	(72.5%)	102	(100%)	0	(0.0%)
Age at first symptom (years)		1.0	[0.0–39.0]	3.0	[0.5–34.0]	22.0	[20.0–34.0]	0.1	[0.0–4.0]
Infancy		51	(31.7%)	3	(3.9%)	0	(0.0%)	9	(3.7%)
Childhood		99	(61.4%)	10	(13.1%)	0	(0.0%)	3	(1.2%)
Adulthood		5	(3.1%)	4	(5.2%)	7	(7.4%)	0	(0%)
Free carnitine ratio	(N samples)	87		86		84		213	
	(Ratio)	0.12	[0.00–1.00]	0.44	[0.00–0.83]	0.20	[0.00–1.00]	0.41	[0.00–0.92]
Transporter activity	(N samples)	50		3		11		4	
	(% of control)	5.0	[0.0–22.0]	5.0	[5.0–6.0]	3.7	[0.1–9.0]	5.0	[5.0–16.0]
Genetic analysis performed		143		100		97		249	
MS.IF/MS.IF		52	(36.4%)	37	(37.0%)	65	(67.0%)	121	(48.6%)
MS.IF/NS.FS.SS		24	(16.8%)	11	(11.0%)	25	(25.8%)	90	(36.1%)
NS.FS.SS/NS.FS.SS		55	(38.5%)	5	(5.0%)	2	(2.1%)	16	(6.4%)
MS.IF/missing		3	(2.1%)	0	(0.0%)	1	(1.0%)	7	(2.8%)
NS.FS.SS/missing		3	(2.1%)	0	(0.0%)	0	(0.0%)	3	(1.2%)
Missing/missing		6	(4.2%)	47	(47.0%)	4	(4.1%)	13	(5.2%)
Cardiac symptoms		118[a]	(72.7%)	22	(20.2%)	3	(2.9%)	6[b]	(2.4%)
Neurological symptoms		38	(23.6%)	3	(2.8%)	0	(0.0%)	3	(1.2%)
Muscle symptoms		55	(34.2%)	3	(2.8%)	4	(3.9%)	2	(0.8%)
Hepatic symptoms		49	(30.4%)	1	(0.9%)	1	(1.0%)	1	(0.4%)
Metabolic symptoms		49[a]	(30.4%)	1	(0.9%)	2	(2.0%)	5	(2.0%)
Only unspecific symptoms		4[c]	(2.5%)	48[d]	(44.0%)	9[e]	(8.8%)	11[f]	(4.4%)
Asymptomatic		0	(0.0%)	33	(30.3%)	85	(83.3%)	224	(90.0%)
Deceased		21	(13.1%)	1	(0.9%)	0	(0.0%)	3	(1.2%)

Note: Data presented as: N (%) or median [Min–Max]. For age at diagnosis, age period at diagnosis. Age at first symptom and age period at first symptom, the number of missing data were, respectively: symptomatic presentation: 29, 3, 45, 6; risk screening: 11, 5, 65, 59; maternal NBS: 9, 0, 14,10; NBS: 5, 0, 15, 13. Missing (under genetic analysis), Variant identified, but type was not reported.

Abbreviations: Maternal NBS, mothers identified through NBS of their child; MS.IF, missense/in-frame variant; NBS, newborn screening; NS.FS.SS, nonsense/frameshift/splice-site; PCD, primary carnitine deficiency.

[a]Includes one patient that was also diagnosed with NAGS deficiency.

[b]Includes one patient that was also diagnosed with NICCD.

[c]Failure to thrive without cardiac symptoms ($N = 2$) | fatigue ($N = 1$) | diabetes ($N = 1$).

[d]Fatigue ($N = 17$) | abdominal symptoms ($N = 10$) | respiratory infection ($N = 4$) | palpitations ($N = 3$) | fever ($N = 2$) | chest pain ($N = 2$) | slow weight gain ($N = 1$) | diabetes ($N = 1$) | carotid dissection ($N = 1$) | dizzynes ($N = 1$) | oligomenorrhea ($N = 1$) pneumothorax ($N = 1$) | psoriasis ($N = 1$) | hypertension ($N = 1$) | sinusitis ($N = 1$) | testicular cancer ($N = 1$).

[e]Decreased stamina during pregnancy, spontaneous miscarriages ($N = 1$) | sinus tachycardia, palpitations, and preeclampsia ($N = 1$) | hyperemesis gravidarum ($N = 1$) | fatigue ($N = 6$).

[f]Growth retardation ($N = 6$) | cleft palate ($N = 2$) | abdominal pain ($N = 1$) | congenital double thumb ($N = 1$) | fatigue ($N = 1$).

