GPT2 mutations cause developmental encephalopathy with microcephaly and features of complicated hereditary spastic paraplegia

running title: Phenotypic spectrum of GPT2 mutations

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Conflicts of Interest:

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Abstract:

Various genetic defects can cause intellectual and developmental disabilities (IDD). Often IDD is a symptom of a more complex neurodevelopmental or neurodegenerative syndrome. Identifying syndromic patterns is substantive for diagnostics and for understanding the pathomechanism of a disease. Recessive GPT2 mutations have recently been associated with IDD in four families. Here, we report a novel recessive GPT2 stop mutation p.Gln24* causing a complex IDD phenotype in a homozygous state in five patients from two consanguineous Arab families. By compiling clinical information of these individuals and previously described GPT2 patients a recognizable neurodevelopmental and potentially neurodegenerative phenotype can be assigned consisting of intellectual disability, pyramidal tract affection with spastic paraplegia, microcephaly and frequently epilepsy. Due to the consistent presence of pyramidal tract affection in GPT2 patients, we further suggest that GPT2 mutations should be considered in cases with complex hereditary spastic paraplegia.

Keywords: GPT2, intellectual and developmental disability, hereditary spastic paraplegia, microcephaly

Introduction:

Intellectual and developmental disability (IDD) is a frequent symptom in a large group of highly heterogeneous, often genetic diseases. IDD can occur as an isolated symptom in non-syndromic IDD or in combination with additional features like epilepsy, various types of movement disorders or non-neurological manifestations like dysmorphic signs constituting syndromic forms of IDD. Such syndromic features help for the better assignment within the disease group and sometimes can even point directly to the molecular cause of disease when clear genotype-phenotype correlations have been established. With a continually growing group of rare and ultra-rare neurogenetic disorders, it is of utmost importance to best identify and sharpen the clinical features that are frequently associated with a specific genetic defect.

Recessive mutations in Glutamate Pyruvate Transaminase 2 (GPT2) have recently been associated with intellectual disability in five independent families[1-4]. Here we present five patients from two consanguineous families with Palestinian ancestry with a novel homozygous stop mutation in GPT2. By comparing the common clinical features with previously published GPT2 patients the picture of a recognizable neurodevelopmental and probably neurodegenerative phenotype becomes apparent, consisting of severe IDD, spastic paraplegia, microcephaly and often epilepsy.

Materials and Methods

Patient and ethical issues

All patients were enrolled in the Palestinian Authority. Written informed consent was obtained from the parents for diagnostic procedures and next-generation sequencing as well as for the publication of this case report including the accompanying images. The study was approved by the local ethical review board (vote 180/2010BO1).

Whole Exome Sequencing (WES)

WES was performed for the patients F1-II.5 and F2-II.4 using the SureSelectXT Human All Exon V5 enrichment kit (Agilent, Santa Clara CA). Sequencing was performed on a HiSeq2500 platform (Illumina, San Diego CA) in paired-end mode according to the manufacturers' protocol. For F1-II.5 a total of 64852816 sequence reads of 101 bp length were produced. The mean sequencing depth was 73 and 89% of the target sequence were covered for at least 20 times. For F2-II.4 a total of 72736640 reads of 101 bp length were produced. The average sequence depth was 83 while 91% of the target sequence were covered for at least 20 times. Further analysis was performed through an in-house pipeline including BWA-MEM for alignment and freebayes for variant calling. Variant annotation was performed with SnpEff. After excluding well known genetic defects using the HGDM database, variants were then filtered for rare (< 0.1% in ExAC) homozygous variants that were not present in more than 20 families within our in-house database. Rare homozygous variants that resulted from this analysis can be found in the Supplementary Table S1 for F1-II.5 and Supplementary Table S2 for F2-II.4.

Sanger Sequencing

Sanger sequencing was performed using standard methods and chemicals. Primer sequences are available on request.

Magnetic resonance imaging (MRI)

Cranial MRIs have been recorded on a 1.5 Tesla Siemens MAGENTOM Aera scanner. Sagittal, transversal and coronal images of the brain have been acquired with standard sequences including T1, T2 and Flair images.

