Published in final edited form as:

Mol Genet Metab. 2019 November ; 128(3): 363–366. doi:10.1016/j.ymgme.2018.11.013.

Porphyria Cutanea Tarda and Hepatoerythropoietic Porphyria: Identification of 19 Novel Uroporphyrinogen III Decarboxylase Mutations

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Abstract

Porphyria Cutanea Tarda (PCT) is a hepatic cutaneous porphyria due to the hepatic inhibition of the heme biosynthetic enzyme uroporphyrinogen decarboxylase (UROD), and can occur either in the absence or presence of an inherited heterozygous *UROD* mutation (PCT subtypes 1 and 2, respectively). A heterozygous *UROD* mutation causes half-normal levels of UROD activity systemically, which is a susceptibility factor but is not sufficient alone to cause type 2 PCT. In both Types 1 and 2 PCT, the cutaneous manifestations are precipitated by additional factors that lead to generation of an inhibitor that more profoundly reduces hepatic UROD activity. PCT is an iron-related disorder, and many of its known susceptibility factors, which include infections (e.g. hepatitis C virus, HIV), high alcohol consumption, smoking, estrogens, and genetic traits (e.g. hemochromatosis mutations) can increase hepatic iron accumulation. Hepatoerythropoietic Porphyria (HEP) is a rare autosomal recessive disease that results from homozygosity or compound heterozygosity for *UROD* mutations and often causes infantile or childhood onset of both erythropoietic and cutaneous manifestations.

During the 11-year period from 01/01/2007 through 12/31/2017, the Mount Sinai Porphyrias Diagnostic Laboratory provided molecular diagnostic testing for 387 unrelated patients with PCT and four unrelated patients with HEP. Of the 387 unrelated individuals tested for Type 2 PCT, 79 (20%) were heterozygous for *UROD* mutations. Among 26 family members of mutation-positive PCT patients, eight (31%) had the respective family mutation. Additionally, of the four unrelated HEP patients referred for *UROD* mutation analyses, all had homozygosity or compound heterozygosity for *UROD* mutations, and all eight asymptomatic family members were

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Competing Interest Statement

All authors declare no conflicts of interest for PCT or HEP diagnosis or treatment.

heterozygotes for *UROD* mutations. Of the *UROD* mutations identified, 19 were novel, including nine missense, one nonsense, one consensus splice-site, and seven insertions and deletions.

These results expand the molecular heterogeneity of PCT and HEP by adding a total of 19 novel *UROD* mutations. The results document the usefulness of molecular testing to confirm a genetic susceptibility trait in Type 2 PCT, confirm a diagnosis in HEP, and identify heterozygous family members.

Keywords

Porphyria cutanea tarda; Hepatoerythropoietic porphyria; Uroporphyrinogen decarboxylase; Cutaneous porphyrias; Mutation analysis

1. Introduction

Porphyria Cutanea Tarda (PCT) is a hepatic porphyria with prominent, chronic cutaneous manifestations that develops after UROD is reduced to less than about 20% of normal activity in the liver [1]. Hepatic UROD activity is reduced by a possible inhibitor identified as a uroporphomethene that is generated from uroporphyrinogen probably by one or more cytochrome P450 enzymes in the presence of iron and oxidative stress [2, 3]. About 80% of patients have no mutation of *UROD* and are classified as Type 1 (sporadic) PCT. Another 20% of patients are heterozygous for *UROD* mutations and are classified as Type 2 (familial) PCT [1, 4]. Type 2 PCT is a genetic disease with low penetrance, in that the great majority of those who inherit heterozygous *UROD* mutations never develop symptoms. These types of PCT are clinically indistinguishable, except that some Type 2 patients may experience earlier onset of symptoms or occasionally have relatives with overt PCT. Susceptibility factors that contribute in both types of PCT include excessive alcohol consumption, hepatitis C or HIV infection, estrogens, smoking, and common *HFE*-related familial hemochromatosis mutations p.C282Y and p.H63D [1, 4–7].