TABLE 3 Overview of cardiac symptoms

	Cardiac function				Cardiac events							
	Cardiomyopathy $N = 112$ (82)		**Cardiac involvement NOS** $N = 15$ (11)[a,b]		**Sus-VT** $N = 2$		**VF** $N = 7$		**Cardiac arrest** $N = 6$		**Sudden death** $N = 8$	
Arrhythmia NOS[c]	6	(1 tachy, 4 brady, 1 ns-VT)	0		0		2	(1 brady, 1 ns-VT)	0		0	
Conduction disorder NOS[d]	4	(1 HB, 3 short QT)	2	(2 short QT)	1	(long QT)	1	(short QT)	0		0	
Deceased	6		3		1		2		4		8	
Age at first symptom (years)	1.0	[0.0–39.0]	1.0	[0.0–22.0]	23.0	[20.0–26.0]	28.0	[15.0–39.0]	0.5	[0.3–1.0]	5.0	[0.3–6.0]
Infancy	35		3		0		0		4		1	
Childhood	72		6		0		1		2		6	
Adulthood	3		3		2		6		0		0	
Detected through												
Symptoms	103		7		1		3		5		7	
Family screening	8		3		0		2		1		0	
Maternal NBS	1		1		1		2		0		0	
NBS	0		4		0		0		0		1	
Free carnitine ratio (*N* samples)	66		9		1		4		4		3	
(Ratio)	0.10	[0.00–1.00]	0.40	[0.11–0.76]	0.08	[−]	0.11	[0.04–0.20]	0.25	[0.09–0.41]	0.16	[0.14–0.16]
Transporter activity (N samples)	**35**		**2**		**1**		**1**		**4**		**1**	
(% of control)	**5.00**	**[0.00–20.80]**	**3.47**	**[2.48–5.00]**	**4.8**	**[−]**	**9.6**	**[−]**	**5.0**	**[2.0–10.0]**	**5.00**	**[−]**
Genetic analysis performed	99		13		2		6		5		7	
MS.IF/MS.IF	27	(27.3%)	6	(40.0%)	2	(100%)	4	(66.7%)	2	(40.0%)	2	(28.6%)
MS.IF/NS.FS.SS	17	(17.2%)	5	(33.3%)	0	(0.0%)	2	(33.3%)	1	(20.0%)	1	(14.3%)
NS.FS.SS/NS.FS.SS	44	(44.4%)	1	(6.7%)	0	(0.0%)	0	(0.0%)	2	(40.0%)	4	(57.1%)
MS.IF/missing	2	(2.0%)	0	(0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
NS.FS.SS/missing	3	(3.0%)	0	(0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Missing/missing	6	(6.0%)	1	(6.7%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)
Carnitine treatment started	91		12		1		4		2		1	
Clinical improvement	86		7		1		3		2		0	

Note: Data presented as: *N* total (confirmed)/*N* (%) or median [Min–Max]. For age at first symptom and age period at first symptom, the number of missing data were, respectively: cardiomyopathy: 30, 2; cardiac involvement NOS: 8, 18; sus-VT: 0, 0; VF: 1, 0; cardiac arrest: 1, 0; sudden death: 3, 1. Missing (under genetic analysis), variant identified, but type was not reported.

Abbreviations: brady, bradycardia; HB, first degree heart block; Maternal NBS, mothers identified through NBS of their child; MS.IF, missense/in-frame variant; NBS, newborn screening; NOS, not otherwise specified; NS.FS.SS, nonsense/frameshift/splice-site; ns-VT, nonsustained ventricular tachycardia; PCD, primary carnitine deficiency; sus-VT, sustained ventricular tachycardia; tachy, tachycardia; VF, ventricular fibrillation.

[a]Includes one patient that was also diagnosed with NAGS deficiency.

[b]Includes one patient that was also diagnosis with NICCD.

[c]Total ($N = 11$): brady ($N = 5$) | atrial fibrillation ($N = 3$) | ns-VT ($N = 1$) | tachy ($N = 1$) | arrhythmia, not otherwise specified ($N = 1$).

[d]Total ($N = 13$): short QT ($N = 7$) | right bundle branch block ($N = 3$) | left bundle branch block ($N = 1$) | HB ($N = 1$) | Long QT ($N = 1$).

ventricular septal defect and/or mitral valve insufficiency), metabolic signs ($N = 1$; metabolic decompensation), metabolic signs and neurological symptoms ($N = 3$; hypoglycemia and coma/convulsions), hepatic symptoms ($N = 1$; elevated alanine aminotransferase (ALAT)), muscle symptoms ($N = 2$; mild muscle weakness, elevated creatine kinase), and death ($N = 3$).[6,27-32] In patients identified through high-risk screening, the majority of individuals did not experience any (PCD-related) symptoms (30.3% asymptomatic and 44.0% only nonspecific symptoms). But the prevalence of severe symptoms (cardiomyopathy, cardiac events, and sudden death) was higher in this group than in patients identified through newborn screening (NBS and maternal cases). Genetic analysis of the *SLC22A5* gene revealed more biallelic nonsense, frameshift, or splice-site variants in patients that were diagnosed after symptomatic presentation, than in patients detected through high-risk screening, maternal, and NBS cases (38.5% vs 5.0, 2.1 and 6.4%, respectively, P value < 0.001). Symptoms across all symptom groups were more prevalent in those that carry biallelic nonsense, frame-shift, and/or splice-site variants compared to those that carry missense or in-frame variants (Supporting Information Table S5).

Treatment was initiated upon diagnosis in 133 of 161 (82.6%) of the clinically diagnosed patients (no treatment $N = 14$ and missing $N = 14$), in 93 of 109 (85.3%) of the high-risk screening patients (no treatment $N = 1$ and missing $N = 15$), in 69 of 102 (67.6%) of the maternal cases (no treatment $N = 11$ and missing $N = 22$), and in 200 of 249 (80.3%) of the newborns (no treatment $N = 7$ and missing $N = 42$). The clinically detected patients and high-risk screening patients that did not receive treatment died prior to diagnosis.