Results:

Clinical features:

Five similar affected patients from two consanguineous Arab families were identified at the Caritas Baby Hospital in Bethlehem. All five patients were delivered on full term without complications after an uneventful pregnancy. Either hypotonia (2/5) or a global developmental delay (3/5) were noted as first symptoms and became apparent within the first year of life (Table 1). At the time of examination, the patients were between 7 and 15 years old. All presented with severe intellectual disability including limited development of language skills, either being able to communicate with single words and a limited comprehension (2/5) or not being able to speak at all (3/5). While being hypotonic or normotonic after birth, all developed pyramidal tract signs (5/5) with hyperreflexia (5/5), a persistent positive Babinski sign (5/5) and spastic paraparesis (4/5) over time (Table 1). A deterioration of walking abilities and clear progression of spastic paraplegia was noted in one patient. Patient F1-II.5 was able to walk on his own at the age of 3 years with an ataxic gait; at the age of 8 years, the physical examination revealed severe spastic paraparesis with fixed contractures of ankles and inability to walk or even stand without support. The other four patients were still able to walk and shared a spastic-ataxic gait pattern. Epilepsy with generalized myoclonic and tonic seizures was noted in two patients starting in the first two years of life and partly responding to medication with Valproate. All patients were microcephalic with a standard deviation of -1.9 to -3.3 compared to controls. Mildly dysmorphic features with a short philtrum and deep frontal hairline (5/5), prominent crus helix of the ears (5/5), epicanthus medialis (2/5) and synophrys (3/5) were seen in several patients (Figure 2b). One patient presented with strabismus convergence.

Cranial MRIs were available in two patients and showed no malformations but some thinning in the posterior third of the corpus callsoum in both patients, mega cisterna magna in one patient and no obvious signs of cerebellar or cerebral atrophy (Figure 2a).

Identifying a novel GPT2 mutation

To unravel the genetic basis of the disease, we performed whole exome sequencing in one patient of each family. After filtering sequencing data as described in the Methods section, we identified a novel homozygous GPT2 stop mutation NM_133443: c.70C>T; p.Gln24* in both exomes, which was the only rare homozygous variant shared across both families. The

mutation segregated with the disease (Figure 1a) and was very rare in public databases (not present in ExAC[5] or Greater Middle East Variome[6], one single allele present in gnomAD, MAF of $7.3x10^{-6}$). Further, there was a noticeable phenotypic overlap of previously described GPT2 patients and the patients presented in this study. Therefore we concluded that the GPT2 stop mutation is the causative mutation in both families. No formal relationship could be established between the two families but both came from the same village in Palestine. On a genetic basis the two exomes share a homozygosity stretch with identical haplotype of about 15 Mb around the p.Gln24* mutation (Supplementary Figure S1, Supplementary Table S3). Of interest, in the first notion of family 2 individual F2-II.3 was reported to be also affected. However, in terms of genetics this patient is only heterozygous for the GPT2 mutation. On detailed clinical examination of the family, it became clear that he is suffering from a different disease. He had no phenotypic similarities to the other patients, especially no IDD, a normal head circumference for his age (56 cm), and no spasticity or epilepsy but was suffering from deafness only. Extended family history revealed that there were several patients with isolated deafness in both families (Figure 1a). We assume that two independent genetic diseases are segregating within these families. The presence of more than one genetic disease within one family is not unusual in consanguineous populations [7].

In our families, other additional individuals were reported to suffer from cerebral palsy which was also the previous external diagnosis of the five identified GPT2 patients. Unfortunately, these patients were not available for physical or genetic examination, but it may be hypothesized that they are GPT2 patients as well.

