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# Expanding the clinical spectrum of Fowler syndrome: three siblings with survival into adulthood and systematic review of the literature

*Short running title: Fowler syndrome*

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## **Author Contributions**

All authors were involved in the conception or design of the study, analysis and interpretation of the data, and/or drafting the article or revising it critically.

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## **Competing Interest Statement**

The authors have declared no competing interests.

### ***Data Deposition and Access***

Raw data are available by contacting the authors.

Deposition of brain tissue: Biobank Antwerpen, Antwerp, Belgium; ID: BE 71030031000”

### **ABSTRACT**

Proliferative Vasculopathy and Hydranencephaly-Hydrocephaly syndrome (PVHH, OMIM 225790), also known as Fowler syndrome, is a rare autosomal recessive disorder of brain angiogenesis. PVHH has long been considered to be prenatally lethal. We evaluated the phenotypes of the first three siblings described with survival into adulthood, performed a systematic review of the Fowler syndrome literature and delineated genotype-phenotype correlations using a scoring system to rate the severity of the disease. Thirty articles were included, describing 69 individual patients. To date, including our clinical reports, 72 patients have been described with Fowler syndrome. Only 6/72 (8%) survived beyond birth. Although our three patients carry the same mutations (c.327T>A – p.Asn109Lys and c.887C>T – p.Ser296Leu) in *FLVCR2*, only two of them presented with the same cerebral features, ventriculomegaly and cerebral calcifications, as affected fetuses. The third sibling has a surprisingly milder clinical and radiological phenotype, which is suggestive for intrafamilial variability. Although no clear phenotype-genotype correlation exists, some variants appear to be associated with a less severe phenotype compatible with life. As such, it is important to consider Fowler syndrome in patients with gross ventriculomegaly, cortical malformations and/or cerebral calcifications on brain imaging.

**Keywords:** FLVCR2, Fowler syndrome, PVHH syndrome, brain angiogenesis

### **INTRODUCTION**

Proliferative vasculopathy and hydranencephaly–hydrocephaly syndrome (PVHH, OMIM#225790), also known as Fowler syndrome, is a rare autosomal recessive disorder of brain angiogenesis first described in 1972 by Fowler et al<sup>1</sup>. PVHH is characterized by hydrocephaly ranging from ventriculomegaly to severe hydranencephaly with cortical thinning and fetal akinesia, leading to pulmonary hypoplasia and multiple joint contractures.

The pathognomonic hallmark of PVHH is a cerebral proliferative glomeruloid vasculopathy (PGV), also called encephaloclastic proliferative vasculopathy (EPV), representing a severe vascular malformation of the developing central nervous system (CNS) that is associated with varying degrees

of calcification and necrosis in the white matter, basal ganglia, brainstem, cerebellum and spinal cord.

The first correlation between the clinical phenotype of Fowler syndrome and the underlying genetic defect was made by Meyer et al. in 2010<sup>2</sup>. They demonstrated disease-linkage to chromosome 14q24.3 by identifying biallelic germline mutations in *FLVCR2* gene (OMIM\*610865) in seven probands from five families. To date, 28 molecularly-confirmed patients have been described in the literature<sup>2-8</sup>. Although there is a strict correlation between the vascular and CNS lesions, the pathophysiology remains elusive<sup>1,8-12, 3</sup>. Three hypotheses have been proposed for the molecular pathogenesis of the syndrome: 1) impairment of calcium trafficking<sup>13</sup>, affecting the proliferation and motility of endothelial cells and determining altered angiogenesis, 2) heme deficiency, which affects neuronal migration<sup>3</sup> and, 3) a deficit in pericytes, interfering with normal stabilization and remodeling during CNS angiogenesis<sup>6,12</sup>. Further studies are essential to explore these hypotheses, and to explain why the disease is confined only to the CNS.

