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Clinical and molecular epidemiology of erythropoietic protoporphyria in Italy

Background: Erythropoietic protoporphyria (EPP) is a rare inherited disease associated with heme metabolism, characterized by severe life-long photosensitivity and liver involvement. *Objective:* To provide epidemiological data of EPP in Italy. *Materials & Methods:* Prospective/retrospective data of EPP patients were collected by an Italian network of porphyria specialist centres (Gruppo Italiano Porfiria, GrIP) over a 20-year period (1996-2017). *Results:* In total, 179 patients (79 females) with a clinical and biochemical diagnosis of EPP were assessed, revealing a prevalence of 3.15 cases per million persons and an incidence of 0.13 cases per million persons/year. Incidence significantly increased after 2009 (due to the availability of alfa-melanotide, which effectively limits skin photosensitivity). Mean age at diagnosis was 28 years, with only 22 patients (12.2%) diagnosed ≤10 years old. Gene mutations were assessed in 173 (96.6%) patients; most (164; 91.3%) were *FECH* mutations on one allele in association with the hypomorphic variant, c.315-48C, on the other (classic EPP), and nine (5.2%) were *ALAS2* mutations (X-linked EPP). Only one case of autosomal recessive EPP was observed. Of the 42 different *FECH* mutations, 15 are novel, three mutations collectively accounted for 45.9% (75/164) of the mutations (c.215dupT [27.2%], c.901_902delTG [11.5%] and c.67 + 5G > A [7.2%]), and frameshift mutations were prevalent (33.3%). A form of light protection was used by 109/179 (60.8%) patients, and 100 (56%) had at least one α-melanotide implant. Three cases of severe acute liver involvement, requiring OLT, were observed. *Conclusion:* These data define, for the first time, the clinical and molecular epidemiology of EPP in Italy.

Key words: cutaneous porphyrias, erythropoietic protoporphyria, ferrochelatase, photodermatosis, protoporphyrin, X-Link EPP

Erythropoietic protoporphyria (EPP, OMIM 177000) is a rare inborn metabolic disease characterized by significant and life-long severe acute photosensitivity (often strongly negatively impacting the quality of life of patients) as well as possible haematological and severe liver involvement (2-3% of patients), due to accumulation in blood and tissues of protoporphyrin IX (PPIX) [1-4]. The disease affects at least one in 100,000 of the population in Europe and usually presents in early childhood or infancy with severe painful burning and pruritus within minutes of sunlight exposure. Onset of symptoms in adult age is very rare and is mostly a consequence of an acquired somatic mutation within the *ferrochelatase (FECH)* gene, secondary to haematological malignancy [1, 2, 4-8]. Four different inheritance patterns of EPP have

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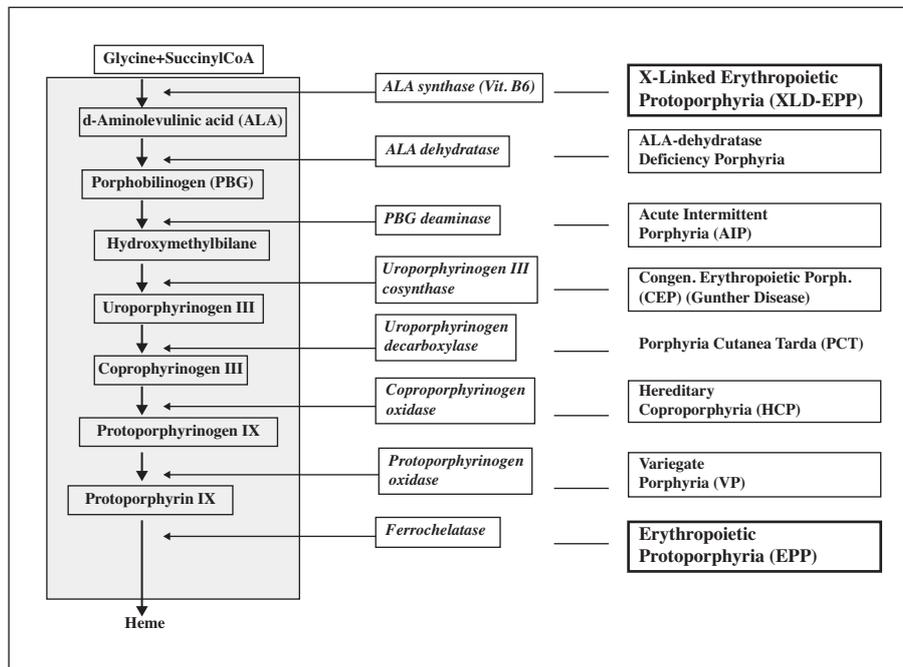
Figure 1. Heme synthesis pathway, enzymes involved and corresponding diseases.