Hepatoerythropoietic Porphyria (HEP) is a rare disorder resulting from homozygosity or compound heterozygosity of *UROD* mutations. HEP can cause hematologic and severe photosensitive cutaneous manifestations in infancy or childhood, but mild cases may become manifest in adult life [1, 8].

PCT is initially diagnosed clinically by prominent cutaneous friability and blistering on sun exposed areas, as well as milia, hypertrichosis, and areas of hyper- and hypopigmentation. A biochemical diagnosis is made by urinary or plasma porphyrin analyses, which demonstrate the accumulation of porphyrins that are mostly highly carboxylated in urine and plasma, including uroporphyrin and heptacarboxylporphyrin. Fecal total porphyrins may be normal or elevated with a predominance of isocoproporphyrin. Management includes removal of disease-inducing susceptibility factors and effective and specific treatment either by phlebotomy to reduce hepatic iron levels or a low-dose regiment of hydroxychloroquine (or chloroquine) to deplete excess porphyrins in the liver [1, 4, 9]. In PCT patients with hepatitis C, direct acting antiviral agents may also be effective in treating PCT [9, 10], but whether

this leads to PCT remission as quickly as treatment by phlebotomy or low dose hydroxychloroquine is under study.

The Mount Sinai Porphyrias Diagnostic Laboratory specializes in molecular testing for the diagnosis of all the porphyrias. PCT patient samples are often submitted for molecular testing for *UROD* mutations to identify patients with the Type 2 familial subtype. The identification of Type 2 patients permits the genetic testing of relatives to detect those with the family mutation who are at increased risk for PCT, if they also have or acquire additional susceptibility factors that can lead to inhibition and further lowering of hepatic UROD activity. Here, we report results of testing for *UROD* mutations in the Mount Sinai porphyria laboratory over the 11-year period from January 1, 2007 through December 31, 2017. Resulting from this effort we now describe previously reported *UROD* mutations and 19 novel mutations which have not been reported previously or listed in the Human Gene Mutation Database (HGMD), version 2018.2 [11]. These newly identified mutations expand the molecular heterogeneity of Type 2 PCT and HEP.

2. Methods and Materials

Patients with PCT were initially diagnosed by characteristic friability and blistering lesions affecting sun exposed areas of skin, especially on the dorsal hands, and by characteristic elevations in urinary or plasma porphyrins.

For *UROD* mutation analyses, genomic DNA was isolated from blood samples or saliva using QIA Symphony Technology or the PureGeneTM® Genomic DNA purification kit. Full gene analysis was performed by PCR amplification of the isolated DNA followed by exon-specific primer extension analysis of all exons, exon-intron boundaries (20–30 base pairs from both boundaries), and promoter regions, essentially as previously described [12]. All sequencing was done bi-directionally. Variant nomenclature and exon numbering used reference transcript NM_00374.3. The DNA was amplified by PCR using the Bio-Rad C1000 Touch Thermal Cycler. All PCR primers and sequencing primers were designed in the Mount Sinai Genetics Diagnostic Laboratory using the MacVector program and checked for common single nucleotide polymorphisms (SNPs) by the SNPCheck program (ngrl.org.uk/Manchester/projects/snpcheck). Takara PrimeSTAR® GXL DNA Polymerase from Clontech Laboratories was used for PCR amplification. Sanger sequencing of the PCR products was then performed. Sequencher (Gene Codes Corporation) software was used for alignment and analysis of the sequences. Once a specific mutation was identified in a proband, at-risk family members could be diagnosed by targeted mutation testing for the specific family mutation.

3. Results

During the 11-year period from January 1, 2007 through December 31, 2017, samples from 413 PCT patients and 12 HEP patients were referred by physicians to the Mount Sinai Porphyrias Diagnostic Laboratory for *UROD* molecular diagnostic testing. These included 387 unrelated PCT-manifesting individuals whose *UROD* genes were sequenced as described in the Methods section (Table 1). Among these 387 patients, 79 (20%) had *UROD*

mutations and were classified as having Type 2 PCT. Of their 26 family members, eight (31%) had the respective family mutation. The four unrelated HEP patients were homozygous or compound heterozygous for *UROD* mutations while eight of their asymptomatic parents or siblings were heterozygous for the *UROD* family mutation, as expected. In the remaining 308 unrelated PCT-manifesting patients, no pathogenic mutations or benign variants of *UROD* were detected, indicating the diagnosis of sporadic Type I PCT.