Fowler syndrome is a rare disease. Since its first description, only 69 patients have been reported on the basis of clinical, histological and/or molecular criteria<sup>1-12,14-23</sup>. The vast majority of previously described cases ended with voluntary termination of pregnancy due to the severe prenatal phenotype. Indeed, the disorder was previously considered to be prenatally lethal<sup>8</sup>, until Kvarnung et al. in 2016 reported two siblings who survived beyond the neonatal period<sup>5</sup>. Here, we describe the clinical and molecular features of three cases of Fowler syndrome, with survival up to adolescence-young adulthood, the longest survival described so far. We also perform a systematic review of published clinical and molecular data of all previously reported patients with Fowler syndrome. Thus, we further delineate the phenotype associated with *FLVCR2* mutations, during the gestational period and in particular after birth.

## **MATERIALS AND METHODS**

### **Search strategy**

This systematic review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) guidelines<sup>24</sup> in the PubMed database. We searched for all articles up to November 2019, using key terms related to Fowler syndrome and the *FLVCR2* gene: "proliferative vasculopathy and hydranencephaly-hydrocephaly syndrome" OR "fowler syndrome[title]" OR "PVHH" OR "cerebral proliferative glomeruli vasculopathy" OR "encephaloclastic proliferative vasculopathy" OR "Hydrocephalus-Hydrancephaly" OR "FLVCR2" OR "Fowler-like" OR "hydranencephalic-hydrocephalic syndrome" OR "hydrocephaly/hydranencephaly" OR "hydranencephaly-hydrocephaly" OR "Fowler-type". Only articles in English language were included in the review. Articles were excluded by title, abstract, or full text for irrelevance to the investigated

issue. References and citations of relevant articles were searched by hand. Data were independently extracted by 2 reviewers (C.D.L. and A.R.) according to the Cochrane guidelines<sup>25</sup>. The following information was extracted: article author names, patients age, gender, clinical presentation, genotype when available and history of the disease, as well as *FLVCR2* function and hypotheses on the mechanism of the disease.

### **3 new cases with *FLVCR2* bi-allelic variants and survival into adulthood**

Three new cases of Fowler syndrome were recruited and clinically characterized through the Department of Pediatric Neurology in collaboration with the Center for Human Genetics of the Department of Human Genetics at University Hospitals Leuven in Belgium and molecularly analyzed by the Laboratory of Neurogenetics and Neuroinflammation at the Imagine Institute in Paris, France. Written informed consent was provided by the parents. Whole-exome sequencing was performed on genomic DNA from the three affected individuals using SureSelect Human All Exon kit (Agilent Technologies) for targeted enrichment and Illumina HiSeq2000 for sequencing. Variants were assessed using the in silico programs SIFT (<http://sift.jcvi.org>) and Polyphen2 (<http://genetics.bwh.harvard.edu/pph2/>), and population allele frequencies obtained from the gnomAD (<https://gnomad.broadinstitute.org/>) database. Variants were confirmed by standard Sanger sequencing using the transcript NM\_017791.2 (**Supplemental Table 1**). The brain and spinal cord of case 1 was neuropathologically examined at the neuropathological institutes of the Radboud Hospital in Nijmegen (The Netherlands) and the Born-Bunge Institute in Antwerp (Belgium). The slides reside at “Biobank Antwerpen”, Antwerp, Belgium; ID: BE 71030031000. No BB20004<sup>26</sup>. The brain and spinal cord were fixed in a 4 % aqueous solution of formaldehyde. After external examination coronal slices were performed. 5 µm thick slides were made from selected blocks from representative regions and stained with hematoxylin and eosin (H&E) and H&E-Luxol Fast Blue.

### **Genotype – phenotype correlation**

In order to define the severity of manifestation of the disease in each patient, and to be able to correlate it to the type of mutation detected, we defined a score, which was applied only to the patients with a molecular diagnosis. First, we divided the clinical manifestations into three groups: major criteria, supporting criteria and minor criteria. The major criteria include the main features as well as the pathognomonic hallmarks of the syndrome, such as ventriculomegaly/hydrocephalus, fetal akinesia deformation sequence (FADS), cerebral PGV and/or calcification and spinal PGV and/or calcification. The supporting criteria include the remaining and less disease-specific neurologic manifestations, such as thin cortical parenchyma, absent or hypoplastic midline structures and cystic

posterior fossa, and the minor criteria comprehend all the other clinical features less frequently observed as macrocephaly, generalized edema/cystic hygroma, cleft palate, microretrognathism, ocular involvement and microcephaly. One point has been assigned for each feature present. Each point has then been added to the other from the same category, generating a composed score of three numbers for each patient. The cases have then been ordered from the highest score to the lowest, for each category (Supplemental Table 1).