3.2 | Cardiac symptoms

Cardiomyopathy was the most prevalent symptom, reported in 112 of 621 cases (18.0%) (see Table 3). In 59 (9.5%) patients, cardiac dilation was described, with results of cardiac imaging available for 53 patients. Data on left ventricular ejection fraction (LVEF) were available for 23 patients, with a median LVEF of 34% (IQR: 25–39.5) without treatment. Fifteen of these received carnitine treatment with improvement of ejection fraction (median 66%; IQR: 61.5–73). Cardiomyopathy with hypertrophy was reported in 31 patients (5.0%) (imaging results available for 23 patients). For the remaining 22 (3.5%) patients with cardiomyopathy, imaging results were available for six patients, but no information of cardiac morphology was provided. Most patients that developed cardiomyopathy presented with symptoms in childhood ($N = 102$; 95.3%; median age 1 year). Two patients (1.9%) developed cardiomyopathy in adulthood without any prior symptoms; one mother that was diagnosed through NBS of her child and one patient was diagnosed through family screening in childhood. The latter remained asymptomatic whilst on carnitine supplementation and developed cardiomyopathy after discontinuation in adulthood.[33,34] None of the PCD patients detected through NBS developed cardiomyopathy. In 15 (2.4%) patients other cardiac signs or symptoms were described, namely: cardiomegaly ($N = 3$), cardiac dilation with normal ejection fraction ($N = 3$), mitral/aortic valve insufficiency ($N = 2$), myocardial infarction ($N = 2$), atrial septal defect ($N = 2$), ventricular septal defect ($N = 1$), cardiac fibroelastosis ($N = 1$), and intramyocardial lipid deposition ($N = 1$).

Twenty-three patients (3.7%) experienced cardiac events (Table 4), namely: VF ($N = 7$; 1.2%), sus-VT ($N = 2$; 0.3%), cardiac arrest ($N = 6$; 1.0%), and sudden cardiac death ($N = 8$; 1.3%). In 14 of 23 patients with a cardiac event (60.8%), this event was the presenting symptom. In eight of 23 patients (34.8%), the first cardiac event occurred in adulthood. Five of these adults (CE-1, CE-2, CE-4, CE-18, and CE-23) were completely asymptomatic before the cardiac event occurred. They had not received carnitine supplementation prior to the event. The three remaining patients had the following medical history prior to the cardiac event: coma 2 months before event (CE-14), laparotomy 4 days prior to event (CE-15), and NAGS deficiency diagnosed at 5 years of age (CE-9) (dubious diagnosis, more details on case description in "deceased patients").[35] One patient (CE-6) experienced a cardiac event whilst on carnitine treatment; he died at the age of 26 years due to asystole after a history of multiple cardiac arrests in childhood, hypoglycemic coma with seizures, hyperammonemia, hepatomegaly, weakness, and developmental delay.

3.3 | Neurologic, hepatic, and metabolic signs and symptoms

Neurologic, hepatic, and/or metabolic symptoms were reported in a total of 89 patients (14.3%), with more than one of these symptoms in 47 (52.8%) patients (Table 5). Coma or encephalopathy occurred in 35 (5.6%) patients and was the presenting feature in 27 patients. In 14 (40%) of 35 patients, an underlying cause was reported: hypoglycemia ($N = 8$), hyperammonemia ($N = 1$), or both ($N = 5$). In 21 of 35 cases, an underlying cause was not reported, but 13 of 21 did have a history with hypoglycemia and/or hyperammonemia. Hepatic symptoms

TABLE 4 Patient characteristics of patients that suffered a cardiac event

Patient ID	Reference		Sex	Country	Detected through	Cardiac event	Age at first cardiac event	Age at diagnosis (years)	Cardiac event presenting symptom	Variant allele 1	Variant allele 2	Deceased
CE-1	Schimmenti et al.22	2007	F	USA	Maternal NBS	sus-VT	20 years	25	Yes	c.95A>G	c.136C>G	
CE-2	Mazzini et al.[53]	2011	F	USA	Maternal NBS	VF	22 years	22	Yes	c.424G>T/ c.1463G>A	**c.1586+1G>T**	
CE-3	Rijlaarsdam et al.[54]	2003	F	Iran	Symptomatic presentation	VF	15 years	15	Yes	NP. Transporter activity 9.6% of control		
CE-4	Rasmussen et al.[55]	2014	F	Faroe	Symptomatic presentation	VF	39 years	39	Yes	c.95A>G	c.95A>G	
CE-5	Rasmussen et al.[55]	2014	F	Faroe	Symptomatic presentation	Sudden cardiac death	NA	Postmortem	Yes	c.95A>G	c.95A>G	†
CE-6	Chapoy et al.[56]	1980	M	Mexico	Symptomatic presentation	Cardiac arrest	3 months	3	Yes	**c.806delT**	**c.806delT**	†
CE-7	Tang et al.[57]	1998	M	China	Symptomatic presentation	Cardiac arrest	6 months	Postmortem	Yes	**c.396G>A**	c.1433C>T	†
CE-8	Vaz et al.[58]	1999	F	Cape Verde	Symptomatic presentation	Cardiac arrest	20 months	1	No	c.632A>G	c.632A>G	
CE-9	Hwu et al.[35]	2007	M	Taiwan	Symptomatic presentation	Sudden cardiac death	20 years	Postmortem[a]	No	**c.760C>T**	**c.652+1G>A**	†
CE-10	Kishimoto et al.[59]	2012	M	Japan	Symptomatic presentation	Sudden cardiac death	8 years	Postmortem	No	**c.-91_22del**	**c.-91_22del**	†
CE-11	Melegh et al.[60]	2004	M	Hungary	Symptomatic presentation	Sudden cardiac death	6 years	Postmortem	Yes	**c.844delC**	**c.844delC**	†
CE-12	Han et al.[61]	2014	NA	China	Symptomatic presentation	Sudden cardiac death	6 months	Childhood	No	**c.760C>T**	c.338G>A	†
CE-13	Grünert et al.[62]	2020	M	Macedonia	Family screening	Cardiac arrest	4 years	7	No	c.1319C>T	c.1319C>T	
CE-14	Rasmussen et al.[63]	2013	F	Faroe	Adult screening	VF	34 years	55	No	c.95A>G	c.95A>G	
CE-15	Rasmussen et al.[63]	2013	F	Faroe	Symptomatic presentation	sus-VT	26 years	Postmortem	No	c.95A>G	c.95A>G	†
CE-16	Rasmussen et al.[63]	2013	F	Faroe	Symptomatic presentation	VF	NA	Postmortem	Yes	c.95A>G	c.95A>G	†
CE-17	Dobrowolski et al.[64]	2005	M	Australia	Symptomatic presentation	Sudden cardiac death	18 months	Postmortem	No	NP. Transporter activity < 5% of control		†
CE-18	Gélinas et al.[65]	2019	F	China	Symptomatic presentation	Sudden cardiac death	26 years	Postmortem	Yes	c.51C>G	c.51C>G	†
CE-19	Rasmussen et al.[63]	2013	F	Faroe	Symptomatic presentation	VF	NA	Postmortem	Yes	c.95A>G	c.95A>G	†
CE-20	Tang et al.[57]	1998	M	China	Symptomatic presentation	Cardiac arrest	6 months	Postmortem	Yes	NP. Transporter activity 5% of control		†
CE-21	Lin et al.[29]	2020	F	China	NBS	Sudden cardiac death	2 months	Infancy	Yes	**c.497+1G>T**	**c.517delC**	†
CE-22	Lin et al.[29]	2020	F	China	NBS	Cardiac arrest	1 years	Infancy	NA	**c.760C>T**	**c.824+1G>A**	†
CE-23	Roussel et al.[66]	2016	F	France	Family screening	VF	30 years	28	Yes	c.1411C>T	**Exon 2 deletion**	

