RESEARCH ARTICLE

The impact of gender, puberty, and pregnancy in patients with POLG disease

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Abstract

Objective: To study the impact of gender, puberty, and pregnancy on the expression of POLG disease, one of the most common mitochondrial diseases known. **Methods**: Clinical, laboratory, and genetic data were collected retrospectively from 155 patients with genetically confirmed POLG disease recruited from seven European countries. We used the available data to study the impact of gender, puberty, and pregnancy on disease onset and deterioration. **Results**: We found that disease onset early in life was common in both sexes but there was also a second peak in females around the time of puberty. Further, pregnancy had a negative impact with 10 of 14 women (71%) experiencing disease onset or deterioration during pregnancy. **Interpretation**: Gender clearly influences the expression of POLG disease. While onset very early in life was common in both males and females, puberty in females appeared associated both with disease onset and increased disease activity. Further, both disease onset and deterioration, including seizure aggravation and status epilepticus, appeared to be associated with pregnancy. Thus, whereas disease activity appears maximal early in life with no subsequent peaks in males, both menarche and pregnancy

appear associated with disease onset or worsening in females. This suggests that hormonal changes may be a modulating factor.

Introduction

Mitochondria are essential organelles found in almost all human cells. The most fundamental function of mitochondria is energy production through the process of oxidative phosphorylation (OXPHOS).[1] The respiratory chain conserves the energy released through intermediary metabolism and the ATP synthase then uses it to generate adenosine triphosphate (ATP). Thirteen of the protein subunits of the OXPHOS system are encoded by mitochondrion's own DNA (mtDNA), while all the remaining subunits are encoded by the nuclear DNA (nDNA).[2] Mitochondrial dysfunction can present at any age with heterogeneous clinical manifestations and multi-organ involvement. Nevertheless, mitochondrial disease appears predominantly to affect tissues with high energy requirement, such as the brain, liver, and heart.[3–4]

Polymerase gamma is the enzyme responsible for replicating and repairing mtDNA. Variants in *POLG*, the nuclear gene encoding the catalytic subunit of this enzyme, are among the most frequent causes of mitochondrial disease[5,6] and cause a variety of clinical phenotypes ranging from infantile refractory epilepsy and liver failure to juvenile and adult-onset epilepsy, myopathy and ataxia, and to late-onset myopathies with progressive external ophthalmoplegia.[7–10] Epilepsy has a major impact on morbidity and mortality. Data from a large cohort of individuals with POLG disease, which was recently published, showed that epilepsy is significantly associated with poor outcome.[11]

Mitochondrial diseases have a minimum prevalence of ca. 1:5000.[12] Despite the fact that they are considered the most common form of inborn error of metabolism, information on the impact of gender on these diseases remains limited and stems mainly from case reports and small case-series of pregnant women with disorders, such as mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes (MELAS).[13–18] Since mutations in *POLG* are one of the most common causes of mitochondrial disease, we wanted to study the impact of gender, puberty, and pregnancy on POLG disease with the aim of providing insights to guide physicians responsible for their management.

Patients and Methods

Study design and population

We conducted a multicenter retrospective study of 155 patients with confirmed pathogenic *POLG* variants from 12 centers in seven European countries: Norway, Finland, Sweden, Denmark, The Netherlands, Spain, and the United Kingdom. Detailed demographic, clinical, laboratory, neuroimaging, and genetic data were obtained. Age of disease onset was defined as age in months when the patient first required medical evaluation. The age of onset of each individual symptom was also identified. End of follow-up was defined as the patient's last visit to the hospital or death. Secondary amenorrhea was defined as cessation of menstruation for six consecutive months in a woman who has previously had regular menstruation. A detailed description of the clinical, laboratory, and neuroimaging data for the whole cohort has been published.[11] The mean age of thelarche and menarche in the population of each country participating in the study[19–27] is provided in File S1.

Data analysis

In order to study the effect of gender, individuals were stratified into two groups according to their gender and further grouped into those with disease onset before and after onset of puberty (12 years). To study the effect of pregnancy, females of childbearing age (18–50 years) were identified and their data were analyzed separately.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Package of Social Sciences), Version 23.0. A two-sided P value less than 0.05 was considered to be statistically significant. For survival analysis, the end-point was time to death, which was defined as the time in months from the date of disease onset to the date of death. Univariate survival analysis was performed using log-rank test (Kaplan–Meier) to compare differences in survival time between the subgroups defined above.