Discussion:

We identified five patients with complex IDD from two families with a novel homozygous GPT2 mutation. This p.Gln24* mutation is an early truncating mutation that affects only one of two known GPT2 transcripts. While this mutation leads to a substantial truncation or degradation of the longer isoform NM_13443, the shorter transcript NM_001142466 remains unaffected. Similarly, the most recently published mutation p.Gly96Arg affects only the longer isoform (Figure 1b). This suggests that the shorter splice variant do not essentially contribute to the disease. This is also supported by the lack of apparent phenotypic differences between patients with mutations affecting both or only the longer transcript. In accordance with this notion, functional analyses demonstrated that the shorter splice variant does not possess any enzymatic activity[8] but the proposed pathomechanism of GPT2-related disease is a loss of function of the enzymatic activity of GPT2[3].

Clinical characterization of the five patients from this study revealed striking similarities to previously published cases[1-4] and illustrate a broad phenotypic spectrum beyond cognitive deficits including microcephaly, epilepsy, corticospinal tract affection and mild dysmorphic features. In fact, even in the report linking the p.Gly96Arg variant only to non-syndromic IDD[2], substantial similarities have been named.

Almost all GPT2 patients with reported head circumferences have a microcephaly (27/28, Table 1) that developed only after birth in most cases. Furthermore, pyramidal tract signs with spastic paraplegia have been mentioned in 25 of 32 cases (Table 1). Further, a history of seizures (12/32) and some mild dysmorphic features like epicanthic folds are frequently observed. We therefore suggest to regard the phenotype of GPT2-related disease as syndromic rather than non-syndromic intellectual disability.

Of interest, a detailed patient history and thorough examination revealed, that most patients where initially hypotonic and developed spastic paraplegia only later in the course of the disease. While such a development from hypotonia after birth to hypertonia can also be seen in patients with cerebral palsy, a clear progression of spasticity over several years as seen in patient F1:II-3 might indicate an additional neurodegenerative process. In fact, the patients reported here could phenotypically as well be regarded as a complicated form of early-onset hereditary spastic paraplegia (HSP). Indeed, several subtypes of early-onset HSP like SPG47, SPG48, SPG50, SPG51 and SPG52 with mutations in adapter proteins like AP4B1, AP5Z1, AP4M1, AP4E1 and AP4S1, typically present with an initially hypotonic variant of HSP complicated by intellectual disability or epilepsy in addition to progressive spasticity becoming apparent only in the course of disease[9-12]. Given this overlap between phenotypes, GPT2 should be on the list of HSP genes. The other way round, some spastic paraplegia genes should be considered in cases with syndromic intellectual disability.

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Figure legends:

