Loss-of-function mutations in \textit{UDP-Glucose 6-Dehydrogenase} cause recessive developmental epileptic encephalopathy

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Developmental epileptic encephalopathies are devastating disorders characterized by intractable epileptic seizures and developmental delay. Here, we report an allelic series of germline recessive mutations in \textit{UGDH} in 36 cases from 25 families presenting with epileptic encephalopathy with developmental delay and hypotonia. \textit{UGDH} encodes an oxidoreductase that converts UDP-glucose to UDP-glucuronic acid, a key component of specific proteoglycans and glycolipids. Consistent with being loss-of-function alleles, we show using patients’ primary fibroblasts and biochemical assays, that these mutations either impair UGDH stability, oligomerization, or enzymatic activity. In vitro, patient-derived cerebral organoids are smaller with a reduced number of proliferating neuronal progenitors while mutant \textit{ugdh} zebrafish do not phenocopy the human disease. Our study defines UGDH as a key player for the production of extracellular matrix components that are essential for human brain development. Based on the incidence of variants observed, \textit{UGDH} mutations are likely to be a frequent cause of recessive epileptic encephalopathy.

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evelopmental epileptic encephalopathies are a clinically and genetically heterogeneous group of devastating disorders characterized by severe epileptic seizures that are accompanied by developmental delay or regression. In several cases, a genetic etiology has been identified. Germline mutations in these genes lead to different pathophysiological defects, including ion channel dysfunction, synaptic impairment, transporter defects and metabolic abnormalities, such as deficiencies in glycosylation pathways. However, the genetic cause of many epileptic encephalopathies remains unknown.

Defects of glycosylation are causing more than 100 rare human genetic disorders, most of these affecting the central and/or peripheral nervous systems. Patients typically show developmental delay or intellectual disability, seizures, neuropathy, and metabolic abnormalities in multiple organ systems. Adding the correct sugar chains (glycans) to proteins and lipids significantly impacts their function. UGDH (MIM603370) codes for an enzyme that converts UDP-glucose (UDP-Glc) to UDP-glucuronic acid (UDP-GlcA) through the concomitant reduction of NAD+ into NADH. UDP-GlcA is not only needed for detoxification via glucuronidation, but is also an obligate precursor for the synthesis of glycosaminoglycans (GAGs), and therefore an important component of proteoglycans of the extracellular matrix.

In this study, we establish UGDH as a gene responsible for autosomal recessive developmental epileptic encephalopathy in humans. We catalog a series of 30 patients from 25 families with biallelic germline UGDH variants. Using patients’ primary fibroblasts and biochemical assays, we demonstrate that these are loss-of-function alleles. While mutant ugdh zebrafish did not phenocopy the disease, we bring evidence that patient-derived cerebral organoids, which were smaller due to a reduced number of proliferating neuronal progenitors, can serve as an alternative disease-in-a-dish model for in vitro functional studies.

Results

Biallelic mutations in UGDH cause developmental epileptic encephalopathy. To identify the genetic cause of a developmental epileptic encephalopathy in a consanguineous Palestinian family with three affected siblings (Fig. 1a, F1), we performed exome sequencing on two affected siblings. No mutations in genes known to be associated with neurological disorders (either recessive or dominant) were found. As the consanguineous background and the pedigree suggested autosomal recessive inheritance, we focused on homozygous or compound heterozygous variants shared by the affected siblings. A rare homozygous variant c.131C>T in UDP-Glucose 6-Dehydrogenase (UGDH), which changes alanine into valine at position 44 of the UGDH protein, was the only segregating candidate variant. The UGDH p.A44V missense affects a highly conserved residue (Suppl. Fig. 1b and phyloP 100-way8 score 9.43), is extremely rare in public databases (not present in EVS65008, MAF of 0.0017% in ExAC10) and is a good candidate according to in silico prediction scores (CADD score11 of 33) (Suppl. Table 1). We then (i) screened the GENESIS12 database for additional patients with recessive UGDH variants, (ii) contacted the EuroEPINOMICS RES Consortium, and (iii) searched with the help of GeneMatcher13 for additional families with germline UGDH mutations. We uncovered 27 additional patients from 24 families carrying either compound heterozygous or homozygous UGDH variants (Fig. 1a and Suppl. Fig. 1a). All variants were absent or had an extremely low frequency (<0.01%) in the public databases ExAC/gnomAD14 and EVS6500 (Suppl. Table 2). Nineteen of the 20 identified missense variants are in highly conserved residues (Suppl. Fig. 1b and phyloP 100-way between 3.81 and 9.43). The A44V variant, identified in the Palestinian index family, was also found in two additional families from Puerto Rico (F11) and from Spain (F13) indicative of independent but recurrent mutation in this residue. In ExAC the A44V variant is observed in African (MAF 0.0096%) and European (Non-Finish) populations (MAF 0.0015%), however, it is not present in the Greater Middle East Variome.