so far been identified. In most cases, the accumulation of PPIX is due to a partial deficiency of FECH, the last enzyme in the heme biosynthesis pathway [1] (*figure 1*). In the majority, the disease is inherited in a pseudodominant manner (EPP) and photosensitivity is normally overt only in those subjects in whom a deleterious *FECH* mutation (significantly decreasing or abolishing FECH activity) is inherited together with the hypomorphic variant, c.315-48C (also described as FECH IVS3-48C) [9, 10]. Other patients have autosomal recessive EPP (arEPP), which is characterized by the presence of a mutation on both alleles responsible for reduced activity of FECH [11, 12]. In a few cases, PPIX accumulation has been reported to be a consequence of a gain-of-function mutation in an erythroid variant of 5-aminolaevulinate synthase gene (*ALAS2*), the first enzyme of erythroid haem biosynthesis (X-linked Protoporphyria, XL-EPP; OMIM 300752) [13]. More recently, an increase in activity of ALAS2 protein, responsible for PPIX accumulation and photosensitivity, has been reported to result from a point mutation in the *caseinolytic mitochondrial matrix peptidase chaperone subunit (CLPX)* gene, which encodes for a regulatory protein, responsible for ALAS2 activation [14, 15].

The clinical course of EPP as well as application of an adequate treatment and clinical monitoring justify prompt and accurate diagnosis, requiring specific tests, often supported only by porphyria specialistic centres. As with most rare diseases, healthcare resources and preventative strategies required for support of EPP patients are strictly related to the prevalence of overt cases, however, data concerning the epidemiology of EPP in Italy are so far not available, except for some small studies [16]. The present study aimed to provide clinical and molecular epidemiological data of patients affected by EPP in Italy.

Patients and methods

Patients

A multi-centre cross-sectional national study of EPP in Italy over a 20-year period, 1997-2017, was conducted by Gruppo Italiano Porfiria (GrIP), the Italian Porphyria network including the six reference centres for Porphyrias (Brescia, Milan, Modena, Padova, Roma, and San Giovanni Rotondo). Clinical, biochemical and genetic data of patients with a diagnosis of EPP and XL-EPP were collected. The diagnosis was made in patients with life-long photosensitivity, consistent with EPP, and an increase in total erythrocyte protoporphyrin (usually from 4x to 100x the normal values) with a >50% increase in erythrocyte metal-free protoporphyrin rather than zinc protoporphyrin [1, 2, 17, 18]. In EPP, metal-free protoporphyrin generally represents >85% total porphyrins. In XL-EPP, metal-free protoporphyrin is generally about 50% of total porphyrins. When available, the genetic analysis confirmed the diagnosis. All patients or their parents gave informed consent to be included in this observational survey.

Biochemical analyses

Light-protected collected samples were analysed in the laboratories of porphyria centres for measurement of erythrocyte total PPIX and PPIX fractions that were metal-free and zinc-chelated, as previously described [17, 19, 20].

Molecular analyses

Genomic DNA (gDNA) was extracted from buffy coat by both manual and automated systems. As previously

Table 1. Distribution of EPP patients according to each Italian porphyria centre database.