Note: Nonsense/frameshift/splice-site variants are in bold.

Abbreviations: †, deceased; F, female; M, male; maternal NBS, mothers identified through NBS of their child; NA, not available; NBS, newborn screening; NP, not performed; PCD, primary carnitine deficiency; sus-VT, sustained ventricular tachycardia; VF, ventricular fibrillation.

[a]Diagnosed with NAGS deficiency (9% residual activity) at age 5 years, PCD was confirmed postmortem, and no variants in *NAGS* gene could be identified.

TABLE 5 Overview of neurologic, hepatic, and metabolic symptoms

	Only neurologic $N = 10$	Only hepatic $N = 20$	Only metabolic $N = 12$	Neurological and hepatic $N = 2$	Neurological and metabolic $N = 15$	Hepatic and metabolic $N = 13$	Neurological, hepatic and metabolic $N = 17$	Total $N = 89$
Neurologic								
Coma[a]	4 (2 seizure)	-	-	1	5	-	11 (3 seizure)	21 (5 seizure)
Encephalopathy[b]	2	-	-	1	7 (1 seizure)	-	4	14 (1 seizure)
Neurological involvement NOS[c]	2	-	-	0	0	-	1	3
Seizure[d]	2	-	-	0	3	-	1	6
Coma/encephalopathy presenting feature (yes)	4	-	-	1	9	-	13	27
Hepatic								
Elevated liver enzymes	-	4	-	0	-	5	9	18
Impaired liver function	-	2	-	0	-	1	1	4
Hepatic steatosis	-	1 (1)	-	2 (2)	-	2 (1)	6 (4)	11 (8)
Hepatomegaly	-	16 (0)	-	1 (0)	-	10 (1)	14 (0)	41 (1)
Hepatic failure	-	0 (0)	-	0 (0)	-	1 (0)	0 (0)	1 (0)
Metabolic								
Hypoglycemia	-	-	8 (4)[e]	-	14 (2)	9 (0)	15 (4)	46 (10)
Hyperammonemia	-	-	4 (3)[e]	-	6 (1)	5 (2)	10 (5)	25 (11)
Acidosis	-		4 (1)	-	3 (0)	2 (2)	3 (1)	12 (4)
Deceased	3	5	2	2	0	1	2	15
Age at first symptom (years)	2.0 [0.0–34.0]	0.8 [0.3-26.0]	0.4 [0.0-5.0]	1.0 [1.0-1.0]	1.0 [0.1-12.0]	1.0 [0.3-39.0]	0.8 [0.3-5.0]	1.0 [0.0–39.0]
Infancy	1	10	6	0	5	4	11	37
Childhood	8	6	5	1	10	7	6	43
Adulthood	1	2	1	1	0	1	0	6
Detected through								
Symptoms	8	18	9	2	11	12	17	77
Risk screening	2	1	0	0	1	0	0	4
Maternal NBS	0	0	1	0	0	1	0	2
NBS	0	1	2	0	3	0	0	6

TABLE 5 (Continued)

	Only neurologic $N = 10$		Only hepatic $N = 20$		Only metabolic $N = 12$		Neurological and hepatic $N = 2$		Neurological and metabolic $N = 15$		Hepatic and metabolic $N = 13$		Neurological, hepatic and metabolic $N = 17$		Total $N = 89$	
Genetic analysis																
MS.IF/MS.IF	6	(60.0%)	7	(41.2%)	4	(33.3%)	2	(100%)	5	(33.3%)	2	(20.0%)	8	(53.3%)	35	(43.2%)
MS.IF/NS.FS.SS	0	(0.0%)	2	(11.8%)	2	(16.7%)	0	(0.0%)	3	(20.0%)	3	(30.0%)	1	(6.67%)	13	(16.0%)
NS.FS/NS.FS.SS	2	(20.0%)	5	(29.4%)	4	(33.3%)	0	(0.0%)	4	(26.7%)	4	(40.0%)	6	(40.0%)	22	(27.2%)
MS.IF/missing	0	(0.0%)	2	(11.8%)	1	(8.33%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	3	(3.7%)
NS.FS.SS/missing	1	(10.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	1	(1.2%)
Missing/missing	1	(10.0%)	1	(5.9%)	1	(8.3%)	0	(0.0%)	3	(20.0%)	1	(10.0%)	0	(0.0%)	7	(8.6%)