All 30 patients carrying biallelic mutations in UGDH presented with a common core phenotype consisting of marked developmental delay, epilepsy, mild dysmorphism, and motor disorder with axial hypotonia (Table 1 and Suppl. Data 1). Dysmorphic facial features such as short and flattened philtrum, outward protruding earlobes, ptosis, or blepharophimosis were mild but frequently present (Fig. 1b and Suppl. Data 1). Most patients have severe epilepsy ranging from neonatal onset developmental epileptic encephalopathy to infantile developmental epileptic encephalopathy (27 patients, 90%), of which 16 (53%) had infantile spasms (Table 1). Three patients have developmental encephalopathy, of which two had seizures in the setting of fever (F5-II:1 and F5-II:2). Only these two patients were seizure-free on sodium valproate. All other patients, except for one patient who seemed to benefit from ketogenic diet, did not respond to antiepileptic treatment. All patients had a severe motor disorder with axial hypotonia, while some patients presented with limb spasticity (43%), dystonia (17%), ataxia, chorea, and tremor, which were often present prior to onset of seizures. Twenty-four out of the 30 (80%) were noted to have swallowing difficulties and gastrostomy tubes were required for feeding in 12 infants. None but two patients (F5-II:1 and II:2) achieved sitting ability. A moderate to severe intellectual disability was observed in all patients. Three patients were deceased between 4 months and 6 years of age (Table 1 and Suppl. Data 1). Electroencephalography (EEG) was markedly abnormal with a burst suppression pattern in the neonatal period, hypsarrhythmia in affected children with infantile spasms, and focal and/or generalized spike-wave complexes in childhood (Suppl. Data 1). MRI revealed a spectrum of abnormalities with delayed myelination and enlarged ventricles probably due to cerebral and cerebellar atrophy in more severely affected patients without any signs of maldevelopment (Fig. 1c and Suppl. Data 1).

UGDH mutations behave as hypomorphic alleles. The UGDH oxidoreductase consists of three distinct domains: the NAD-binding (N-terminal) and UDP-binding (C-terminal) domains, and an internal domain that bridges the two termini together. The UGDH enzyme assembles into a disc-shaped double layer composed of a trimer of dimers (Suppl. Fig. 2a, b). This hexameric structure is a prerequisite for proper UGDH enzymatic function. The 23 germline mutations presented in this study are distributed throughout the UGDH gene and its encoded protein (Fig. 2a). One of the variants in Family 12 mutates the first nucleotide of exon 8 (c.907 G > A; p.Val303Ile, Fig. 2a, b), which is predicted to affect the splice donor site16. Three different nonsense mutations were found in a compound heterozygous state with a missense mutation (Fig. 2a and Suppl. Table 2). All identified missense mutations are anticipated to be destabilizing according to DUET17 (Suppl. Table 2, ΔΔG). The missense mutations in residues Y14, I42 and A44, which are close to the NAD-binding site (Fig. 2c and Suppl. Fig. 2c) are expected to impair NAD+ reduction. Alteration of residues in the central domain such as I255, G271, M306, and R317 are expected to affect homo-dimerization18 (Fig. 2b and Suppl. Fig. 2d). The I116 residue (located in the NAD-binding domain), as well as the R393 and A410 residues (UDP-Glc binding domain) sit at the dimer-dimer interface19 (Fig. 2d), suggesting that these variants may...
To better understand the effect of the mutations on UGDH, we then derived and biobanked primary dermal fibroblasts from patients F3-II:1 (R393W/A410S), F4-II:1 (Y14C/S72P), F5-II:1 (A82T/A82T) and F6-II:1 (R65*/Y367C), and a non-affected parent F5-I:1 (WT/A82T). Endogenous UGDH messenger RNA (mRNA) levels were not significantly different in patients’ primary cells as compared to control fibroblasts (Fig. 3a, top panels). In contrast, we observed significant changes in endogenous UGDH protein levels for three of the four alleles studied. Fibroblasts with compound heterozygous R393W/A410S mutations displayed comparable UGDH levels relative to wild-type (WT) cells, while patients’ cells with R65*/Y367C,
Y14C/S72P, or homozygous A82T mutations showed dramatically reduced endogenous UGDH levels (Fig. 3a, bottom panels). In contrast to the three nonsense mutations and the missense mutation potentially affecting splicing, which are likely to cause nonsense-mediated decay of the endogenous UGDH transcript, the nonsense mutations are most likely impacting the stability of the enzyme and/or its oxidoreductase activity. Consistently, we observed a significant decrease in the UGDH-catalyzed reduction of NAD+ to NADH in patients’ primary fibroblasts (R393W/A410S, Y14C/S72P, or homozygous A82T mutations) while the non-affected parent’s cells heterozygous for the A82T mutation showed intermediate level of NAD+ reduction (Fig. 3b, left panels). Patient’s cells with the homozygous A82T mutation also exhibited a reduction in the synthesis of hyaluronic acid (HA), which requires UDP-glucuronate, a product of UGDH enzymatic activity (Fig. 3b, right panel). When produced in bacteria, mutant UGDH had altered stability, kinetic, and biochemical properties as compared to WT-UGDH. Compared to the wild-type enzyme, mutant A44V and A82T UGDH (mutations found at the homozygous state in the patients from Families 1 and 5, respectively) were more susceptible to partial proteolysis by trypsin (Fig. 3c). The stability of UGDH44V could be partially rescued upon incubation with substrate, product, or cofactor, while the UGDH82T remained strongly sensitive to proteolysis regardless of the presence of any cofactor or substrate (Fig. 3c). A thermal stability study showed that the melting temperature of UGDH44V was significantly reduced relative to WT and could only partially be rescued upon addition of substrate, product, reduced or oxidized cofactor, or any combination thereof (Fig. 3d). Notably, UGDH82T was so intrinsically unstable that a melting temperature was unable to be ascertained. By gel filtration chromatography, we investigated the effect of the A44V and A82T mutations on UGDH oligomerization. When compared to UGDHWT, UGDH132 (an obligate hexamer15), and UGDH325D (an obligate dimer15), we observed that UGDH44V and UGDH82T proteins were mainly eluted as dimer and monomer species, respectively, with virtually no stable hexameric population (Fig. 3e). This suggests that the A44V and A82T mutations may affect UGDH function by altering its capacity to form active hexamers. Finally, using equal amounts of recombinant enzyme, we determined that UGDH44V and UGDH82T were respectively 75% and 50% less efficient at reducing NAD+ to NADH as compared to UGDHWT (Fig. 3f). Similarly, comparison of the steady state Michaelis–Menten kinetic constants (summarized in Table 2) showed that UGDH44V Vₘₐₓ was only ~50% of the value of UGDHWT for both cofactor and substrate. In contrast, Kₘₐₓ was not significantly different from UGDHWT for either cofactor or substrate, revealing that the mutation results in a reduced ability of the enzyme to catalyze the reaction, while still being able to associate with NAD+ and UDP-Glc. Taken together, our biochemical findings indicate that these missense mutations mainly impact the enzymatic function of UGDH by altering its quaternary structure and/or directly impairing its oxidoreductive activity.