	Brescia	Milan	Padua	Modena	Rome	San Giovanni Rotondo	Total included	True total*
EPP	24	77	17	12	111	11	252	170
XL-EPP	0	9	0	0	4	0	13	9
Total	24	86	17	12	115	11	265	179

After excluding multiple referrals (see text for details).

reported [21, 22], for the manual method, gDNA was extracted using the PUREGENE DNA purification system blood kit (Gentra Systems Minneapolis, MN, USA); for the automated systems, the Maxwell 16 DNA Blood Kit with the automatic extractor Maxwell®16 was used (Promega Corp., Madison City, WI). The promoters and the entire coding regions of the human *FECH* and *ALAS2* genes were amplified with specific primers pairs. For the *FECH* and *ALAS2* genes, 11 and 10 primer pairs were used, respectively. gDNA, 100-150 ng, was amplified using BIOTAQ DNA polymerase (Bioline, London, UK) with the specific PCR primer pair for each exon or fragment. Primer sequences and melting temperatures are available from the authors on request. The PCR products were then subjected to automated direct sequencing on an ABI Prism 310 Genetic Analyser (Thermo Fisher Corporation Inc., San Francisco, CA, USA). When no *FECH* mutation was detected by sequencing, multiplex ligation dependent probe amplification (MLPA) technique analysis to identify large deletions and characterization of deletion breakpoints was carried out, as previously described [23]. In the absence of long deletions, a custom enrichment panel for *FECH* gene resequencing was designed; the panel covers 40 kb upstream to 10 kb downstream of the *FECH* gene, including all exons and introns. DNA libraries were produced by a standard Haloplex target enrichment system procedure (Agilent Technologies, Santa Clara, USA) and 150 bp paired-end reads were generated using a MiSeq sequencer (Illumina, San Diego, USA), as already described [24]. Sequence variant nomenclature is according to Human Genome Variation Society (HGVS) recommendations (version 19.01). Nucleotides are numbered in the cDNA sequence of the human *FECH* gene (NCBI reference sequence NM_000140.3) and human *ALAS2* gene (NM_000032.4) with the A of the ATG initiation codon as '+1'.

Statistical methods

Data were analysed using Stata 15.1 statistical software (StataCorp, Texas USA). Measurements were expressed as mean (± standard deviation), median and range, or percentage, when appropriate. The significance of differences between quantitative variables was assessed by the Mann-Whitney or Kruskal-Wallis tests, when appropriate. For all statistical analysis, $p < 0.05$ was considered significant.

Results

A total of 179 patients (79 females) among 140 unrelated families were analysed. The cases included in the original

Table 2. General characteristics of studied patients.

Sex	
Male	100 (55.4%)
Female	79 (45.6%)
Prevalence (cases/million inhabitants)	3.15
Incidence (new diagnosis/ year/million inhabitants) *	0.13
Mean age (years)	38.2 (±16.1)
Male	38.4 (±15.6)
Female	37.9 (±16.3)
Mean age at diagnosis (years) (min-max)	28.3 (±15.1) (3-71)
Male	28.8 (±15.8)
Female	29.5 (±15.4)
Treatment	
None/unknown	67
Photoprotectors[1]	21
Alfa-melanotide[2]	100
Heme arginate	2
Liver transplant	3
Erythrocyte protoporphyrin (μmol/L) median (range)	27.1 (8.3-91.6)
Metal-free protoporphyrin (%) (range)	89 (78-91)

Mean rate within the considered period (see text for details).[1]At least one period during a life-time (see text for details).[2]At least one subcutaneous implant during a life-time (see text for details).