Ethical statement

The ethical approval for the study was obtained from the Regional Committee for Medical and Health Research Ethics, Western Norway (REK 2014/1783-4). Each participating country had obtained approval by the local ethical committee. The study was registered as an audit at Great Ormond Street Hospital, London, UK (Registration Number 1675).

Results

Demography

In the whole study cohort of 155 individuals, age of disease onset extended from birth to 71 years with median age of onset 10 years (interquartile range: 19 years). Fifty-four percent of individuals ($n = 83$) had disease onset before the age of 12 years and 46% ($n = 72$) after this age. Fifty-one percent ($n = 79$) were females and 49% ($n = 76$) were males.

Major clinical features and gender differences

Median age of disease onset for males was 5 years, while for females it was 13 years. Further, the percentage of males and females presenting prior to or after the age of 12 years differed; before age 12, we found 55% males ($n = 46$) and 45% females ($n = 37$) while after 12 years, it was 42% ($n = 30$) and 58% ($n = 42$). When we analyzed age of onset for the whole cohort, we found a bimodal distribution with the first peak early in life (before the age of 4 years) and the second around 12–16 years. This bimodal pattern was, however, gender

dependent; it was apparent only in females and was not seen when we analyzed data only from males (Fig. 1).

Seizures occurred in 69% ($n = 107$), and acute episodes of worsening (previously called stroke-like episodes (SLE)) in 37% ($n = 52$) of individuals. Once again there was a trend toward a gender difference; a bimodal distribution with one peak in early life and the second around puberty was seen in females but not males for both seizures and SLE (Fig. 1). Further, regardless of the age of disease onset, status epilepticus was reported in 56% ($n = 44/79$) of females and 44% ($n = 35/79$) of males.

When we looked at other features of POLG disease, such as ataxia and liver dysfunction, these manifested more often in males prior to the age of 12 years than females under 12, while in those with disease onset after the age of 12 (Fig. 2), it was the reverse. Features such as peripheral neuropathy, progressive external ophthalmoplegia, and ptosis did not show any clear gender differences. A detailed description of the frequency of each individual symptom before and after the onset of puberty is provided in Figure 2 and in File S2.

Secondary amenorrhea was reported in three patients (one homozygous for p.Trp748Ser, one compound heterozygous for p.Trp748Ser/p.Gln497His, and one heterozygous for the dominant p.Tyr955Cys).
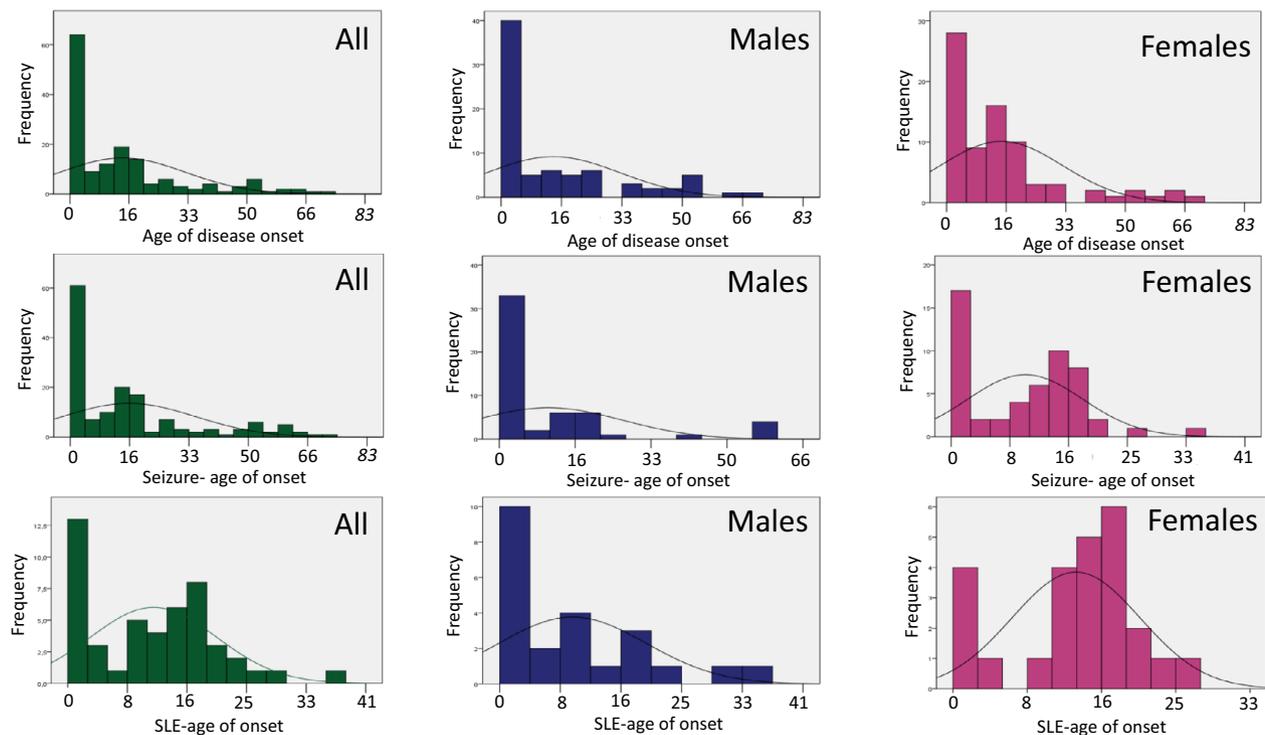