Patient-derived cerebral organoids partially phenocopy human disease. Our attempts to model this developmental epileptic encephalopathy using the existing zebrafish hypomorphic loss-of-function ugdh (c.992 T > A; p.I331D) allele known as jekyll m1520–22 were unsuccessful (Suppl. Fig. 3). The behavioral activity of homozygous jekyll mutant larvae were recorded in presence or absence of the seizure-inducing drug pentylenetetrazol (PTZ)23. By quantitative PCR (qPCR), c-fos expression, which marks neural activity23,24, similarly increased in a dose-dependent manner upon PTZ treatment in all larvae regardless of genotypes (Suppl. Fig. 3a–d). Homozygous mutant larvae did not show signs of increased c-fos expression at basal state, suggesting that fish depleted of Ugdh activity do not exhibit spontaneous seizure and are equally responsive to PTZ treatment. As noted by reviewers, ugdh mutant fish do not have fully-inflated swim bladders, which may contribute to their reduced locomotor activity and demise before 14-dpf (Suppl. Fig. 3e). These in vivo experiments suggest that zygotic ugdh depletion in zebrafish does not satisfactorily model the human disease.

UGDH has been extensively studied in vertebrate model organisms where its complete knockout causes embryonic lethality around gastrulation25,26. To address its role in the context of central nervous system (CNS) development in humans, we attempted instead to model this disease in vitro by developing cerebral organoids27,28 from several patients with compound heterozygous R65*/Y367C, Y14C/S72P or homozygous A82T mutations, and from a non-affected parent (WT/A82T). After 10 weeks of differentiation, the volume of cerebral organoids from patients with biallelic UGDH mutations was on average 50% smaller and showed rougher edges than that of WT or carrier WT/A82T cerebral organoids (Fig. 4a, b and Suppl. Fig. 4a). Quantitative reverse transcription PCR (RT-qPCR) analysis revealed decreased levels of the early and intermediate neuronal progenitors markers PAX6 and TBR2, respectively, while the levels of neuronal marker TUJ1 were unchanged (Fig. 4c and
Table 1 Simplified clinical findings and course of disease in patients with UGDH mutations from families F1 to F10.