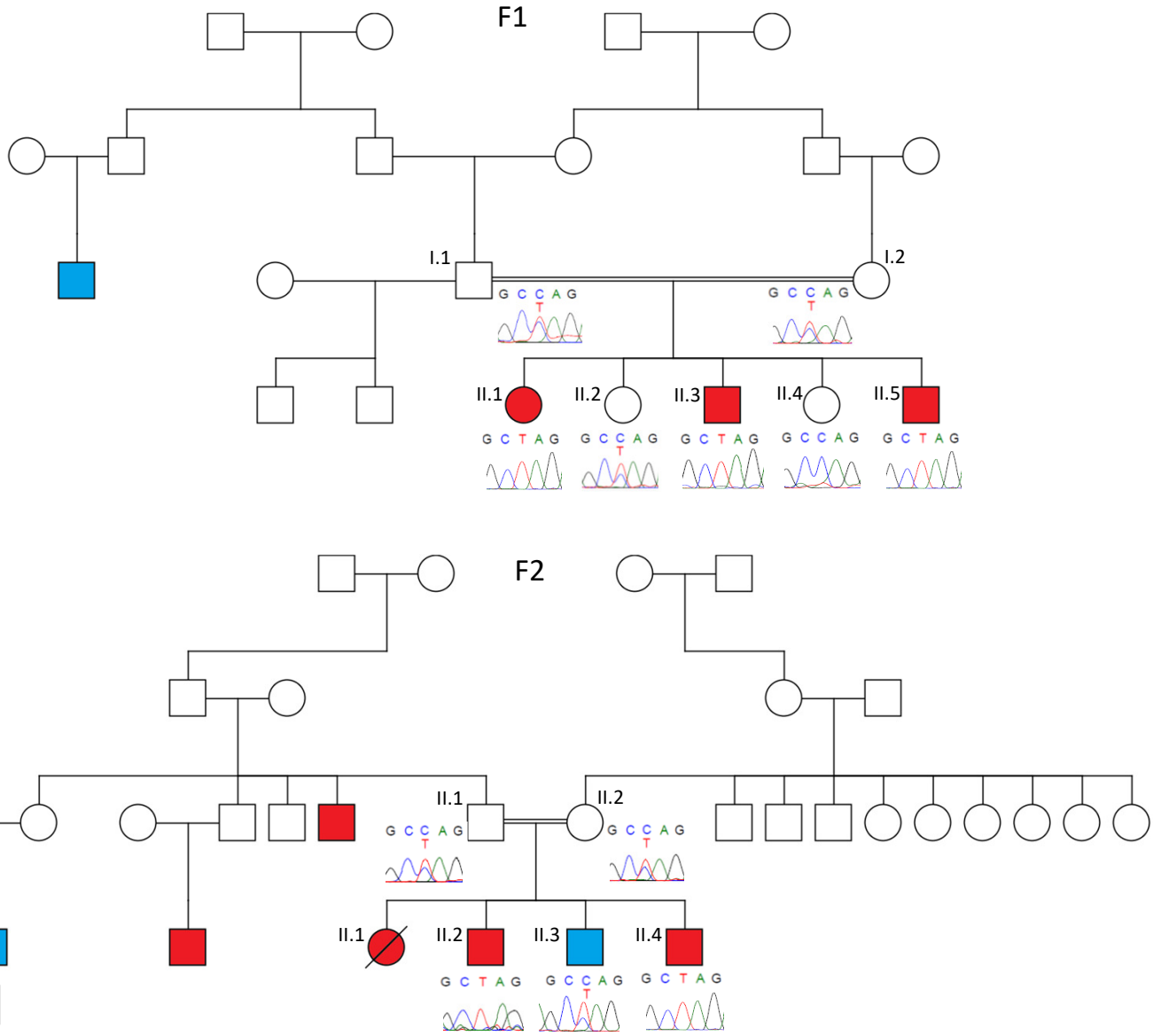
Figure 1: Pedigrees and overview of GPT2 mutations.
(a) Pedigrees and segregation analysis of families F1 and F2. Affected patients with IDD are depicted in red. Patients with deafness are depicted in blue and were not affected with IDD, microcephaly, spasticity or epilepsy. **(b)** Distribution of previously reported mutations in black[1-4] and the new GPT2 mutation in red on the two expressed isoforms. The conserved Aspartate Aminotransferase domain is depicted in blue.

Figure 2: Cerebral MRI of GPT2 patients and mild dysmorphic features
(a) Cerebral MRIs of two GPT2 patients (F2-II.2 and F2-II.4) and a sibling with deafness but no intellectual disability (F2-II.3). No clear cerebral malformation was seen. Some thinning of the posterior part of the corpus callosum was noticed in F2-II.2 and F2-II.4, however, this was similar in the brother without intellectual disability (F2-II.3). A mega cisterna magna was present in F2-II.4. No clear cerebellar or cerebral atrophy was seen neither in F2-II.2 nor F2-II.4. **(b)** Photos of Patients from Family F2. Family F1 didn't consent to publish identifying photos. Microcephaly and minor dysmorphic features like a short philtrum and deep frontal hairline were noticed in all patients. Synophrys was present in F1-II.1, F2-II.2 and F2-II.4. Epicanthus medialis was present in F1-II.1 and F1-II.3. F1-II.1 was the only patient with strabismus convergence.