Figure 1. Age of disease onset, onset of seizure, and onset of stroke-like episodes (SLE) in years for the whole study cohort of individuals with POLG disease and also according to the gender. The figure shows clearly that the age of disease onset, seizure and stroke-like episode (SLE) onset in females had a bimodal distribution with the first peak in childhood and the second peak around puberty, while males were presented earlier, and the age of disease onset, seizures and SLE onset had a unimodal distribution with peak of onset during the childhood.
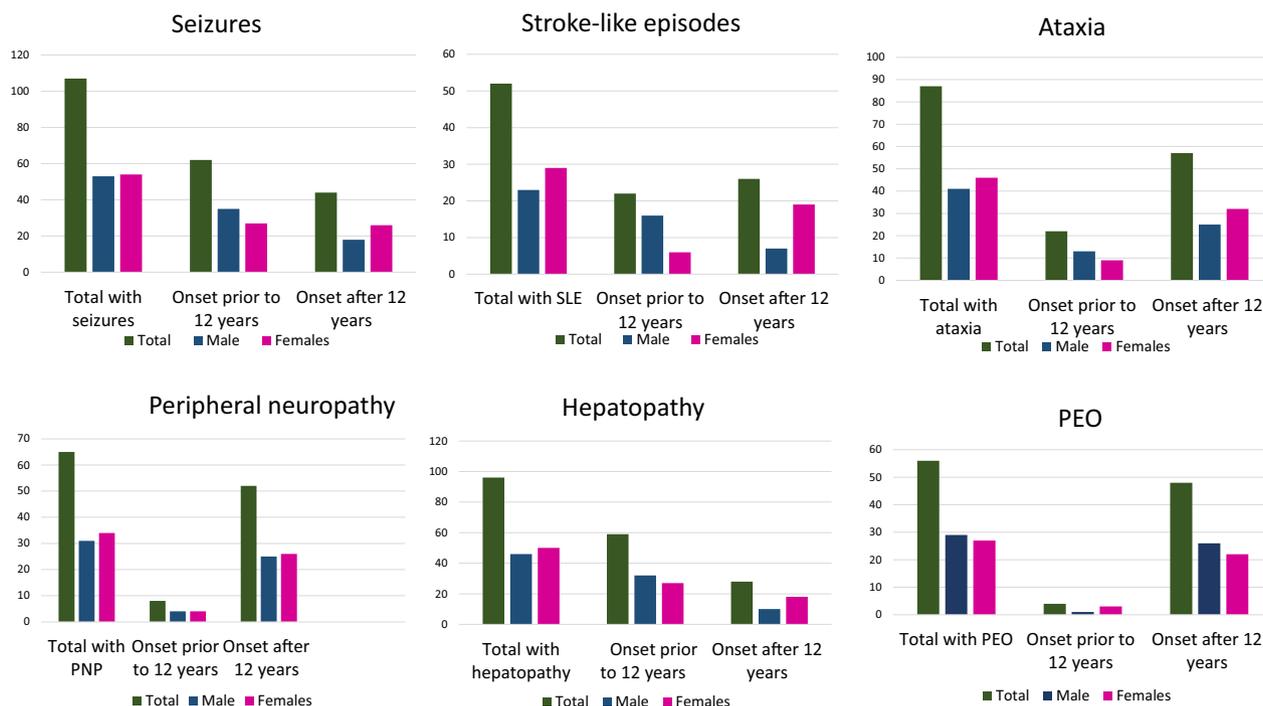
Figure 2. Major clinical features of POLG patients stratified according to gender and puberty onset. SLE, stroke-like episodes, PEO, progressive external ophthalmoplegia, PNP, peripheral neuropathy.