| Family | Patient | Gender, age at last follow-up | Main phenotype | Age at seizure onset | Epilepsy, seizure types | Drug sensitivity | Motor development at last follow-up | Intellectual disability | Speech | Swallowing/feeding difficulties |
|--------|---------|------------------------------|----------------|---------------------|------------------------|------------------|-----------------------------------|----------------------|--------|---------------------------------|
| F1     | II:4    | M, 13 yrs IDEE              | 9 mths         | Epileptic spasms    | Resistant              | Absence          | Severe                            | Absence              | Yes    | Yes                             |
| F1     | II:6    | M, 5 yrs IDEE              | 15 mths        | Epileptic spasms    | Resistant              | Absence          | Severe                            | Absence              | Yes    | Yes                             |
| F1     | II:7    | M, 4 yrs IDEE              | 6 mths         | Epileptic spasms    | Resistant              | Absence          | Severe                            | Absence              | Yes    | Yes                             |
| F2     | II:1    | F, 23 mths IDEE            | 5 mths         | Epileptic spasms    | Resistant              | Absence          | Severe                            | Absence              | ND     |                                 |
| F3     | II:1    | M, 6 yrs* IDEE             | 8 wks          | Epilepsy with focal seizures | Absent               | Absence          | Severe                            | Absence              | Yes    | Yes                             |
| F3     | II:2    | M, 2 yrs IDEE              | 4 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Absence                            | Severe               | Moderate | Yes                             |
| F4     | II:1    | M, 5 yrs IDEE              | 3 yrs          | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F4     | II:1    | F, 14 years ID, MD        | 3 yrs          | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F5     | II:2    | F, 6 yrs ID, MO            | 3 yrs          | Infrequent seizures in the setting of fever | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Moderate             | Yes    | Yes                             |
| F6     | II:1    | M, 4 mths* IDEE            | 3 yrs          | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F7     | II:1    | M, 7 yrs IDEE              | 3 yrs          | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F7     | II:1    | M, 7 yrs IDEE              | 3 yrs          | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F8     | II:1    | F, 25 mths IDEE            | 12 mths        | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F9     | II:1    | F, 4 yrs IDEE              | 8 wks          | Epileptic spasms    | Myoclonic spasms, tonic seizures, tonic seizures, tonic seizures, tonic seizures | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F10    | II:1    | M, 5 mths* IDEE            | 4 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F11    | II:1    | M, 16 yrs IDEE             | 3 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Absence | Yes                             |
| F11    | II:1    | M, 16 yrs IDEE             | 3 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F12    | II:1    | M, 4 mths* IDEE            | 3 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F13    | II:1    | M, 16 yrs IDEE             | 3 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F14    | II:1    | F, 8 yrs IDEE              | 4 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F15    | II:1    | F, 8 yrs IDEE              | 4 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F16    | II:1    | F, 11 yrs IDEE             | 12 mths        | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes    | Yes                             |
| F17    | II:2    | F, 5 yrs IDE, MD          | 20 mths        | No epilepsy         | Daily generalized tonic and myoclonic seizures | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | No     | Yes                             |
| F18    | II:1    | F, 8 yrs IDEE              | 20 mths        | No epilepsy         | Daily generalized tonic and myoclonic seizures | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | No     | Yes                             |
| F19    | II:1    | F, 5 yrs IDEE              | 30 mths        | Recurrent generalized tonic convulsions | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | No     | Yes                             |
| F19    | II:2    | F, 3 yrs IDEE              | 18 mths        | Daily generalized tonic seizures with eye fluttering | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | No     | Yes                             |
| F20    | II:2    | M, 6 yrs IDEE              | 3 yrs          | Epileptic spasms, myoclonic seizure, tonic seizure, myoclonic seizure | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes with difficulty | Yes |
| F21    | II:1    | F, 4 yrs IDEE              | 18 mths        | Epileptic spasms, myoclonic seizure, tonic seizures, myoclonic seizure | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes with difficulty | Yes |
| F20    | II:2    | M, 6 yrs IDEE              | 3 yrs          | Epileptic spasms, myoclonic seizure, tonic seizures, myoclonic seizure | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes with difficulty | Yes |
| F21    | II:2    | F, 4 yrs IDEE              | 18 mths        | Epileptic spasms, myoclonic seizure, tonic seizures, myoclonic seizure | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes with difficulty | Yes |
| F22    | II:5    | F, 9 yrs IDEE              | 20 mths        | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes     | Yes                             |
| F23    | II:5    | F, 7 yrs IDEE              | 5 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Delay   | ND                             |
| F24    | II:1    | M, 7 yrs IDEE              | 6 mths         | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes     | Yes                             |
| F25    | II:4    | M, 8 yrs IDEE              | 11 mths        | Epileptic spasms    | Seizures in the setting of fever | Absent               | Seizures sitting at 12 mths, walking at 3 yrs | Severe               | Yes     | Yes                             |

M male, F female, IDEE infantile developmental epileptic encephalopathy, NDEE neonatal onset developmental epileptic encephalopathy, MD motor disorder, n/a not applicable, ND non-determined, ID intellectual disability, g-tube gastrostomy tube, NJ nasojugal, wks weeks, mths months, yrs years.

*Age at death.

Multiple congenital anomalies in F16-II:1: prenatal polyhydramnios; multiple ocular anomalies (bilateral cataracts, multiple bilateral lens colobomas, bilateral microphthalmia, hypoplastic iris, iris and bicornular vascularization, bilateral anterior segment dysgenesis, posterior synchiae bilaterally secondary to neovascularization); megacystis; genoplastic bladder; moderate hiatal hernia; camptodactyly of 3rd and 4th fingers and overriding 2nd and 4th digits on the right hand; long tapered fingers; skeletal survey showed overlapping of the parietal bones and mild elongation of the 2nd through 5th fingers.
Fig. 2 Mutations in UGDH enzyme possibly affect critical amino-acids. a UGDH genomic and protein domain structures. Type and positions of 22 germline UGDH mutations. 5’ and 3’ UTRs are shown in dark gray. NAD-binding (blue), central (pink), and UDP-binding (orange) domains are highlighted. Homozygous mutations are shown in bold. Compound heterozygous mutations that are in trans are linked by a line below the UGDH domain structure. b–d Three close-up views ribbon diagrams of the UGDH protein bound to UDP-Glc and NADH. b Interface between the central domains of subunits A and B. c NAD-binding site in NAD-binding domain of subunit A. Distances between NADH and mutated residues in patients are measured in Angström (Å). d Interface between the subunit A NAD-binding domain with the subunit C UDP-Glc-binding domain. In all the structures, residues carrying missense mutations in the patients are highlighted as 3D backbone. Residues Q110 and T325 known to interact together for dimer formation15 and residue V132, which is important for hexamerization15 are highlighted in black backbone. In all the structures, NAD-binding (blue), central (light/dark pink), and UDP-binding (orange) domains are shown. UDP-Glc (dark red) and NADH (midnight blue) are represented as colored carbon backbones. Adapted from PDB code 2Q3E6 using the Swiss-Pdb Viewer software67. For gels and graphs source data, please refer to the source data files 1 and 2.
Suppl. Fig. 4b). Immunofluorescence revealed similar amounts of peripheral neurons marked by TUJ1, and astrocytes marked by GFAP while ventricular zones marked by SOX2-positive neuronal progenitors were appreciably less proliferative. This was evidenced by reduced PCNA staining in mutant cerebral organoids relative to WT and WT/A82T sections (Fig. 4d and Suppl. Fig. 4c). These results argue that reduced UGDH activity is associated with impaired neuronal development in vitro, causing atrophy of patient-derived cerebral organoids. Even though our cerebral organoid data is congruent with our patients’ phenotype and biochemistry data, replicative studies with additional WT and complete UGDH knockout lines are warranted in light of the known variability in induced pluripotent stem cells (iPSCs) response to differentiation protocols. To understand whether mutations in UGDH directly affect neuronal function, we also differentiated WT, non-affected parent (WT/A82T), and patient (Y14C/S72P) iPSCs into neuro-precursor cells (NPCs), which were subsequently matured into neurons over a period of 21 days. Using a multi-electrode array (MEA) system, and in contrast to neurons mutant for CAMK2A29, no significant differences between controls and mutant UGDH neurons were recorded for either the total number of spontaneous spikes or the mean firing rate (Suppl. Fig. 4d). Altogether, these in vitro experiments suggest that while UGDH
**Discussion**