collected database by the six different Italian porphyria centres participating in the study are presented in *table 1*. The sum of patients included by each single centre is higher than the true number of patients as a consequence of a "over-sampling bias" (see below in the discussion section for explanation). The mean age at diagnosis was 28 years (with no significant difference between genders) (*table 2*). No EPP diagnosis was made under three years of age. The frequency of EPP diagnosis was 4/179 (2.2%) and 22/179 (12.2%) under five and under 10 years of age, respectively. Eighteen (10.4%) patients were diagnosed after 50 years of age. None had haematological malignancies. The mean age at diagnosis for patients with XL-EPP ($n = 9$) was older than that of patients with classic EPP (36.7 ± 14.9 vs.28.6 ± 15.6, $p = 0.109$) even though the difference was not statically significant. Three patients affected by the classic form of EPP underwent liver transplantation due to severe hepatic complications (in all cases, acute liver failure with significant increase in markers of cholestatic disease). In all cases, liver pathology revealed a diffuse precipitation of protoporphyrin microaggregates into the intrahepatic biliary canaliculi. One patient died during the

Figure 2. EPP diagnosis per year in Italy during the observational study period.

observation study due to infectious complications. None had simultaneous liver bone marrow transplantation. Considering that only one patient died during the study period, we estimated that the prevalence of EPP in Italy is about 3.15 cases per million inhabitants, although the prevalence of EPP among the different Italian regions (*figure 2*) shows that most of the patients live in the North of Italy (Lombardia).

The incidence has been estimated at about 0.13 cases per million/year in Italy, however, new diagnoses of EPP per year, over the considered observation time (*figure 3*), indicate an increase in diagnosis from 2009.

All clinical details such as data on incidence, prevalence and treatment of studied patients are summarized in *table 2*. As reported in *figure 4*, the majority of EPP families (106) presented only one patient; 29 families had two related patients, and one family had three related patients and another six related patients.

Mutations were identified in all patients for whom it was possible to analyse DNA ($n = 173$). Of these, 158 patients had a *FECH* null mutation *in trans*, c.315-48C (IVS3-48C), and were classified with EPP. The hypomorphic *FECH* IVS3-48C allele was not assessed in one patient. Four patients with biochemical and clinical features of EPP (increased total erythrocyte protoporphyrin and increased percentage of erythrocyte metal-free protoporphyrin) were also considered to have EPP, although at the genetic level, they only presented a heterozygous hypomorphic allele. For these patients, it was not possible to exclude *FECH* long deletions or *ALAS2* mutations, nor intronic deep mutations. Only one patient had *FECH* mutations on both alleles (arEPP), and nine patients had *ALAS2* mutations (XL-EPP). The frequency and type of mutation detected in the *FECH* gene in the studied population is presented in *table 3*.

We reported 42 different mutations, including 27 already reported and 15 novel mutations. The 15 novel variants included six small deletions, four splicing defects, three nonsense and two missense mutations. Three mutations were found in one or more families that together accounted for 45.9% (75/164) of *FECH* gene mutations: c.215dupT (27.2%), c.901_902delTG (11.5%), and c.67 + 5G > A (7.2%).

Considering the possible effects of detected mutations in our survey, 13 of 42 (31%) were frameshift mutations, nine (21.4%) were missense mutations, nine (21.4%) splicing mutations, seven (16,7 %) non-sense mutations, three (7.1%) long deletion mutations, and one (2.4%) was a regulatory gene mutation (*figure 5*).

The autosomal recessive patient was a *FECH* compound heterozygote for two missense mutations but neither of these mutations were found in other EPP patients.

Among the nine patients with XL-EPP, only the c.1706_1709delAGTG mutation was found in the *ALAS2* gene. These patients came from four unrelated families and among them, five were females and four males. The five females in these families presented with a high degree of phenotypic variability, ranging from classic EPP symptoms to very limited clinical or biochemical signs. As already published, although not statistically significant, a very high level of PPIX accumulation was demonstrated in female patients, even higher than that in four male patients [20].

The median concentration of erythrocyte protoporphyrin did not differ significantly among patients with EPP even after stratification for different kinds of mutation (*table 4*). Median erythrocyte protoporphyrin concentration in the six patients with biochemical and clinical signs suggestive of classic EPP, in whom no mutation was detected, did not differ from that in EPP patients and no distinctive clinical features were observed (data not reported).