Impact of pregnancy

Our cohort included 14 women between 18 and 50 years whom we considered potentially fertile and of childbearing age. Ten of these became pregnant and in all, pregnancy had a clear negative impact: five patients presented with the first signs of their disease in pregnancy and five with a clinical deterioration. The most critical period for these women appeared to be the second trimester of pregnancy (Table 1). Pregnancy was associated with status epilepticus in six individuals and in three there was evidence of new-onset liver dysfunction (study cases 6, 7, and 9).

All women who experienced pregnancy-associated disease onset/worsening were homozygous or compound heterozygous for the common founder variants p.Ala467Thr and p.Trp748Ser apart from one who was homozygous for the p.Gly268Ala variant (Table 1).

Table 1. Summary of pregnancy data on POLG patients included in the current study.

Patient no.	Variants	Age at disease onset	Age at pregnancy	Parity[1]	Impact of pregnancy	Gestational age at disease debut or exacerbation in weeks	Symptoms at disease debut/ exacerbation	Survival status	Age at death
1	p.Ala467Thr/p.Ala467Thr	18	18	G1P1	Debut	18	Seizure	Deceased	54
2	p.Ala467Thr/p.Ala467Thr	23	23	G1P1	Debut	20	Seizure	Alive	
3	p.Trp748Ser + p.Glu1143Gly[2]/ p.Trp748Ser + p.Glu1143Gly[2]	19	33	G1P1	Exacerbation	19	Seizure/SE[3]	Alive	
4	p.Trp748Ser/p.Trp748Ser	15	20	G1P1	Exacerbation	17	Ataxia	Alive	
5	p.Gly268Ala/p.Gly268Ala	39	39	G1P1	Debut	16	Ataxia	Deceased	45
6	p.Trp748Ser/p.Trp748Ser	18	24	G3P2	Exacerbation	18	Seizure/SE[3]	Deceased	44
7	p.Ala467Thr/p.Ala467Thr	20	20	G1P1	Debut	19	Seizure/SE[3]	Deceased	39
8	p.Ala467Thr/p.Trp738Ser	14	23	G1P1	Exacerbation	32	Seizure/SE[3]	Deceased	24
9	p.Ala467Thr/p.Trp738Ser	27	27	G1P1	Debut	17	Ataxia	Alive	
10	p.Trp748Ser/p.Trp748Ser	18	18	G1P1	Debut	16	Seizure/SE[3]	Deceased	21

1. G: number of pregnancies, P: number of deliveries after 24 weeks. 2. Polymorphism. 3. SE: status epilepticus.

Survival and gender differences

There was no significant difference in the overall survival between males and females. The analysis also showed that the onset of puberty did not have an impact on survival, regardless of gender. Interestingly, however, time to death was clearly shorter in females (median = 77 months) as compared to males (median = 108 months) regardless of the age of disease onset.

Discussion

Our study shows that there are differences in the timing and in the manner that POLG disease manifests between males and females. Timing of disease onset showed a bimodal distribution in females with the first peak in childhood and the second around the time of puberty. Indeed, approximately half of the females in our cohort remained asymptomatic until they reached puberty. In addition, our study shows that pregnancy is a potential risk for women with POLG disease. Two thirds of women of childbearing age in our cohort either had disease onset or experienced disease deterioration during pregnancy. In contrast, the peak age of onset in males was unimodal and early in life.

The presenting feature of the disease also showed gender differences. Seizures, SLE, hepatopathy, and ataxia are major features of POLG disease and their presence reflects severity of the disease. In our cohort, seizures and SLE were more common in males prior to the onset of puberty, while in females, they appeared more common after the onset of puberty, particularly SLE. Hepatopathy and ataxia showed similar age-related trends, albeit less dramatic. In contrast, the frequency of peripheral neuropathy and PEO showed similar gender frequencies before and after the age of puberty (Fig. 2, File S2).