Note: Data presented as: *N* total (confirmed)/*N* (%) or median [Min–Max]. For age at first symptom and age period at first symptom, the number of missing data were, respectively: only neurologic: 2, 0; only hepatic: 5, 2; only metabolic: 2, 0; neurological and hepatic: 1, 0; neurological and metabolic: 4, 0; hepatic and metabolic: 4, 1; neurologic, hepatic, and metabolic: 1, 0; total: 19, 3. Missing (under genetic analysis), variant identified, but the type was not reported.

Abbreviations: MS.IF, missense/in-frame variant; NBS, newborn screening; NOS, not otherwise specified; NS.FS.SS, nonsense/frameshift/splice-site.

[a]Hypoglycemic ($N = 5$), hyperammonemic ($N = 1$), hypoglycemic hyperammonemic ($N = 3$), and NOS ($N = 12$).

[b]Hypoglycemic ($N = 3$), hypoglycemic hyperammonemic ($N = 2$), and NOS ($N = 9$).

[c]Encephalitis; hydrocephalus; and patchlike abnormalities brain MRI.

[d]The number of cases where seizure was the only reported neurological symptom.

[e]Includes one patient that was also diagnosed with NAGS deficiency.

TABLE 6 Patient characteristics of deceased patients

Patient ID	Reference		Country	Sex	Consanguinity	Family death	Age at diagnosis	Carnitine at diagnosis (ref)		Transporter activity
16/CE-6	Chapoy et al.[67]	1980	Mexico	M	No	1 brother	3.5 years	Unsp.	4.82 (28.11–44.57)	2% of control
52/CE-9	Hwu et al.[35]	2007	Taiwan	M	NA	NA	NA[a,b]	Free	4 (29–45)	
086	Melegh et al.[60]	2004	Hungary	M	No	2 cousins (180)	NA[a]	NP		
162_A/CE-10	Kishimoto et al.[59]	2012	Japan	M	Yes	1 brother (163_A)	NA[a]	NP		
163_A	Kishimoto et al.[59]	2012	Japan	M	Yes	1 brother (162_A)	NA[a]	NP		
164	Koizumi et al.[68]	1999	Japan	M	No	0	NA[a]	NP		
168	Lund et al.[69]	2007	Faroe	F	NA	0	NA[a]	Free	3.4 (median 27.8)	
169	Lund et al.[69]	2007	Faroe	F	NA	0	NA[a]	Free	4.4 (median 27.8)	
182/CE-11	Melegh et al.[60]	2004	Hungary	M	No	1 sister	NA[a]	NP		
186/CE-5	Rasmussen et al.[55]	2014	Faroe	F	NA	0	NA[a]	NP		
293	Lee et al.[33]	2010	Taiwan	F	NA	NA	1 year	NP		
90	Ohkuma et al.[70]	2009	Japan	F	No	NA	Childhood	NP		
123/CE-7	Tang et al.[57]	1998	China	M	No	1 sister	NA[a]	Free	9 (22–50)	5% of control
180	Melegh et al.[60]	2004	Hungary	M	No	2 cousins (86)	NA[a]	NP		
301/CE-12	Han et al.[61]	2014	China	NA	NA	1 sibling	Infancy	Free	1.62 (>10)	
98_B/CE-15	Rasmussen et al.[63]	2013	Faroe	F	NA	1 sister (414_B)	NA[a]	NP		
414_B/CE-16	Rasmussen et al.[63]	2013	Faroe	F	NA	1 sister (98_B)	NA[a]	NP		
408/CE-17	Dobrowolski et al.[64]	2005	Australia	M	NA	0	NA[a]	Free	3 (−)	5% of control
412/CE-18	Gélinas et al.[65]	2019	China	F	No	0	NA[a]	NP		
415	Rasmussen et al.[63]	2013	Faroe	F	NA	NA	NA[a]	NP		
416/CE-19	Rasmussen et al.[63]	2013	Faroe	F	NA	NA	NA[a]	NP		
427/CE-20	Tang et al.[57]	1998	China	M	No	1 sister	NA[a]	Free	9 (22–50)	5% of control
507/CE-21	Lin et al.[29]	2020	China	F	NA	NA	1 months	Free	2.3 (10.3-54.2)	
572/CE-22	Lin et al.[29]	2020	China	F	NA	NA	1 months	Free	2.8 (10.3–54.2)	
724	Lin et al.[27]	2021	China	NA	NA	NA	1 months	Free	2.8 (9–50)	

Patient ID	Variant allele 1	Variant allele 2	Symptoms	Age at death	Comments on death
16/CE-6	**c.806delT**	**c.806delT**	CA(childhood), CiNOS, HG, coma, Seiz, HA, HepM, W, Dev	26 years	Asystole
52/CE-9	**c.760C>T**	**c.652+1G>A**	CiNOS, HA, HG	20 years	Sudden death
086	**c.844delC**	**c.844delC**	Asymptomatic	6 months	Admitted with mild respiratory tract infection, found dead 1 day after admission

TABLE 6 (Continued)