## RESULTS

### Study selection

The study selection process is summarized in Figure 1. Abstracts for 41 unique records were screened and 8 were excluded. Thirty-three studies were reviewed, 3 additional studies were excluded. For example, one article fulfilled the inclusion criteria, but was not included in the phenotypic characterization of the syndrome because the patient had only one heterozygous variant in *FLVCR2* together with exposure to cocaine during pregnancy<sup>27</sup>. Finally, thirty studies were included in this review.

### Clinical reports

The patients are born to non-consanguineous parents originating from Belgium. A pedigree of the family is shown in Figure 2. The first child (P1) was born at term. Prenatal ultrasound revealed hydrocephaly and microcephaly at 35 weeks of gestation. Initial workup for congenital infections was negative. She developed refractory seizures from the age of 3 months. Computed tomography (CT) at the age of 5 months revealed supratentorial hydrocephaly and multiple calcifications in the cerebral cortex, thalami and brain stem (Figure 3a). She was treated with multiple anti-epileptic drugs. From an early age she had feeding problems, treated with tube feeding through gastrostomy and she suffered from recurrent respiratory tract infections with obstructive respiratory difficulties and exacerbations during episodes of infections. At the age of 12 months, she was severely disabled, with clinical examination revealing axial hypotonia, spastic quadriplegia and severe psychomotor retardation. Her head circumference remained within the normal range. She died at the age of 18 years due to a respiratory exacerbation with multi-organ failure. Macroscopy autopsy showed severe hydrocephalus (occipital > frontal), thin cortical windings with almost no white matter left and a thin corpus callosum (fig 3c-d). Microscopic examination confirmed a global loss of white matter although myelin formation was normal, and the subcortical fibers were spared (fig 3e-f). In addition, there were multiple microcalcifications, most pronounced in the white matter of the cerebral convolutions, the brainstem and the thalamus. Around the calcifications there was loose glial tissue without myelin. There were no signs of a migrational disorder in the neocortex, but in several places (insular

cortex, occipital cortex) an important neuronal loss with spongiosis and gliosis, most pronounced in cortical layers 2-3 was observed. Glomerular vascular malformations were not present. The spinal cord was remarkably spared except for Wallerian degeneration of the long tracts. The optic nerve was atrophic.

The second child (P2) was born at full term after an unremarkable pregnancy and normal prenatal ultrasounds. She had initially normal neurologic development. She presented at the age of 2 years because of toe-walking and frequent falling. Clinical examination at that time revealed hyperreflexia of the lower limbs with clonus and spastic diplegia. CT at the age of 28 months showed multiple calcifications both in a periventricular distribution and in the brain stem (Figure 3b). At the present age of 29 years, she has microcephaly (head circumference 50 cm, -4 SD) and spastic diplegia, but remains ambulatory (GMFCS 2) and lives independently. She has only mild developmental impairment, with severe dyslexia, dyspraxia and dysorthographia. She does not have epilepsy.

The third child (P3) was born at full term after an unremarkable pregnancy. CT of the brain was performed at the age of 5 months because of hypertonicity of the lower limbs, which showed supratentorial hydrocephaly, and intracerebral calcifications in the cerebral cortex, thalami and brain stem. At the age of 3 years he developed refractory epilepsy and was treated with multiple anti-epileptic drugs. Clinically, he was severely disabled with axial hypotonia, spastic quadriplegia and severe psychomotor retardation. He had feeding problems, treated with tube feeding through gastrostomy and he suffered from recurrent respiratory tract infections with obstructive respiratory difficulties with exacerbations during episodes of infections. He also suffered from severe scoliosis, which was surgically corrected at the age of 17 years. He died at 23 years of age due to status epilepticus and multi-organ failure. Autopsy was not performed.