In addition to previously published mutations recorded in HGMD (version 2018.2), 19 novel *UROD* mutations were identified, including nine missense [c.27G>C (p.Q9H), c.50A>G (p.D17G), c.55T>C (p.F19L), c.131C>T (p.P44L), c.284G>C (p.G95A), c.616C>A (p.Q206K), c.629G>A (p.G210D), c.766G>A (p.V256M), and c.994C>T (p.R332C)], two nonsense [c.430C>T (p.R144X), c.1082C>G (p.S361X)], one consensus splice-site (c.20+2T>G), and seven insertions or deletions [c.118_119del (p.G40Pfs*7), c.186dup (p.E63X), c.327_328delinsC (p.E110Sfs*3), c.645_646del (p.N215Hfs*3), c.655_656delinsAA (p.S219N), c. 819_828del (p.Q274Mfs *23), and c.1020del (p.L341Ffs*42)] (Figure 1). Notably, one six-year-old male from India was homozygous for a novel mutation, c.766G>A (p.V256M), and had a porphyrin profile and symptoms compatible with HEP [1]. His parents were both asymptomatic heterozygotes.

These findings have increased the number of *UROD* mutations reported by HGMD by 15.6% (Table 2).

4. Discussion

UROD mutation analyses of 387 unrelated patients with PCT documented both clinically and biochemically identified 79 (20%) with familial (type 2) disease, a prevalence similar to that reported by other investigators [4]. This enabled detection and counseling of asymptomatic family members found to be heterozygous for the familial mutations. These individuals could then be counseled to avoid PCT precipitating factors and to request diagnostic biochemical confirmation should symptoms occur in the future. Mutation analysis in the four HEP patients confirmed the diagnosis of a disease that is often misdiagnosed as Congenital Erythropoietic Porphyria (CEP) and led to the identification of family members with heterozygosity for pathogenic *UROD* mutations. These results confirm the molecular heterogeneity of PCT and HEP and add an additional 19 novel pathogenic *UROD* mutations, which included nine missense, one nonsense, one consensus splice-site, and seven insertion and deletion mutations.

Among 79 unrelated PCT probands, 52 *UROD* mutations were identified, including 33 that were previously published. Among them, the most common were c.952G>A (p.G318R) in five unrelated probands, c.238G>T (p.A80S) in four unrelated probands, and c.399_401delinsCCA (p.V134Q) in four unrelated probands (Table 4). The complex rearrangement g.645del1053ins10 GATCGCCAGA was identified in three unrelated probands of Hispanic ancestry. Furthermore, the same rearrangement was identified in two other unrelated probands of Hispanic ancestry at the Mount Sinai Porphyria Diagnostic Laboratory in 2006 and 2018, before and after the study period reported here. This mutation

was also previously identified in a Spanish patient [13], for a total of six patients, presumably unrelated, all of Hispanic origin, suggesting a possible founder effect.

Three of the four HEP patients and their *UROD* mutations have been previously reported [14–16]. The fourth case, reported here for the first time, was a six-year-old male from India and was homozygous for a novel *UROD* mutation, c.766G>A (p.V256M). He experienced typical early onset of disease manifestations, including anemia, marked photosensitivity, and severe cutaneous damage [8]. He was initially thought to have CEP, but had no *UROS* mutations, and his biochemical findings and molecular studies that identified homozygous *UROD* mutations document that he had HEP.