Table 1: Clinical characteristics of GPT2 patients
Severity of intellectual disability was rated according to skills in daily activities and need of support according to the definition of DSM-5 Criteria and AAIDD Criteria. (https://www.ncbi.nlm.nih.gov/books/NBK332877/table/tab_9-1/?report=objectonly)

Figure 1

a



b

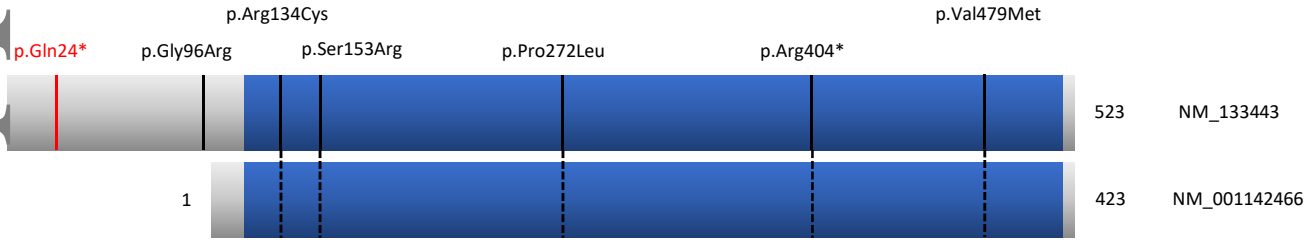
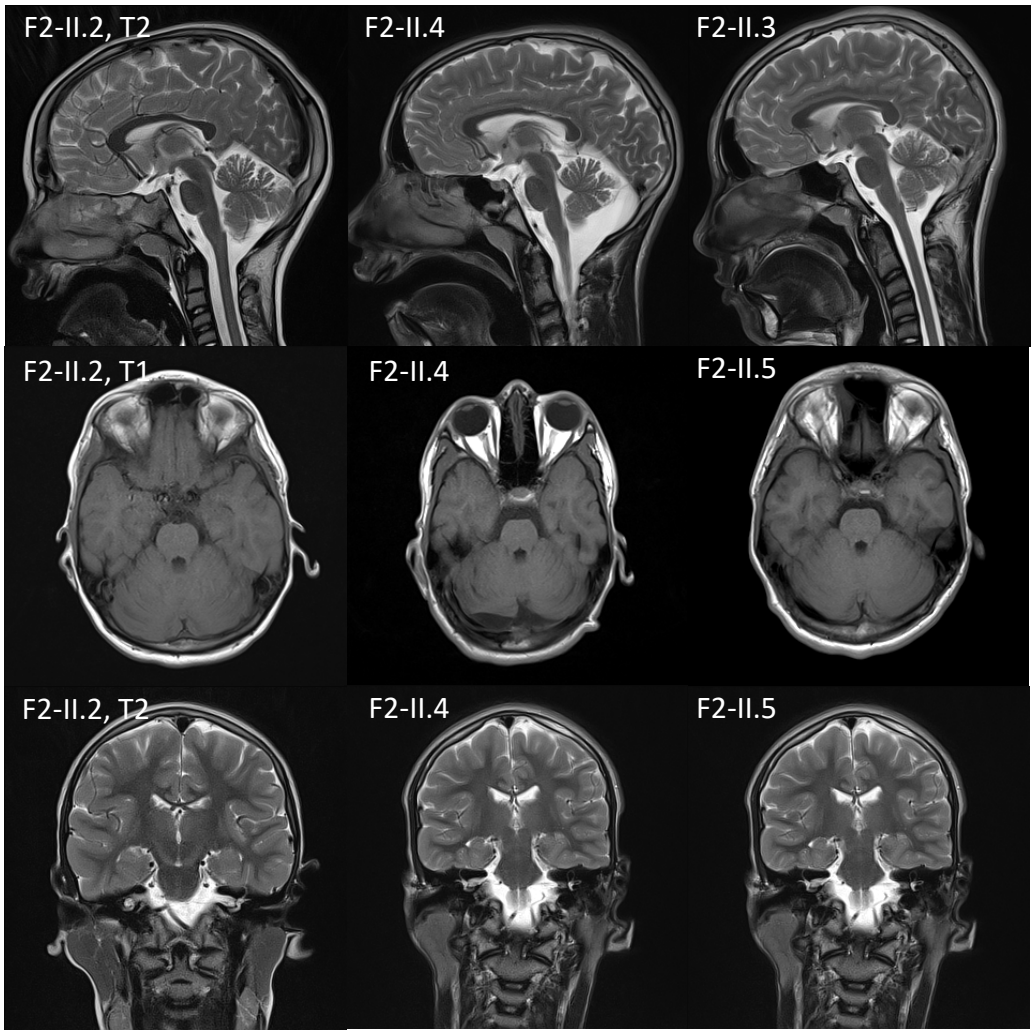