All three siblings had a comprehensive workup for congenital infections and metabolic investigations including serum lactate, pyruvate, amino acids, and cerebrospinal fluid amino acids, which did not identify an etiology for their presentation. Genetic investigations included karyotyping, also after G-banding, T-banding and prometaphases, FISH investigation of 17p13.3 deletion for Miller-Dieker syndrome (OMIM#247200) and tests for c.3243A>G (for MELAS OMIM #540000) and c.8344A>G (for MERRF OMIM #545000) in mitochondrial DNA. These investigations were normal for all the patients. Haplotype analysis at the AGS1 locus on chromosome 3q21 for Aicardi Goutières syndrome (OMIM#225750) was also performed, showing only one haplotype shared by the three siblings. In addition, microarray analysis performed for P1 was normal.

Finally, whole exome sequencing analysis revealed that the three children were compound heterozygous for a paternally inherited (NM\_017791.2, c.327T>A, p.Asn109Lys) variant and a

maternally inherited (NM\_017791.2, c.887C>T, p.Ser296Leu) variant in *FLVCR2*. These variants were confirmed by Sanger sequencing. Both variants are absent in the general population, demonstrate a high degree of evolutionary conservation [\(see Supplement 3\)](#), and are predicted to be deleterious according to different *in silico* tools of pathogenicity prediction (PolyPhen2, SIFT and Mutation tester) [\(Supplemental Table 2\)](#).

### Phenotypic spectrum of Fowler syndrome

Overall we listed 72 individuals with Fowler syndrome: 66 fetuses and 6 patients surviving beyond birth<sup>1-12,14-23</sup>. Their phenotypes and genotypes, when available, together with assigned scores are reported in [Supplemental Table 1](#). We compared the phenotypes of our 3 patients with the 69 patients with Fowler syndrome reported in literature [\(Table 1 and Supplemental Table 1\)](#)<sup>1-12,14-23</sup>. Ventriculomegaly and/or hydrocephaly was present in almost all patients (n=68/72, 94%). Cerebral and spinal lesions manifesting with PGV and/or calcifications were present in respectively 90% (n=65/72) and 60% (n=43/72) of the cases. FADS was present in 74% (n=53/72), thinning of cortical parenchyma in 54% (n=39/72), absence or hypoplastic appearance of midline structures, ranging from agenesis of corpus callosum to severely hypoplastic brain stem cerebellum and spinal cord, manifesting in 31% (n=22/72), and cystic posterior fossa in 14% (n=10/72). Some additional features were also present in a smaller portion of patients: skin or generalized edema and cystic hygroma were present in 21% (n=15/72), cleft palate and/or microretrognathism in 10% (n=7/72) and ocular involvement in 7% (n=5/72). Head size was variable, ranging from severe microcephaly in 4% (n=3/72) to severe macrocephaly in 31% (n=22/72). Macrocephaly was more frequent in fetuses than in living patients [\(Table 1\)](#).

#### *Patients with survival beyond birth*

Strikingly, only six patients (8.3%) survived beyond birth. Therefore, data on the phenotype associated with *FLVCR2* mutations after birth are still limited. [Table 2](#) summarizes the clinical features of this subgroup of individuals. Kvarnung et al reported two siblings with a homozygous mutation in *FLVCR2* (c.1289C>T, p.Thr430Met). The first sibling died at 3 years of age, the other was 6 years old and still alive at the time of the report<sup>5</sup>. Castro-Cago et al described another family with three siblings with only a clinical diagnosis of PVHH. One of these three siblings survived until the age of 4 years<sup>16,19</sup>. The clinical spectrum of Fowler syndrome in these patients is characterized by developmental delay, presenting in 100% of the cases, severe in 5 of 6 patients and mild in one patient (P2), early onset epilepsy in 83% (n= 5/6), feeding problems in 67% (n= 4/6) and recurrent respiratory infections in 83% (n=5/6). P2 from our report has the mildest presentation and the

longest survival. All our patients survived into adulthood, two of them (P1 and P3) died respectively at the age of 18 and 23 years, but P2 is still alive at the age of 29 years. Respiratory infection has been the cause of death in 3 of 4 cases (P1, P4 [patient III:3 in Kvarnung et al.<sup>5</sup>] and P6 [first pregnancy in Castro-Cago et al.<sup>19</sup>]). Status epilepticus with multi-organ failure was the cause of death of the other patient (P3).