Patient ID	Variant allele 1	Variant allele 2	Symptoms	Age at death	Comments on death
162_A/CE-10	**c.-91_22del**	**c.-91_22del**	CMP	8 years	Sudden death
163_A	**c.-91_22del**	**c.-91_22del**	Asymptomatic	2 years	Died suddenly at 2 years of age after 1 day of diarrhea and vomiting
164	c.849G>T	c.849G>T	Asymptomatic	18 months	Sudden death after 1 day episode of vomiting
168	c.95A>G	c.95A>G		3 years	Encephalopathy with cerebral oedema and death
169	c.95A>G	c.95A>G	Enc, Seiz, CMP	14 months	Febrile illness with encephalopathy, seizures, and cardiac failure. Had been treated with pivampicillin
182/CE-11	**c.844delC**	**c.844delC**	Asymptomatic. CMP and HepS at autopsy	6 years	Died suddenly at age 6 years without any previous alarming sign
186/CE-5	c.95A>G	c.95A>G	Asymptomatic	NA	Died suddenly while sleeping
293	**c.760C>T**	**c.760C>T**	Enc	NA	No additional information
90	**c.396G>A**	**c.844C>T**	HepM, HA coma	NA	Died to heart failure after an infection
123/CE-7	**c.396G>A**	c.1433C>T	HepM	6 months	Cardiac arrest after 1 day of poor feeding due to URTI
180	**c.844delC**	**c.844delC**	HepM, CiNOS	2 years	Respiratory tract infection followed by cardiac decompensation
301/CE-12	**c.760C>T**	c.338G>A	HG, HepM	6 months	Sudden death at 6 months of age
98_B/CE-15	c.95A>G	c.95A>G	HepS	26 years	Underwent laparatomy and treatment with pivampicilin. 4 days later, developed ventricular tachycardia, then asystole and died
414_B/CE-16	c.95A>G	c.95A>G	VF, coma, HepS	43 years	After treatment with pivalic acid: had two cardiac arrests, then myocardial infarction. Circulation worsened and she died
408/CE-17	NP	NP	Asthma, CMP	18 months	Died suddenly
412/CE-18	c.51C>G	c.51C>G	Asymptomatic	26 years	Died suddenly while sleeping
415	c.95A>G	c.95A>G	Coma	3 years	Treated with pivampicilin, developed coma with seizures and died
416/CE-19	c.95A>G	c.95A>G	Bradycardia, CA, VF	30 years	Admitted after treatment with pivampicilin, developed ventricular fibrillation and then asystole. The patient died
427/CE-20	NP	NP	HepM	6 months	Admitted with respiratory tract infection, died due to cardiac arrest shortly after
507/CE-21	**c.497+1G>T**	**c.517delC**	Asymptomatic	2 months	Sudden death

(Continues)

TABLE 6 (Continued)

Patient ID	Variant allele 1	Variant allele 2	Symptoms	Age at death	Comments on death
572/CE-22	**c.760C>T**	**c.824+1G>A**	CA, HG	13 months	Sudden death
724	**c.760C>T**	**c.1161T>G**	Asymptomatic	14 months	Death due to hypoglycemia

Note: Nonsense/frameshift/splice-site variants are in bold.

Abbreviations: CA, cardiac arrest; CiNOS, cardiac involvement not otherwise specified; CMP, cardiomyopathy; Dev, Developmental delay; Enc, encephalopathy; F, female; HA, hyperammonemia; HepM, hepatomegaly; HepS, hepatic steatosis; HG, hypoglycemia; M, male; NA, not available; NP, not performed; PCD, primary carnitine deficiency; Seiz, seizure; Unsp., unspecified; W, weakness.

[a]Not applicable due to a postmortem diagnosis.

[b]Diagnosed with NAGS deficiency (9% residual activity) at age 5 years, PCD was confirmed postmortem, no variants in *NAGS* gene could be identified.

($N = 52$; 8.4%) consisted of hepatomegaly ($N = 41$; 6.6%) and hepatic steatosis ($N = 11$; 1.8%), confirmed by liver biopsy in eight patients. One girl presented in childhood with acute hepatitis, liver dysfunction, and hypoketotic hypoglycemia.

At least 80 (89.9%) of 89 patients experienced their first symptom in childhood with a median age at onset of 1 year [IQR 0.4-2.8]. Adult presentation was reported in six patients, of which four used pivalic acid, a compound known to decrease blood carnitine concentration,[36,37] prior to development of symptoms (CE-14, CE-15, CE-16, and CE-19). Reported symptoms were hepatic steatosis (CE-15), coma (CE-14), hepatic steatosis and coma (CE-16), hepatomegaly (CE-19), metabolic acidosis and elevated liver enzymes (CE-4), and finally, one patient with fasting intolerance.

3.4 | Muscle symptoms

Muscle symptoms occurred in 64 (10.3%) patients. The most frequently reported symptom was muscle weakness ($N = 46$; 7.4%). In 10 (1.6%) patients, rhabdomyolysis-like episodes were reported, with confirmatory laboratory results reported in only four. Muscle biopsies were taken in 15 patients, which revealed lipid accumulation in 12 patients (1.9%). Motor developmental delay occurred in six patients (1.0%). Additional information is provided in Supporting Information Table S6.