Almost all the women who experienced pregnancy-associated disease onset/worsening were homozygous or compound heterozygous for the common founder variants p.Ala467Thr and p.Trp748Ser; this may reflect ascertainment bias as all of these individuals were recruited from Norway where those variants are common[10]. Exacerbation of seizures was the major and predominant complication during pregnancy and was associated with status epilepticus, prolonged hospitalization, and the use of multiple antiepileptic drugs. Further, two of the individuals had disease onset manifesting with ataxia and one had a clear worsening of ataxia during pregnancy (Table 1).

The finding of secondary amenorrhea in three women in our cohort confirms previous reports that documented a link between pathogenic *POLG* variants and premature ovarian failure.[28,29]

Changes in estrogen and progesterone levels clearly provide a link between menarche and pregnancy. Experimental animal models show that both hormones can alter neuronal excitability.[30,31] Estrogen increases the excitatory neurotransmitters and may alter the structure of the synaptic area by increasing the number of dendritic spines leading to increased cell-to-cell contact. Estrogen may also contribute to increased brain-derived neurotrophic factor, which in turn may lead to excess glutamate release and decreased seizure threshold. Estrogen is therefore considered to be pro-convulsant.[31,32] Progesterone appears to have a seizure inhibitory effect. Metabolites, such as allopregnanolone, a GABAA receptor-modulating neurosteroid, appear to regulate hippocampal neuronal excitability by exerting a positive allosteric effect on GABAA receptors.[33,34] Studies have, moreover, demonstrated clustering of seizures in association with cyclic ovarian hormonal changes also in humans.[32,35] The estrogen peak occurring with puberty and the menstrual cycle may also explain the timing of disease onset or deterioration in females with POLG disease.

Pregnancy is a high-demand metabolic state that requires optimal energy production to maintain normal physiological adaptation and fetal growth. Increased levels of serum lactate during pregnancy, even in healthy women,[36] suggest, therefore, that pregnancy is associated with metabolic adaptions and potentially a degree of mitochondrial stress. It is possible to hypothesize that the increased energy demands affect all tissues, including neurons, and since these are already metabolically compromised, seizure threshold may be reduced. Our study showed that pregnancy is indeed a high-risk period for women with POLG disease, particularly the second trimester.

Another potential risk factor is folate deficiency. This can result in mitochondrial dysfunction by reducing the expression of genes encoding essential proteins of the electron transport chain such as cytochrome c oxidase.[37–41] The 10 pregnant women included in our study (Table 1) were Norwegian and would most probably have followed Norwegian pregnancy guidelines including the recommended daily supplement of 400-microgram folate. This is, however, only in the first trimester (www.helsenorge. no). Thus, any potential protective effect given by folate supplementation in the first trimester may be lost following discontinuation and could contribute to the disease onset in the second trimester.

Given the results of our study, it is pertinent to ask whether women with POLG disease, especially those reaching childbearing age, should be counseled on the potential risk of disease deterioration during pregnancy. Clearly, proper planning and discussion of issues, such as sexual health, contraception, and pregnancy, are important.

Nevertheless, our data suggest that there is a definite risk associated with pregnancy and that this is the case both in those with previously recognized disease and in those who first manifest their disease during pregnancy. Given this, we feel that women with POLG disease should be informed of the risks involved and, should they wish to proceed, we would suggest that they be managed both prior to and during pregnancy by a multispeciality team including obstetricians and neurologists/epileptologists.

Identifying those at greatest risk and, thus, those requiring maximal follow-up is challenging. Individuals with POLG-related epilepsy show EEG changes particularly involving the occipital regions, and these are present before the onset of clinically apparent seizures.[42–44] While this is not a particularly robust biomarker, it may be useful in some patients. In the light of the second trimester worsening, the use of folic acid supplementation beyond the first trimester may also be a relevant option. Further studies will be needed to define the risk profile and show how best to manage fertile women with POLG disease; however, our data provide a clear indication that pregnancy and menarche are times of increased risk for females with *POLG* mutations.

Acknowledgments

Conflict of Interest

The authors declare no financial or other conflict of interest.

Authors Contributions

O.H and L.B designed the study, were responsible for data collection, analyzed the data and drafted the initial manuscript, and approved the final manuscript as submitted. K.N, M. E., C. K., M.R., C.ME. T., C.S, E.B., T.F., E.O., R. d.C, L.P., P.I., J.U., N.D., and S.R. were responsible for data acquisition and analysis, revising the manuscript critically, and approving the final manuscript as submitted. All authors are responsible for accuracy and integrity of the work.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. Timing of thelarche and menarche in the population of each participating country in the study.

File S2. Major clinical features stratified according to the gender and puberty onset.