The surviving patients present with the same cerebral hallmarks as the fetuses, such as ventriculomegaly/hydrocephalus (83%), cerebral and spinal calcifications (100% and 33% respectively), thin cortical parenchyma (83%) and ocular involvement (50%) but, notably, these alterations manifest in a later stage of pregnancy (i.e. from the 28th week of gestational age on) or only after birth, as described in P2 and P3 of our report and P4 [patient III:3 in Kvarnung et al.<sup>5</sup>], which have history of unremarkable pregnancy. Microcephaly was only present in living patients (50%).

### **Genotype-phenotype correlation**

Sixteen different pathogenic mutations spread throughout the *FLVCR2* gene have been reported, including 11 missense variants, 2 nonsense mutations, 2 deletions and one splice site mutation. Here, we add 2 new missense mutations: c.327T>A (p.Asn109Lys) and c.887C>T (p.Ser296Leu) (Fig 2, Table 2 and Supplemental Table 1). In order to classify the patients, we applied a severity score based on their clinical and molecular characteristics (See methods and Supplemental Table 1). Overall, the variants described in less severely affected patients were not recorded in more severely affected patients. There is no relation between the type of mutation and severity of presentation. All the mutations described so far are shown in Figure 4.

### ***FLVCR2* overview and possible pathogenic mechanisms**

The mechanism by which mutations in *FLVCR2* cause Fowler syndrome is not well defined, because the function of the gene itself is still controversial<sup>28,29</sup>. Some hypotheses have been postulated.

First, Brasier et al. proposed that *FLVCR2* has a role in calcium trafficking<sup>13</sup>. Proliferation and motility of endothelial cells are calcium-dependent events. Therefore a dysregulation of calcium homeostasis could explain the disturbed angiogenesis in Fowler syndrome. The varying degrees of calcification throughout the central nervous system in patients with Fowler syndrome support this hypothesis<sup>30</sup>. However, the involvement of *FLVCR2* in calcium transport has not been demonstrated, thus this hypothesis needs further evidence to be confirmed.

Second, it is hypothesized that impaired heme import activity is involved in the pathogenesis of Fowler syndrome<sup>31</sup>. Heme is a major component of hemoproteins and plays a pivotal role in many cellular processes (e.g., cytochromes, nitric oxide synthase, hemoglobin, etc.). It is also known that

heme deficiency interferes with the normal assembly of complex IV of the mitochondrial respiratory chain<sup>32</sup>. Since young neurons that migrate to the neocortex are dependent on oxidative phosphorylation, impaired oxidative phosphorylation would subsequently lead to neurodegeneration and developmental abnormalities<sup>2,31</sup>. This hypothesis is supported by a case of Fowler syndrome with deficiency of mitochondrial complexes III and IV in a muscular biopsy<sup>16,19</sup>. Moreover, some fetuses have fetal fluid accumulation, leading to cystic hygroma and generalized edema<sup>2,3,8,12,20–22</sup>, which could be secondary to severe anemia due to heme deficiency. All our patients had anemia, which might be explained by feeding problems in two of them, but this does not explain the anemia in our still living patient. Heme import impairment could also result in iron accumulation, possibly accounting for the severe bone abnormalities observed in some fetuses<sup>31</sup>. Overall, a role for *FLVCR2* as a heme importer<sup>28,29</sup> remains to be confirmed.

Third, mutations in *FLVCR2* could cause a deficit in pericytes, cells essential for capillary stabilization and remodeling during brain angiogenesis<sup>6</sup>, that results in abnormal vessels in the fetal CNS. This assumption is based on reduced smooth muscle actin (SMA) immunostaining in the PGV within the CNS of fetuses affected by Fowler syndrome. SMA is a marker of pericytes and vascular smooth muscle in larger vessels<sup>6,12</sup>. In addition, alterations in pericytic coverage of blood vessels result in calcification<sup>33</sup>, which is also observed in brain sections of patients with PGV. Moreover, Sharma et al. found *FLVCR2* to be overexpressed in astrocytes and precursor cells of pericytes from brain of a rat model of stroke with enhanced heme content, suggesting that *FLVCR2* could be able to play a role in neoangiogenic events during stress<sup>34</sup>. It is therefore not clear whether the alterations on pericytes observed in fetuses affected by Fowler syndrome are the primary cause or an effect of the disease.