Demonstrating a marked elevation in urinary or plasma porphyrin levels, with a predominance of highly carboxylated porphyrins, remains essential for the diagnosis of active PCT, and also for monitoring improvement during treatment. As in this series, heterozygous *UROD* mutations are found in only a portion of PCT patients (about 20% of cases in most series), so their presence is not essential in causing PCT. These mutations are additional susceptibility factors in Type 2 patients, but other factors must be present to generate inhibition of hepatic UROD activity. Furthermore, as in other porphyrias, the biochemical data are essential for validating that novel mutations are pathogenic, particularly for missense mutations. The biochemical confirmation of elevated urinary, plasma and fecal porphyrin profiles, which are diagnostic “gold standards” for PCT and HEP, establishes that all the new mutations described here are pathogenic. For HEP, *UROD* mutations are found in all cases, and given the rarity of this porphyria and its close resemblance to CEP, molecular analyses are essential for the accurate diagnosis of HEP [1]. Patients with CEP have uroporphyrinogen synthase mutations and different patterns of porphyrins in erythrocytes, plasma, urine and feces that include marked accumulation of the I isomers of uroporphyrin and coproporphyrins [17].

In summary, the Mount Sinai Porphyria Diagnostic Laboratory has identified 19 novel *UROD* mutations in the past 11 years in 19 affected individuals with marked elevations of porphyrins, including uroporphyrin and heptacarboxylporphyrin, documenting their diagnoses of PCT or HEP. These molecular findings identified individuals with the Type 2 PCT, confirmed the diagnosis of HEP, and enabled identification of relatives who carry *UROD* mutations who were counseled to avoid environmental and behavioral susceptibility risk factors for overt PCT. In addition, the identification of the *UROD* mutations in HEP patients allows the parents to accurately prenatally diagnose subsequent pregnancies at risk for HEP as well as prenatal diagnosis for future pregnancies in HEP families.

Acknowledgements

The authors would like to thank Mrs. Dana Doheny for her assistance in the collection and analysis of the clinical data. This work was primarily supported by the Department of Genetics and Genomic Sciences of the Icahn School of Medicine at Mount Sinai. This work also was supported in part by the Porphyrias Consortium (U54DK083909), which is a part of the NCATS Rare Diseases Clinical Research Network (RDCRN) of the National Institutes of Health. RDCRN is an initiative of the Office of Rare Diseases Research (ORDR), funded through collaboration between the National Center for Advancing Translational Sciences (NCATS) and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and by the Institute for Translational Sciences Clinical Research Center at the University of Texas Medical Branch, supported in part by a Clinical and Translational Science Award (UL1TR001439), from NCATS, National Institutes of Health.

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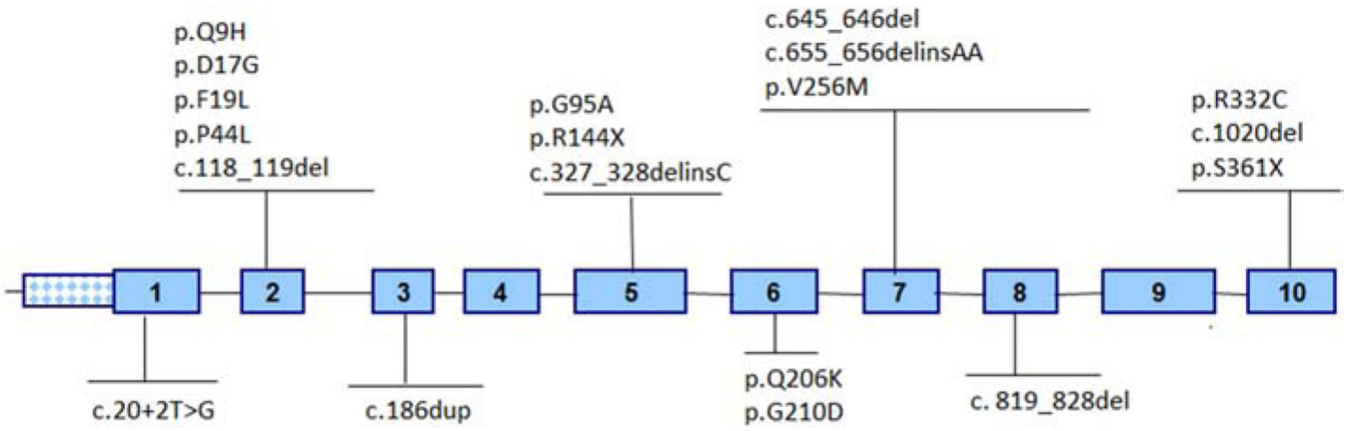

Figure 1 -
Novel *UROD* Mutations by Exon Discovered at Mount Sinai, 2007–2017

Table 1

Molecular Testing Referrals for PCT and HEP at Mount Sinai, 2007–2017

	PCT	HEP
Referred Patients	413	12
Unrelated Individuals	387	4
Mutation-Positive Patients	87	12
Unrelated Probands	79	4

Table 2.