In this study, we described disease-causing mutations in UGDH in humans. These 23 coding variants represent an allelic series of germline mutations, which when inherited recessively are responsible for epileptic encephalopathy with variable degrees of developmental delay. We propose to name this novel Mendelian syndrome, Cantor-Strickler syndrome, in honor of the late Cantor and Strickler families. The genetic, biochemical, cellular and developmental findings reveal that these UGDH germline mutations behave as loss-of-function alleles. This was confirmed in vitro using patient-derived cerebral organoids, which showed marked underdevelopment. In zebra fish, we found that homozygous Ugdh mutant larvae did not show signs of increased seizures at baseline or after PTZ treatment. The brain-specific UGDH phenotype in humans may come as a surprise since in Drosophila, zebrafish, and mouse, complete knockout of Ugdh cause early and lethal gastrulation defects by hindering FGF signaling. One potential explanation for this incongruity is that other proteoglycans not reliant on UGDH activity for the synthesis of UDP-GlcA or UDP-Xylose (UDP-Xyl) may be solubilized by heparan sulfate modification and degradation. For example, EXT1 and CHS1 mutations, which affect heparan sulfate and chondroitin sulfate synthesis, respectively, cause developmental delay and intellectual disabilities (MendelianSyndrome.org). Defects in heparan sulfate modification caused by NDST1 mutations are responsible for intellectual disability associated with epilepsy. Moreover, mucopolysaccharidoses, diseases caused by defects in mucopolysaccharide metabolism, may help to enhance this gene enhancer effect. If a similar route exists in humans, supplementation of glucuronate may help to enhance this alternative pathway and increase levels of UDP-GlcA levels and its essential metabolites. To this day, however, the existence of human homologs of glucuronokinase and UDP-glucuronic acid pyrophosphorylase remains to be proven.

Conservative estimates of disease frequency resulting from germline UGDH mutations projects a prevalence of 1:14,000,000 in the general population. Considering that developmental epileptic encephalopathies are the most common cause of infantile spasms, and that about 1% of all cases of infantile spasms are due to a genetic cause, we estimate that the prevalence of a UGDH-related disease in the general population is about 1:1,000,000. This is a very rough estimate, but it is consistent with the observed disease frequency of 1:2,000,000 (Suppl. Note 1). Considering that developmental epileptic encephalopathy may correlate with the amount of residual UGDH activity, the extent of which may be sufficient to allow gastrulation to take place during early human embryonic stages but may be limiting for neuronal development thereafter.

As UDP-GlcA is the major product of the UGDH enzyme, it is possible that reduced levels of UDP-GlcA may trigger a cascade of secondary pathogenic events resulting in neurodevelopmental delay and encephalopathy. In support of this, is the recent demonstration that a homozygous loss-of-function mutation in the upstream enzyme UGP2 is also responsible for a severe form of developmental epileptic encephalopathy in humans. UDP-GlcA is not only needed for detoxification via glucuronidation, but is also a key component of glycosaminoglycans (GAGs). UGDH deficiency might parallel other neurological diseases with defects in GAG synthesis, modification, and degradation. For example, EXT1 and CHS1 mutations, which affect heparan sulfate and chondroitin sulfate synthesis, respectively, cause developmental delay and intellectual disabilities (MendelianSyndrome.org). In addition, proteoglycans containing GlcA derived from UDP-GlcA are major components of the extracellular matrix (ECM) and key players in neuronal and extracellular matrices (ECMs). They are particularly important in areas important for neuronal migration. In human, various psychiatric and intellectual disorders are caused by mutations in genes involved in ECM homeostasis and may be driven by neuronal migration defects. The central role of UDP-GlcA may open a window for early therapeutic interventions. In plants and lower animals, including zebrafish, UDP-GlcA can be synthesized by two alternative pathways. Apart from UGDH, UDP-GlcA can be generated via the myo-inositol oxygenation pathway from glucuronic acid by glucuronokinase and UDP-glucuronic acid pyrophosphorylase. If a similar route exists in humans, supplementation of glucuronate may help to enhance this alternative pathway and increase levels of UDP-GlcA levels and its essential metabolites. To this day, however, the existence of human homologs of glucuronokinase and UDP-glucuronic acid pyrophosphorylase remains to be proven.
Methods

Ethical approval. Written informed consent was obtained from the parents of the underage patients for diagnostic procedures and next-generation sequencing, as well as for the publication of identifying facial images in Fig. 1b. The study has been approved by the local Institutional Review Board of the Medical Faculty of the University of Tübingen, Germany (vote 180/2010BO1).

Exome sequencing. To unravel the molecular cause of the disease exome sequencing were performed at different genetic institutes using next-generation sequencing techniques according to local standard protocols. Variants were confirmed via Sanger sequencing using standard methods and chemicals (primer sequences are available on request).