Figure 3. Geographical EPP prevalence in Italy.

Figure 4. EPP proband distribution per family.

Discussion

In Italy, most patients with suspected porphyria are referred to expert centres for diagnostic confirmation and clinical management. This study reports, for the first time, data collected by all the leading porphyria expert centres in the country. It should be noted that in Italy some patients consult more than one expert centre, for possible different opinions and clinical management. To reduce this "over-sampling bias", we performed a deep analysis by comparing each single-centre database and excluding all possible iterative inclusions. For this reason, we believe that the data provided by the present study better reflects the true Italian epidemiology of EPP compared to other previously reported data [7].

The analysis of the overall data shows that in Italy the diagnosis of EPP is made later than in the rest of Europe (on average at the age of 28 rather than 21) [7] and, in partic-

ular, that the rate of EPP diagnosis under 10 years of age is still very low (less than 15% of cases). These data suggest that too many Italian patients with EPP are probably still misdiagnosed with "sun allergy", this highlighting the need for a greater awareness of the disease among Italian physicians, in particular, paediatricians and dermatologists, and a higher level of clinical suspicion and improvement in diagnosis [7, 25].

Moreover, our data indicate an incidence of about 0.13 new cases/year/million inhabitants, approximately twice as much as that observed in Europe [7]. However, the incidence in Italy was not constant over the considered period, showing a significant increase from 2009 (*figure 2*). Interestingly, in 2009, alfa-melanotide started to become available and patients taking this medication were enrolled into clinical trials via one Italian centre, in particular [26-28]. One may speculate that the availability of an effective protective drug against PPIX-induced photosensitivity may have favoured the spread awareness of EPP

Table 3. Frequency and type of mutation in the *FECH* gene in the studied population.

Mutation	Number Of Patients	%*	Type	References
Genetic analyses not made	6	–	–	-
None detected (Only HypomorPhic allele)	4	2.4	undefined	-
c.[215dupT]	45	27.4	FS	Wang (1997) J Invest Dermatol 109,688
c.[901_902delTG]	18	11	FS	Schneider-Yin (1994) Hum Genet 93, 711
c.[67 + 5G > A]	12	7.3	SP	Wang (1999) J Invest Dermatol 113, 87
c.[1-251G > C;194 + 4350_463 + 1197del5576]	6	3.6	LD	Di Pierro (2006) Hum Genet 118 776
c.[343C > T]	6	3.6	NS	Henriksson (1996) J Invest Dermatol 106, 346
c.[843delC]	6	3.6	FS	Gouya (1998) J Invest Dermatol 111, 406
c.[599-3C > T]	5	3.0	SP	Aurizi (2007) Mol Genet Metab 90, 402
c.[757_761delAGAAG]	5	3.0	FS	Henriksson (1996) J Invest Dermatol 106, 346
c.[464–1169 A > C]**	4	2.4	SP	Di Pierro (2019) Genet Med 2019
c.[892C > T]	4	2.4	NS	Di Pierro (2004) Hum Genet 114 608
c.[94C > T]	4	2.4	NS	This report
c.[1-251G > C]	3	1.8	RG	Di Pierro (2005) Exp Hematol 33: 584
c.[195-2A > G]	3	1.8	SP	Aurizi (2007) Mol Genet Metab 90, 402
c.[400delA]	3	1.8	FS	Wang (1999) J Invest Dermatol 113, 87
c.[116delG]	2	1.2	FS	This report
c.[286C > T]	2	1.2	NS	Henriksson (1996) J Invest Dermatol 106, 346
c.[315-67G > A]	2	1.2	FS	Aurizi (2007) Mol Genet Metab 90, 402
c.[415C > T]	2	1.2	NS	This report
c.[488_501del]	2	1.2	FS	Di Pierro (2001) Hum Genet 109, 468
c.[706-3C > G]	2	1.2	SP	This report
c.[791C > T]	2	1.2	MS	Martinez (2001) Hum Genet 109, 241
c.[930 G > A]	2	1.2	NS	Di Pierro (2004) Hum Genet 114, 221
c.[942_945delAACA]	2	1.2	FS	This report
c.[1-7887_67 + 2422del10376bp]	1	.06	LD	Brancaleoni (2007) Hum Genet 121, 646
c.[1-9628_67 + 2871del12566bp]	1	.06	LD	Brancaleoni (2008) Hum Genet 123, 546
c.[1041_1047delGCTGGACA]	1	.06	FS	This report
c.[1057delT]	1	.06	FS	This report
c.[1080_1081delTG]	1	.06	FS	This report
c.[1232G > A]	1	.06	MS	Balwani (2013) Mol Med 19, 26
c.[163G > T]	1	.06	MS	Lamoril (1991) Biochem Biophys Res Commun 181, 594
c.[195-16A > G][1]	1	.06	SP	This report
c.[451T > C]	1	.06	MS	Rufenacht (1998) Am J Hum Genet 62, 1341
c.[544delC]	1	.06	FS	This report
c.[680G > A]	1	.06	NS	This report
c.[705 + 1delG]	1	.06	SP	This report
c.[705 + 1G > A]	1	.06	SP	This report
c.[727A > G][2]	1	.06	MS	This report
c.[1231T > G][2]	1	.06	MS	Whatley (2010) Br J Dermatol 162, 642
c.[742T > C]	1	.06	MS	This report
c.[782C > T]	1	.06	MS	Brancaleoni (2008) Hum Genet 124, 296
c.[801G > A]	1	.06	MS	Lamoril (1991) Biochem Biophys Res Commun 181, 594
c.598 + 1G > T(;)599-3C > T[3]	1	.06	SP	Frank (1999) J Investig Med 47, 278