Previously Known and Novel *UROD* Mutations, 2007–2017

	HGMD Mutations*	Novel Mutations	% Increase
Total Reported Mutations	**122**	**19**	**15.6**
Missense	71	10	14.0
Nonsense	8	1	12.5
Splice-sites	13	1	7.7
Insertions/ Deletions	29	7	24.1
Regulatory	1	0	0

* HGMD Professional 2018.2

Table 3.

Novel *UROD* Mutations Detected in Probands at Mount Sinai, 2007–2017

Mutation Type	Exon/Intron	Nucleotide Change	Residue Change	MAF*
Missense	E2	c.27G>C	p.Q9H	5.4e-4
	E2	c.50A>G	p.D17G	<4.0e-6
	E2	c.55T>C	p.F19L	8.1e-6
	E2	c.131C>T	p.P44L	4.1e-6
	E5	c.284G>C	p.G95A	<4.0e-6
	E6	c.616C>A	p.Q206K	<4.0e-6
	E6	c.629G>A	p.G210D	<4.0e-6
	E7	c.766G>A**	p.V256M	<4.0e-6
	E10	c.994C>T***	p.R332C	2.8e-5
Nonsense	E5	c.430C>T***	p.R144X	4.1e-6
	E10	c.1082C>G	p.S361X	<4.0e-6
Small Deletion	E2	c.118_119del	p.G40Pfs*7	<4.0e-6
	E7	c.645_646del	p.N215Hfs*3	<4.0e-6
	E8	c.819_828del	p.Q276Mfs*23	<4.0e-6
	E10	c.1020del	p.L341Ffs*42	7.2e-6
Small Insertion	E3	c.186dup	p.E63X	<4.0e-6
Deletion-Insertion	E5	c.327_328delinsC	p.E110Sfs*3	<4.0e-6
	E7	c.655_656delinsAA	p.S219N	<4.0e-6
Consensus Splice-Site	I1	c.20+2T>G	-	<4.0e-6

* Minor Allele Frequency (MAF) values were calculated based on the total population, irrespective of the ethnic/demographic groups, using the Genome Aggregation Database (gnomAD), a tool that aggregates large-scale sequencing data (gnomad.broadinstitute.org).

** Identified in a patient diagnosed with HEP

*** CpG dinucleotide

Table 4.

Most Frequent *UROD* Mutations Identified in Type 2 PCT Probands

HGMD Accession	Nucleotide Change	Residue Change	Mutation Type	Unrelated Probands	CpG	Population with Greatest MAF*
CG984483	c.25del1053ins10GATCGCCAGA (g.645del1053ins10)	-	Complex Rearrangement	5	-	N/A
CM003170	c.238G>T	p.A80S	Missense	4	-	NFE, 3.2e-5
CX942109	c.399_401delinsCCA	p.V134Q	Small Indel	4	-	N/A
HM971362	c.616C>T	p.Q206X	Nonsense	3	-	NFE, 2.7e-5
CS900261	c.636+1G>C	-	Consensus Splice-site	3	-	NFE, 6.3e-5
CM961406	c.758T>A	p.L253Q	Missense	3	-	FIN, 2.3e-2
CM961408	c.952G>A	p.G318R	Missense	5	+	AFR, 1.7e-2

*Minor Allele Frequency (MAF) values were calculated using the Genome Aggregation Database (gnomAD), a tool that aggregates large-scale sequencing data (gnomad.broadinstitute.org). Mutations not detected in any gnomAD population are indicated with N/A.

**Key: *AFR* African, *FIN* Finnish, *NFE* Non-Finnish European