All these hypotheses imply secondary ischemic changes, that are supported by the autopsy findings in our patient 1. The white matter loss with calcifications and the calcifications in the brain stem are suggestive of previous prenatal tissue destruction as can be seen due to focal ischemia.

*FLVCR2* is expressed in a broad range of human tissues but, in particular, has a strong expression in brain and endothelial cells<sup>2,31,13</sup>, perhaps explaining why the disease is confined only to the CNS. Gene-expression studies from different brain regions of a crab-eating monkey have recently established that *FLVCR2* has effects on brain and eye function and development<sup>35</sup>. This would support a role for *FLVCR2* in the brain and eye alterations found in Fowler syndrome patients.

Further studies are needed to confirm all aforementioned hypotheses.

## **DISCUSSION**

For years, Fowler syndrome has been considered to be prenatally lethal, and in the vast majority of cases pregnancy was prematurely terminated. We present the clinical and genetic findings of the first

three patients with Fowler syndrome demonstrating survival into adulthood. Previously, only two patients have been described who survived into infancy<sup>5</sup>. One other patient was only clinically diagnosed based on a striking phenotypical overlap, but did not have a genetic confirmation<sup>16,19</sup>.

Clinical evaluation of our patients revealed a similar course for two of three patients, with developmental delay, early onset epileptic encephalopathy, feeding problems and recurrent respiratory infections, leading to death in young adulthood. Remarkably, the third patient exhibited a significantly milder presentation and less severe cognitive impairment.

Of the seventy-two patients with Fowler syndrome catalogued here, only six survived after birth (8%). Surviving patients present with the same cerebral hallmarks as fetuses, such as ventriculomegaly, cerebral and spinal calcifications. Interestingly, intracranial calcifications were present in all of them. Moreover, almost all patients had thin cortical parenchyma<sup>15</sup> and two of the six patients have been described to have lissencephaly<sup>5</sup>, already prenatally detected. One of our patients had a postmortem neuropathological examination, which revealed multiple microcalcifications, similar to PGV, although no glomeruloid vessels were seen. None of the surviving patients presented with features secondary to fetal akinesia, nor cleft palate or microretrognathism. Eye involvement seems more frequent: Patient 1 had blindness due to optic atrophy, and Patients 4 and 5 presented with abnormal papillae and central visual impairment. Conversely, only two fetal cases have been described with only mild involvement of the optic tract, but these alterations might be underreported in fetuses.

Since the description of mutations in *FLVCR2* as a cause of Fowler syndrome, 16 different pathogenic mutations spread throughout the gene have been reported (Table 2 and figure 4). Although there is yet no clear correlation between genotype and disease severity, we suggest that some mutations may cause a less severe form of Fowler syndrome which can be compatible with life. Kvarnung et al<sup>5</sup> already described that the mutation p.Thr430Met, present in the homozygous state in two patients surviving into infancy, may cause a less severe phenotype. This mutation was also present in heterozygous state in two fetuses with a less severe phenotype<sup>6</sup>. Since the pregnancies of these fetal cases were voluntarily terminated, we do not have information on the possible survival and natural history of these fetuses. One of the two variants observed in our patients, p.Asn109Lys, seems to have only a moderate effect on the physicochemical characteristics of the amino acid position, and may have a less deleterious effect on protein function. The long survival of our patients after birth is a strong argument suggesting a milder effect, but functional studies are needed to confirm this hypothesis. Some variants seem to have a broad phenotypic heterogeneity. For example, homozygous p.Thr430Arg variants have been described in two fetuses presenting with a very severe phenotype involving brain and spinal cord, but also in three fetuses with less severe neurologic involvement, with sparing of the spinal cord and absence of FADS<sup>2</sup>. Notably, this variant is located at

the same amino acid position as the variants in two patients surviving into childhood (p.Thr430Arg vs p.Thr430Met)<sup>5</sup>, further supporting the milder effect of the latter on the protein structure and/or function. In addition, also our three patients show a broad intrafamilial phenotypic variability, suggesting that other factors influence the clinical manifestations of the disease.