*FS: frameshift; MS: missense; NS: nonsense; SP: splicing; LD: long deletion.*Percentage calculated from the total 164 subjects.**Presence of IVS in both alleles.[1]No IVS analysed.[2]Mutations found in the same patient (ArEPP).[3]Two mutations, probably in cis.*
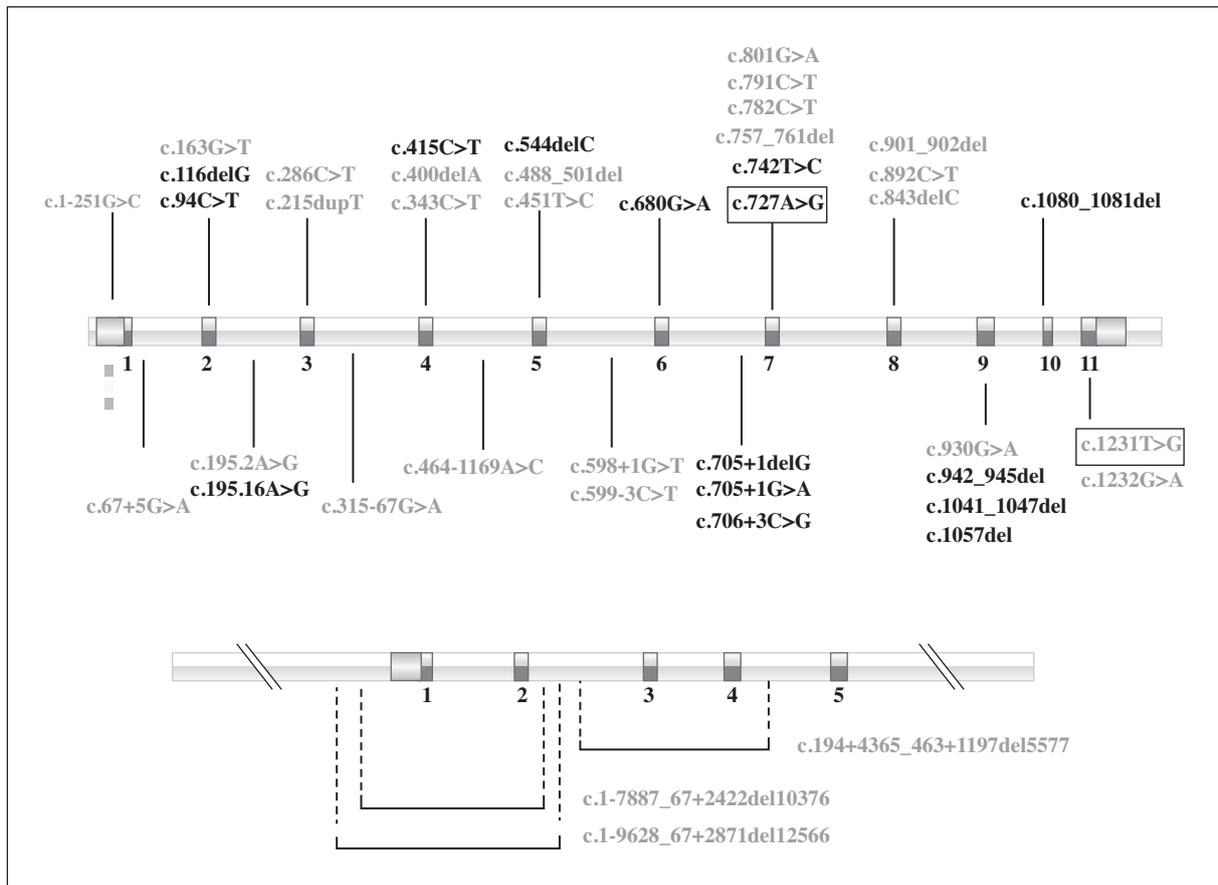
Figure 5. *FECH* mutations observed in Italian patients. Upper panel: point mutations; lower panel: long deletions. Previously unreported mutations are shown in black, while previously described mutations are in grey. The two mutations within a square were found in the only autosomal recessive erythropoietic protoporphyria patient.