Figure 2
a



b

Table 1: Clinical characteristics of GPT2 patients

	F1-II.1	F1-II.3	F1-II.5	F2-II.2	F2-II.4	Lobo-Prada et.al (4 patients)	Ouyang et. al (14 patients)	Celis et.al (3 patients)	Kaymakcalan et al. (6 patients)
GPT2 mutation	p.Gln24* hom	p.Gln24* hom	p.Gln24* hom	p.Gln24* hom	p.Gln24* hom	p.G96R hom	p.P272L hom; p.R404* hom	p.S153R hom	p.R134C /p.V479M compound het
Gender	f	m	m	m	m	1x m, 3x f	9x m, 5x f	2x m, 1x f	1x m, 5x f
first symptoms; age	convulsions and hypotonia at 7 months	global developmental delay; 1 year	hypotonia; 5 months	global developmental delay; 1 year	global developmental delay; 1 year	global developmental delay; early childhood	hypotonia or global developmental delay; infancy or early childhood	global developmental delay during first year of life	hypotonia (index) or global developmental delay
Age at examination	15 years	12 years	8 years	15 years	7 years	26-43 years	11-32 years	4-15 years	12-47 years
Head circumference at examination	50.5 cm (-3.3 SD)	49.0 cm (-3.2 SD)	48.0 cm (-3.1 SD)	51 cm (-2.6 SD)	49.5cm (-1.9 SD)	n/a	all microcephalic (-2.8 to -6.8 SD)	microcephaly 2/3	all microcephalic (-3.2 to -7.0 SD)
Intellectual and Developmental Disability	severe	severe	severe	severe	severe	severe 4/4	severe 14/14	severe 3/3	mild to severe 6/6
Language	no speech	no speech	no speech	only single words	only single words	single phrases, limited comprehension	delayed speech (14/14) with only single words or simple sentences	delayed speech or no speech	delayed speech 6/6
Oral-motor	open mouth, drooling	open mouth, drooling	open mouth, drooling	open mouth, drooling	open mouth, drooling	sialorrhea 2/4	drooling 10/14, dysarthria	sialorrhea	sialorrhea 2/6
Motor examination	hyperreflexia, spastic paraplegia	hyperreflexia, spastic- ataxic gait	hyperreflexia, spastic paraplegia, contractures of ankle joints	hyperreflexia, spastic paraplegia, able to walk with ataxic-spastic gait	hyperreflexia, normal muscle tone, ataxic gait	spastic paraplegia 4/4	progressive spastic paraplegia 10/13; hypertonia 12/13	hypertonia, hyperreflexia or bilateral extensor plantar response 2/3	hypertonia, hyperreflexia in 2/6 patients, 4/6 patients without hypertonia or hyperreflexia

Seizures/Epilepsy	no	about 3 myoclonic and tonic seizures per year under VPA	generalized seizures every 2 months under VPA treatment	no	no	one single status epilepticus 2/4	5/14 history of seizures	2/3 with absences or generalized tonic-clonic seizures	1/6
Brain Imaging	n/a	n/a	n/a	Some thinning of posterior parts of the corpus calosum, otherwise normal MRI	Megacisterna magna and some thinning of posterior parts of the corpus calosum, otherwise normal MRI	MRI: cerebellar atrophy + thin corpus callosum	MRI: normal in 2 patients, one patient with reduced white matter volume and a thin corpus callosum	n/a	MRI: normal in 2 patients
Remarks	aggressive behavior	aggressive behavior	aggressive behavior	looks happy	looks happy	aggressive behavior 2/4	n/a	n/a	aggressive behavior 1/6