## CONCLUSION

We present three cases of Fowler syndrome with survival into young adulthood, the longest described so far, harboring two novel mutations in the *FLVCR2* gene. These cases enrich the still poor casuistry of patients with survival beyond infancy and help to better delineate their clinical characteristics. We also provide an overview of all previously described Fowler syndrome patients and make a systematic review of the clinical presentation and all the mutations described so far. Although as yet there is no clear genotype-phenotype correlation, some variants seem to be associated with a less severe phenotype which can be compatible with life.

The cases here presented further illustrate how the increasingly common use of next generation sequencing in routine molecular diagnostics is leading to the expansion of the phenotypic spectrum of rare diseases. In this context, it is important to consider Fowler syndrome in patients with gross ventriculomegaly, cortical malformations and/or cerebral calcifications on brain imaging.

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## LEGENDS:

### FIGURES:

**Figure 1:** The PRISMA flow diagram, showing the overview of the search process.

**Figure 2:** Pedigree of the family of the present cases. (†) age at death, (‡) current age.

**Figure 3:** Computed tomography (CT) brain imaging of patients 1 and 2. Macroscopic examination of the brain and microphotographs of patient 1. **a)** CT brain of patient 1 at the age of 22 months showing severe ventriculomegaly with only a thin edging of the cerebral cortex. The brain stem is atrophic and has calcifications. Calcifications are also seen periventricular. **b)** CT brain of patient 2 at the age of 30 months showing intracranial calcifications. Normal aspect of the ventricles, cortical gyri and brain stem.

Macroscopic examination of the brain of patient 1. Quite well retained gyral pattern on side view (**c**). The cortex is somewhat falling inside due to hydrocephalus evident at artificial tear (**asterisk, c**). Important hydrocephalus and atrophy of the corpus callosum with severe depletion of the white matter on frontal slices at the level of the head of the caudate nucleus (**d**), the thalamus and the occipital lobe.

Microphotographs at the level of the mesencephalon, with slight dilatation of the aqueduct, atrophic pedunculi cerebri and calcifications (arrows) (HE-Luxol fast Blue); **e, f** occipital cortex and white matter with calcifications (arrows) surrounded by degenerated lightly stained areas of white matter (HE-Luxol fast blue). **e:** detail of black box in **f** (HE).

**Figure 4.** Localization of all the described mutations on a secondary structure prediction of the *FLVCR2* transporter. In red, the amino acid changes identified in surviving patients; in blue, the amino acid changes identified in fetuses.

### TABLES:

**Table 1. Frequency of clinical manifestations of Fowler syndrome in fetal cases and patients that survived beyond birth.** (Abs) absolute number, (Freq) frequencies, (PGV) proliferative glomeruloid vasculopathy, (pt) patients.

**Table 2. Clinical characteristics of the cohort of individuals with clinical and/or molecular diagnosis of Fowler syndrome with survival after birth.** (EE) epileptic encephalopathy, (F) female, (GMFCS) gross motor function classification system, (MRI) magnetic resonance imaging, (M) male, (N/A) not available, (w) weeks of gestational age, (†) age at death.

SUPPLEMENTAL DATA:

**Supplemental Table 1. Summary of the clinical and molecular data of all reported cases of Fowler syndrome.** (ACM) Arnold Chiari Malformation, (ASD) atrial septal defect, (CCA) corpus callosum agenesis, (DWM) Dandy-Walker malformation, (F) female, (hom) homozygous, (IUD) intrauterine death, (IUGR) intrauterine growth retardation, (M) male, (NP) not performed, (PGV) proliferative glomeruloid vasculopathy, (VIP) voluntary interruption of pregnancy, (wga) weeks of gestational age, (Y) years of age, (†) age at death. Patients that survived beyond birth are highlighted in blue.

**Supplemental Table 2. Variant table.** (Chr) Chromosome, (HGVS) Human Genome Variation Society.

**Supplement 3.** Evolutionary conservation. Multiple sequence alignment of *FLVCR2* across species<sup>36</sup>.