Family 1: Exome sequencing for two affected siblings was performed on a HiSeq2500 System (Illumina, CA) after enrichment with SureSelectXT Human All Exon V5 (Agilent, Santa Clara CA). FASTQ files were imported into GENESIS (http://thegenesisprojectfoundation.org/) for further analysis using a pipeline build on BWA1, Picard, and FreeBayes. Variants were filtered for changes that segregated in an autosomal recessive fashion and passed the following filter criteria: (i) frequency in public databases (ExAC10 minor allele frequency (MAF) <0.1%), (ii) present in <5 families within GENESIS (~4,300 exomes), (iii) conserved (PhyloP 100-way score >0.75), (iv)
CADD score >12, and (v) sufficient quality scores (Genotype Quality >75). In addition, variants had to be present in exomes from both siblings. This resulted in a list of seven variants (Suppl. Table 1), out of which only the homoyzous missense variant c.131 C > T in the UGDH gene segregated with the third affected sibling.

Families 2, 9, 10: Using genomic DNA from the proband and parents, the exonic regions and flanking splice junctions of the genome were captured using the Agilent SureSelect Human All Exon V4 (50 Mb) or the Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA). Massively parallel (NextGen) sequencing was done on an Illumina system with 100 bp or greater paired-end reads. Reads were aligned to the human genome build GRCh37/hg19, and analyzed for sequence variants using a custom-developed analysis tool. Additional sequencing technology and variant interpretation protocol has been previously described42. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/). After variant stratification based on population frequencies within an internal database and ExAC10, inheritance, in silico predictors such as PolyPhen, Mutation Taster and CADD, GeneDX reported only the UGDH variants to be the best potentially pathogenic candidates and connected to this project via GeneMatcher entries.

Family 3: Samples of the oldest sibling and both parents were sequenced in context of the EUROCORES project EuroEPINOMICS-RES, for which the technical details have been reported before43. Briefly, the trio underwent exome sequencing at the Wellcome Trust Sanger Institute (Hinxton/Cambridge, UK). Capturing of the exome was performed using the SureSelect Human All Exon 50 Mb exome kit (Agilent). The enriched exome libraries were then sequenced on a HiSeq2000 platform (Illumina) as 75 bp paired-end reads. BWA was used to align the sequenced reads to the reference genome (hg19). De novo analysis of these data did not reveal any variants. As the younger sibling later developed a similar disorder, exome sequencing was also performed locally on the second sibling: for library preparation, genomic DNA was sheared to the average size 150 bp (Covaris) and sequence reads were aligned to the human genome (Hg19) and variant calling were performed with the CLC Genomics Workbench. Subsequent annotation and filtering were executed with GenomeAnalyzer. Homeomonecombosequencingresults of the trio and the second sibling were merged and reannotated and the family was reanalyzed as a quartet. Variants were filtered based on following quality parameters: coverage >7, quality >50 and not located in homoplymeric >8 or tandem repeats. Only variants present with a frequency <0.5% in control population databases ExAC48 and Exome Variant Server, seen <3 times in the local exome sequencing database, and with predicted impact on the encoded protein (missense, nonsense, frameshift, deletions, insertions and (essential) splice site) were retained for further analysis. Remaining variants were filtered under an autosomal recessive (hoemozygous or compound heterozygous) and x-linked hypothesis. The trio and the second sibling were selected for sequencing. The x-linked UGDH gene was found to segregate with the disease.

Family 4: Parent-proband trio exomes were prepared using the SureSelect Target Enrichment System (Agilent, Santa Clara CA) and sequenced on a HiSeq2000 system (Illumina, CA). Data processing, bioinformatics pipeline (for alignment, variant calling, annotation, and genetic model filtering), and analyses were previously described45. The compound heterozygous rare missense alterations in the UGDH gene, c.214T > C and c.41A > G, were interpreted as the only candidate variants.

Family 5: The exome library was prepared on an Ion OneTouch System and sequenced on an Ion Proton instrument (Life Technologies, Carlsbad, CA, USA) using one Ion PI chip. Sequence reads were aligned to the human GRCh37/hg19. Variants were filtered for common SNPs using the NCBI’s “common” and known medical impact databases ExAC10 and ClinVar, c.131 C > T was found to segregate with the disease.

Family 6: Exome sequencing was performed on a NextSeq 500 System (Illumina, CA, USA), with a x 150 bp high-output sequencing kit after enrichment with Seq Cap EZ MedExome kit (Roche, Basel Switzerland). Sequence alignment, variant calling, and variant annotation was performed by Genospech Technology (Paris France) with BWA 0.7.12, picard-tools-1.121, GenomeAnalyzer/TK-2014-3-17-g058318 and SNPeff-4.2 with additional annotations from ClinVar and HGMD. The compound heterozygous UGDH variants were selected to be the most promising candidates and were thus submitted to GeneMatcher.

Families 7, 8, 16–25: Exome sequencing was performed essentially as described before46 for families 7, 8, and 16–25. Target regions were enriched using the Agilent SureSelectXT Human All Exon 50 Mb Kit. Whole-exome sequencing was performed on the Illumina HiSeq platform (BGI, Copenhagen, Denmark) followed by data processing with BWA (read alignment,) and GATK (variant calling) software packages. Variants were annotated using in-house developed pipelines. Prioritization of variants was done by an in-house developed “variant interface” and manual curation. As four families with similar phenotype shared the homoyzous variant p.R317Q as best candidate, a GeneMatcher entry was made and the in-house database was systematically screened for other potentially pathogenic UGDH variants. This allowed the identification of families 18 to 25.