among clinicians. This may explain the higher EPP incidence observed in Italy, where a large proportion of patients [100/179 (55.8%)] received at least one subcutaneous alfa-melanotide implant from 2009 onwards (*table 1*).

Our study reports a prevalence of 3.15 cases/million/inhabitants, which is lower than that reported for Italy in the porphyria European epidemiological study on porphyria conducted in 2012. It should be noted that in this study, the prevalence of different porphyrias was derived from incidence rates over three consecutive years, assuming that the ratio between incidence and prevalence was stable for many years, however, as presented in *figure 2*, this was not the case in Italy [7]. Moreover, the Italian incidence data reported by Elder *et al*. [7] may be biased due to an "over-sampling bias", as mentioned above. Although the data reported in our study, free of this bias, are more likely to be accurate, we cannot exclude that a large number of EPP patients are still undiagnosed.

Moreover, this is supported by the relatively old age at diagnosis, suggesting poor awareness of this disease by clinicians in our country. This hypothesis is also supported by the significant difference in regional distribution of EPP cases (*figure 3*). In fact, most patients seem to live in regions with an active porphyria reference centre, which probably leads to a greater knowledge of the disease, resulting in a greater number of patients diagnosed.

Table 4. Erythrocyte protoporphyrin concentration in patients with erythropoietic protoporphyria, relative to type of mutation.

	Number of patients	Erythrocyte protoporphyrin (μmol/L) median (range)
Total	164	26.7 (8.27-91.6)
Missense mutations	10	18.2 (9.84-54.2)
Splicing mutations	30	19.6 (8.56-91.6)
Frameshift mutations	92	25.8 (8.27-81.9)
Nonsense mutations	20	26.8 (9.82-78.9)
Long-deletion mutations	8	28.9 (9.81-84.5)
Regulatory gene mutations	4	23.6 (10.2-73.9)

In our Italian cohort of EPP patients, 42 different *FECH* gene mutations were detected, confirming the allelic heterogeneity already described in other countries [6, 7, 11, 29-31]. However, in Italy, as well as in other European countries, the level of allelic *FECH* heterogeneity is lower than that observed for autosomal acute porphyria [7], mostly due to the presence of three prevalent mutations accounting for 45.9% of all *FECH* mutations (*table 3*).