3.5 | Deceased patients

An overview of characteristics of the 25 deceased patients (4.0%) is provided in Table 6. In only seven patients, the diagnosis of PCD was established prior to death. The age of death was <1 year in 5 patients, 1-8 years in 11 patients, >20 years in 6 patients, and data on age of death were missing in 3 patients. In seven patients, death was preceded by an episode of infection/vomiting. In five patients, death ensued after treatment with pivampicillin. One third ($N = 8$) of the deceased patients was asymptomatic until death. Three patients identified through NBS died (two sudden death and one due to hypoglycemia) and were reported to have discontinued carnitine supplementation. One case was initially diagnosed with NAGS deficiency (9% residual activity, but no variations in the *NAGS* gene) after presenting with hypoglycemia, hyperammonemia, cardiomegaly, and fatty liver at the age of 5 years, and he was known to have low serum carnitine levels at that time. Several weeks prior to his death, at 20 years of age, he discontinued his carnitine treatment. Postmortem genetic analysis revealed two null

variants in the *SLC22A5* gene, confirming PCD. The diagnosis of NAGS deficiency in this patient remains dubious. Finally, for one case, no additional information on death was provided.

In two of the 25 deceased patients, no genetic analysis was performed, and their diagnosis was based on decreased carnitine transporter activity. In 23 patients, 16 different genetic variants were identified, 11 of which were nonsense, frame-shift or splice-site variants (carried by 14 patients). Out of nine patients that carried only missense variants, seven were homozygous for the c.95A>G variant, a variant highly prevalent on the Faroe Islands,[23] one patient was homozygous for c.51C>G, which is a frequently reported variant in Asia,[29,30] and one patient was homozygous for c.849G>T, a variant only described in one symptomatic Japanese boy.[37] Frequencies of all variants per symptom group are provided in Table S7.

4 | DISCUSSION

We have studied the full spectrum of clinical characteristics, both in PCD patients with a diagnosis based only on low carnitine levels (unconfirmed diagnosis) as well as in PCD patients diagnosed by biallelic variants in the *SLC22A5* gene and/or reduced carnitine transporter activity in fibroblasts (confirmed diagnosis). Overall symptoms were reported less frequently in the confirmed population compared to the unconfirmed population, although not statistically significant in all analyses. As unconfirmed cases were generally published earlier than confirmed cases (IQR 1990–2020 and 2010–2020, respectively), this might be partly explained by its introduction in NBS programs in several countries from 2001 onward. In addition, the group with unconfirmed PCD likely comprises patients that do not meet the current diagnostic criteria for PCD and suffer from a different disorder; as illustrated by the fact that for some cases reported in literature, the diagnosis was reconsidered after reevaluation.[13-17] In disorders that cause secondary carnitine deficiency (eg, fatty acid oxidation disorders and organic acidemias), other organ systems may be more strongly affected than in PCD. This could have increased the prevalence of muscle and neurological symptoms in the unconfirmed population.[38,39] To ensure that the clinical spectrum of PCD cases as described in this study would not be affected by data of non-PCD patients, unconfirmed cases were not included in the further analyses. The most prevalent clinical characteristic in confirmed PCD was cardiomyopathy (18.0%), and this was the only reported clinical characteristic in 8.4% of patients. Neurologic, hepatic, and/or metabolic signs and symptoms occurred in 14.3% and generally developed in early childhood. Four percent of patients died and one third of the deceased patients was completely asymptomatic up until death. Cardiac events in seemingly well patients were often preceded by provoking incidents such as a mild infection and/or initiation of pivalic acid containing antibiotics (13/33; 39.4%).

Symptoms were rarely (5.6%) observed in children identified by NBS. The most obvious explanation for this observation would be early initiation of treatment in NBS-identified patients, thus preventing symptoms. Another explanation could be detection of milder phenotypes in the patients identified by screening programs. The results of this study illustrate that the frequency of nonsense, frame-shift, and splice-site variants was considerably lower in patients identified by screening than in patients identified after a symptomatic presentation. This suggests that the underlying variants may very well play a role in disease severity. In line with this hypothesis, Rose et al.[40] previously reported 14 symptomatic and 14 asymptomatic women with PCD and showed that none of the asymptomatic women were homozygous or compound heterozygous for null-variants, whereas nine of 14 of symptomatic women carried these variants. Indeed, most maternal cases identified after NBS of their child, of whom the majority has remained asymptomatic throughout life, have missense variants on at least one allele. The identification of milder phenotypes by NBS for PCD is in line with phenotypes identified by screening programs for other diseases.[41-43]

The identification of milder or even asymptomatic maternal cases through NBS of their child raised a dilemma since implementation of PCD in NBS programs.[5,22,40] On the one hand, identification enables physicians to monitor and treat patients before they experience potentially lethal events. On the other hand, there are ethical concerns of burdening asymptomatic mothers with a diagnosis of PCD, especially given the uncertainty regarding the health consequences and subsequent need for monitoring and treatment. To see if mothers identified through NBS of their child are at risk for acute events, we looked with particular interest at patients that were completely asymptomatic before suffering a (potentially) lethal event (ie, cardiac event or coma) in adulthood. In our identified cohort, 3.1% ($N = 6$) of adult patients (patients diagnosed in adulthood, $N = 194$) experienced such an event (five cardiac events and one coma) without any prior symptoms (of whom two were mothers identified through NBS of their child). This finding adds to the ethical dilemma of identifying asymptomatic mothers with PCD through NBS of their child, as it shows that the risk of a life-threatening event is small, but not negligible. With this

knowledge, most physicians will likely choose to continue follow-up and treatment in these mothers. However, the question remains whether the burden of lifelong follow-up and treatment and the knowledge of being at risk for a serious event is justified, considering the small risk of this event actually happening.