Family 11: The sequencing was performed at Claritas Genomics (Cambridge, USA). Extracted genomic DNA was amplified using the AmpliSeq protocol and sequenced using an IonTorrent Proton Instrument. Alignment and variant calling of the nuclear DNA was done on Proton data using Torrent Suite 4.4 software. Novel heterozygous variants were filtered using a custom-developed tool. In addition, extracted genomic and mitochondrial DNA was also run on an Agilent Clinical Research Exome capture sequence and then sequenced using an Illumina NextSeq instrument. Alignment and variant calling on NextSeq data was performed by an implementation of GATK Best Practices Pipeline. Genetic DNA results from the two NGS runs of the proband were combined and annotated by an in-house bioinformatics pipeline. Besides a heterozygous SLCEA5 missense variant inherited from the unaffected father, the compound heterozygous UGDH variants were the only candidates reported that had a minor allele frequency (MAF) of <0.01% that passed the laboratory’s quality metrics and were not de novo. X-linked or had biallelic variants.

Family 12: Isolated genomic DNA from peripheral blood leukocytes of proband and parents was captured with the Agilent Sure Select Clinical Research Exome (CRE) kit (v2). Sequencing was carried out with 150 bp paired-end reads on the Illumina HiSeq 4000. Reads alignments to the GRCh37/UCSC hg19 build were achieved using Bowtie (BWA) (version 0.7.7) capturing de novo and variant calls were made using the BWA-mapping option and parallelized bioinformatics pipelines. The compound heterozygous variants in UGDH. The family was linked to this cohort via GeneMatcher. Additional variants not included in this report are available upon request.

Family 14: The patient F14-II:1 was enrolled in the ongoing “Undiagnosed Patients Program” at the Ospedale Pediatrico Bambino Gesu, Rome. Targeted enrichment (SureSelect All Exon V4. Agilent) used genomic DNA extracted from clinical leukocytes for the proband with variable c.214T > C and c.41A > G. Sequencing was performed on an Illumina HiSeq2000 platform, obtaining about 70 million reads. The data analysis was performed using an in-house implemented pipeline, which mainly take advantage of the Genome Analysis Toolkit (GATK V.3.7) framework, as previously reported48. The functional annotation of variants were assessed using SNPeff and dbNSFP (V.3.0)59. The functional impact of variants was analyzed by Combined Annotation Dependent Depletion (CADD) V.1.3, M-CAP V.1.0, and InterVar V.0.16 algorithms41,53,54. Two
compounds from heterogeneous and mixed-sequence variants in the UGDH gene, c.247T > C and c.1328G > A, were interpreted as the only candidate genetic etiology. 

**Family 15** genic DNA from the proband and parents were enriched for exonic sequences with the SureSelect Human All Exon 50 Mb V5 Kit (Agilent Technologies, Santa Clara, California, USA). The HiSeq2500 (Illumina, San Diego, California, USA) was used to generate 125-bp paired-end runs of sequences. Reads were aligned and variant called with DNAseanu (Palo Alto, California, USA) using the reference human genome assembly hg19 (GRCh37). A mean depth of 100x was achieved for the proband. Data analysis was performed using an in-house bioinformatics pipeline. The compound heterozygous UGDH variants were selected to be the most promising candidates and were thus submitted to GenMatcher.

**Brain magnetic resonance imaging.** Magnetic resonance images (MRI) have been acquired with standard sequences, including T1, T2, and Flair images.

**Cell culture.** Primary dermal fibroblast cultures were established from skin biopsies obtained from individuals F3–IL1, F4–IL1, F5–IL1, and F5–IL1A according to standard procedures. In brief, primary fibroblasts were derived from biopsy samples and cultured in Dulbecco’s modified Eagle medium (DMEM; HyClone, SH30243.01) supplemented with 10% fetal bovine serum (Biological Industries) and 2 mM l-glutamine (Biological Industries). Written informed consent of healthy probands and parents of UGDH patients were received prior to biopsy according to the ethical approvals of the local Institutional Review Boards (IRB).

**Reverse transcription (RT-PCR) and quantitative PCR.** Total RNAs were extracted using the RNeasy Mini Kit (Qiagen). RNA (1 µg) was reverse transcribed using the iScript™ complementary DNA (cDNA) Synthesis Kit (Bio-Rad). Quantitative real-time PCRs were performed using Power SYBR green master mix (Thermo Fisher Scientific).

**Protein isolation and analysis.** Cells were lysed using ice-cold RIPA buffer (250 mM Tris, pH 7.5; 150 mM NaCl; 1% NP-40; 0.5% Na deoxycholate; protease inhibitors P2714 [Sigma-Aldrich, USA]). The total protein concentration of cell lysates was determined using the BCA Protein assay Kit (Thermo Fisher Scientific, USA).

**Analysis.** Statistical significance was assessed by Student’s t-test with at least three technical triplicates. Michaelis rate constants, $k_{cat}$, and $K_m$ were determined for UGDH WT using the NADH extinction coefficient of 6220 M$^{-1}$ cm$^{-1}$. UGDH $k_{cat}$ activity was converted to [NADH] in nmol min$^{-1}$ mg$^{-1}$ and subsequently normalized to the fractional purity of UGDH in the sample preparation. Samples were run in triplicate and statistical significance was determined using Student’s t-test. Michaelis rate constants, $k_{cat}$ and $V_{max}$, were determined for UGDH WT and A44V as previously described using a 96-well plate assay to measure the change in NADH ($A_{340}$) with respect to both the substrate, UDP-glucose, and cofactor, NAD$.^+$.