Similar high frequencies of *FECH* mutations among apparently unrelated EPP patients have been reported in other European and non-European countries [6, 7, 29, 31].

Of the 179 patients with EPP, 88.8% had a loss-of-function *FECH* allele and the c.315-48T > C low-expressing variant. This data reinforces the idea that EPP prevalence is strongly influenced by the frequency of the hypomorphic *FECH* IVS3-48C allele [7, 11], which is highly variable in different European countries (ranging from 5-11%) [6, 31]. In Italy, the prevalence is reported to be 3.3% and 3.6%, according to the 1000Genome (https://www.internationalgenome.org/) and GnomeAD (https://gnomad.broadinstitute.org/) databases, for Tuscany and south European populations, respectively. All but 2.4% of our patients underwent complete molecular characterization, excluding patients for whom it was not possible to analyse DNA. In fact, four patients had only the heterozygous hypomorphic *FECH* allele with classic biochemical and clinical features of EPP. In these patients, it was not possible to assess long deletion by MLPA, nor the presence of *ALAS2* mutation, or deep intronic mutation. Thus, it is conceivable that these patients represent classic EPP patients. We identified only one patient with two *FECH* loss-of-function alleles, accounting for only 0.6% of the total number of EPP patients. This percentage is very low when compared to that reported in other countries (about 5%).

Most of the 42 different mutations identified in the *FECH* gene were small deletions and insertions resulting in: a frameshift with creation of a premature stop codon; splicing defects predicted to cause aberrant mRNA processing; or nonsense variants, introducing premature stop codons. Similarly, among the 15 novel variants detected in our population, most were small deletions, splicing defects or nonsense variants, while only two missense mutations were described. Both missense variants (p.K243L, p.F248L) led to replacement of highly conserved amino acids. The most common mutations in the study population were c.215dupT and c.901_902delTG, which have not been reported with such a high frequency in other EPP populations. Among our cases, patients with XL-EPP accounted for approximately 5% of all EPP patients, which is less than that reported in Europe and the US [6, 32, 33]. In contrast to other reports, our XL-EPP patients carried only one mutation, c.[1706_1709delAGTG]. Male patients with XL-EPP reported early onset of symptoms, while females showed significant heterogeneity of symptoms, ranging from onset of symptoms in childhood to being symptomatic until adulthood at the time of the study. This variability is strongly associated with random X-chromosomal inactivation in females patients, as previously described [20].

In contrast to other studies, we did not identify mutations associated with a milder phenotype [34]. However, several patients were reported to carry the same mutation but with different disease severity, suggesting the existence of as yet undefined factors (modifier genes or acquired or environmental factors) which may influence the phenotype of the disease.

In conclusion, to the best of our knowledge, this study represents the largest collection of data on the prevalence and molecular epidemiology of EPP in Italy available so far. Most patients were found to have a *FECH* gene mutation associated with a low-expressing allele (EPP). The autosomal recessive form of EPP appears to be very rare in

Italy, and one patient was identified in our cohort (0.56%), whereas the recently identified X-linked dominant EPP showed a prevalence of 5% [6, 35]. Considering both the availability of an effective treatment for photosensitivity (which negatively impacts on patients' quality of life) and the importance of the clinical follow-up (especially concerning the possibility of severe liver involvement), a significantly greater clinical awareness and knowledge of EPP is required among clinicians [26, 28, 36-41]. In order to reduce the number of misdiagnosed or/and untreated EPP patients, relevant training for clinicians should be implemented. ∎

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