Previously, Rasmussen et al.[44] published a retrospective analysis of sudden death in the Faroese population and uncovered 13 untreated PCD cases, all homozygous for the c.95A>G variant, suggesting a strong association between this variant and sudden death. However, all these cases originate from a confined region with extremely high genetic homogeneity.[45] Therefore, other genetic traits predisposing for sudden cardiac death within this population cannot be ruled out. For PCD in general, it is difficult to establish a clear association between genotype and disease severity.[40,46-48] Typically, missense and in-frame deletion or insertion variants are less detrimental to protein integrity, theoretically resulting in a milder phenotype, as is also suggested by our data. However, in some cases, these types of variants can still substantially affect protein function (eg, when the catalytic site is affected). Therefore, the practical variant categorization used in this review is not suited to truly assess functional consequence of variants, which requires in vitro studies such as gene expression studies. However, these approaches are only able to evaluate the effect of a single variant. Variants exist in numerous allelic combinations, each producing an OCTN2 protein with varying residual activity, making it impossible to predict disease outcome based on genotype alone. Unfortunately, at the time of writing, the other conventional diagnostic tool available—measurement of carnitine transporter activity in cultured fibroblasts—is not sensitive enough to reliably predict disease severity: our data showed a median residual transport activity of 5% of controls in asymptomatic ($N = 15$; range 0.1–16) as well as symptomatic patients ($N = 52$; range 0–22). Recently, a novel method for measuring carnitine transport activity, using D_3-labeled carnitine instead of radioactively labeled carnitine, was published by Ferdinandusse et al.[49] This novel assay is more sensitive and could reliably separate biallelic missense from biallelic nonsense variants (~26 vs ~2% of control, respectively). Further research is required to determine if this novel assay is able to distinguish those patients with symptoms from those individuals that remain asymptomatic.

This review is not without its limitations. First, our approach involved retrieving data from previously reported cases, and is thus susceptible to publication bias. Second, some symptoms or signs may not have been reported in the case description (reporting bias). The latter might have resulted in an overestimation of severe symptoms and/or an underestimation of milder clinical characteristics. However, the case–by-case approach in this study limited the risk of citation bias, and enabled us to approximate the prevalence of certain clinical traits within PCD patients as closely as possible, using only published data. Furthermore, this approach allowed us to focus specifically on severe outcomes and to identify clinical relevant differences between patients presenting with symptoms and asymptomatic individuals identified through screening.

The primary objective of our study was to identify the clinical spectrum of PCD. Although treatment data of PCD patients were collected, these data were often insufficient (eg, no data on treatment duration, treatment dosage and/or treatment adequacy [ie, plasma carnitine levels after treatment]) to draw reliable conclusions on the effect of carnitine supplementation on clinical symptoms. Furthermore, follow-up time could not be reliably collected for included patients. This is particularly important for evaluation of symptoms in the NBS group, as these newborns may have been too young to have developed symptoms when reported. However, the median age at first symptoms for the clinically identified population was 1 year ($N = 117$; IQR 0.5–5 years) suggesting that, if left untreated, PCD-related symptoms generally develop throughout the first years of life. It might well be that part of the population of the NBS group surpassed this age at the time of publication, but this could not be verified based on available data. Research in a controlled setting, preferably with a long follow-up time, is required for assessing symptoms incidence and treatment effect in future studies.

Inherent to the case–by-case approach, studies that only reported pooled data were excluded (four articles; containing 57 cases).[40,44,50,51] Most of these cases had been described in other articles that were included in our study. This will therefore probably not have greatly influenced the outcome of this study. Vice versa, several large cohort studies did meet our inclusion criteria.[24,29,52] As a consequence, there is an overrepresentation of specific geographical regions in our database. This creates a risk of bias in the symptoms reported in groups, based on background genetics, rather than mode of detection. For example, 296 of included cases (48.5%) were Chinese. These cases contribute to 64.4% of the maternal cases identified by NBS of their child and to 79.4% of the NBS cases. Of the 14 symptomatic children identified through NBS, 13 were of Chinese heritage. Moreover, 97 cases (15.9%) are of Faroese heritage, representing 78.7% of the risk screening population.

In summary, this is the most extensive review on PCD to date. By reviewing individual clinical outcomes of patients diagnosed by current diagnostic standards, we provide an extensive overview of the clinical

characteristics associated with PCD. Patients are predominantly at risk for developing cardiomyopathy. Neurological, metabolic, hepatic, and muscle symptoms occurred in approximately 10% of patients. Patients identified through screening rarely developed symptoms, whether this is due to a treatment effect or the detection of milder phenotypes is not yet elucidated. Cardiac events are rare, but they have been reported as the first presenting symptom in a small number of PCD patients, including adults without a remarkable preceding medical history. Cohort studies in genetically diverse populations are needed to compare genetic traits and residual activity on the one hand, and disease outcome on the other hand to better predict disease severity. This information is crucial to improve NBS programs, identifying those individuals with PCD requiring treatment and follow-up (actual disease), while reducing the number of individuals that have a genetic trait leading to reduced carnitine transporter activity, without clinical consequences (healthy individuals). Until more knowledge on the risk of developing serious events in asymptomatic individuals is gained, we recommend monitoring and treatment of all PCD cases identified.

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CONFLICT OF INTEREST

Mirjam Langeveld is involved in premarketing studies with Sanofi-Genzyme, Protalix BioTherapeutics, and Idorsia. Financial arrangements are made through AMC Research BV. No fees, travel support, or grants were obtained from pharmaceutical industry. Loek L. Crefcoeur, Gepke Visser, Sacha Ferdinandusse, Frits A. Wijburg, and Barbara Sjouke declare that they have no potential conflicts of interest.

ETHICS STATEMENT

Ethics approval was not required for this research.

HUMAN AND ANIMAL RIGHTS

This article does not contain any studies with human or animal subjects performed by any of the authors.

ORCID

Loek L. Crefcoeur https://orcid.org/0000-0003-2057-5525

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