**Saturating enzymatic activity and kinetic characterization.** Enzyme activity of recombinant UGDH WT and all point mutants was characterized as described previously with minor alterations. Enzymatic activity was calculated by NADH extinction coefficient of 6220 M$^{-1}$ cm$^{-1}$. UGDH $k_{cat}$ activity was converted to [NADH] in nmol min$^{-1}$ mg$^{-1}$ and subsequently normalized to the fractional purity of UGDH in the sample preparation. Samples were run in triplicate and statistical significance was determined using Student’s t-test. Michaelis rate constants, $k_{cat}$ and $V_{max}$, were determined for UGDH WT and A44V as previously described using a 96-well plate assay to measure the change in NADH ($A_{340}$) with respect to both the substrate, UDP-glucose, and cofactor, NAD$.^+$.

**iPSCs reprogramming.** WT, WT/A82T, and A82T/A82T fibroblasts were reprogrammed using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, A16317) in accordance with the manufacturer’s instructions. Briefly, fibroblasts were transduced and plated after 7 days onto Matrigel Basement Membrane Matrix (Corning, 354234) in mTeSR1 medium (STEMCELL Technologies, 85360). iPSC colonies were picked between days 17–28 and maintained in Matrigel Basement Membrane Matrix and mTeSR1 for expansion. R393W/A410S, R565*/Y367C, and Y14C/S72P iPSCs were reprogrammed using the ReproRNA™ OKSGM kit (Stemcell Technologies, 05930) in accordance with the manufacturer’s instructions. Briefly, fibroblasts were plated onto Matrigel Basement Membrane Matrix (Corning, 354234) and transfected with ReproRNA™-OKSGM cocktail. Puromycin selection was carried out 1 day after transfection. iPSC colonies were picked between 20 and 28 days after transfection and maintained in STEM–OKSGM Bailey Matrix and mTeSR1 for expansion. Between 1 and 3 clones per genotype were maintained for further experiments.

**Neuronal and cerebral organoid differentiation.** Neuronal and cerebral organoid differentiation was performed as previously described. Briefly, on day 0 of organoid culture, iPSCs were dissociated by accutase (STEMCELL Technologies, 07920) treatment to generate single cells. In total, 9000 cells were then plated per well of an ultra-low-binding-96-well plate (Corning) in MEDI medium [Knockout SR 20% (Thermo Fisher scientific, 10828-028)], -glutamine 2 mM (Thermo Fisher scientific, 200 mM, 25030-081), Non-essential amino-acid
Neural induction

Primary antibodies in blocking buffer at the following dilutions: SOX2 (mouse, Triton X-100 and 1% BSA in PBS) for 20 min. Sections were then incubated with 9999, 10×. Sections were then blocked and permeabilized in blocking buffer (0.5% and dehydrated by incubations in Ethanol (70%, 95% then 100%) for 1 h at 4 °C followed by two times 1 h incubation with Xylene 100% at room temperature. The and dehydrated by incubations in Ethanol (70%, 95% then 100%) for 1 h at 4 °C followed by two times 1 h incubation with Xylene 100% at room temperature. The cerebellar organoids were then embedded in Paraplast Plus (Leica, 39602004) and sectioned at 30 µm. Tissue sections were stained with Haematoxylin and Eosin (H&E) or used for immunostaining. For immunofluorescence, antigen retrieval was performed using the Antigen Retriever buffer (Citrate buffer pH 6.0, Sigma, C-9999, 10x). Sections were then blocked and permeabilized in blocking buffer (0.5% Triton X-100 and 1% BSA in PBS) for 20 min. Sections were then incubated with primary antibodies in blocking buffer at the following dilutions: SOX2 (mouse, R&D systems, MBAB18, 1:200), TUJ1 (mouse, Biologend MMS-435P, 1:3000), GFAP (Rabbit, Dako Z0331, 1:2500), PCNA (Rabbit, abcam ab18197, 1 µg/ml). For visualization, an antibody anti-mouse immunoglobulin G (anti-mouse- IgG) Alexa Fluor 594 conjugate (Invitrogen, Molecular Probes) and an anti-rabbit IgG Alexa Fluor 488 conjugate (Invitrogen, Molecular Probes) was applied. DNA was stained by DAPI (1/500) and sections were mounted in ProLong Diamond Antifade mounting medium (Thermo Fisher). Images were collected by using an Olympus FV3000 RS with a 20x objective.

Histology and immunofluorescence of organoids. Cerebral organoids were fixed in 4% paraformaldehyde (PFA) overnight at 4 °C, then washed in PBS for 10 min and dehydrated in Ethanol (70%, 95% then 100%), followed by two times 1 h incubation with Xylene 100% at room temperature. The cerebellar organoids were then embedded in Paraplast Plus (Leica, 39602004) and sectioned at 30 µm. Tissue sections were stained with Haematoxylin and Eosin (H&E) or used for immunostaining. For immunofluorescence, antigen retrieval was performed using the Antigen Retriever buffer (Citrate buffer pH 6.0, Sigma, C-9999, 10x). Sections were then blocked and permeabilized in blocking buffer (0.5% Triton X-100 and 1% BSA in PBS) for 20 min. Sections were then incubated with primary antibodies in blocking buffer at the following dilutions: SOX2 (mouse, R&D systems, MBAB18, 1:200), TUJ1 (mouse, Biologend MMS-435P, 1:3000), GFAP (Rabbit, Dako Z0331, 1:2500), PCNA (Rabbit, abcam ab18197, 1 µg/ml). For visualization, an antibody anti-mouse immunoglobulin G (anti-mouse- IgG) Alexa Fluor 594 conjugate (Invitrogen, Molecular Probes) and an anti-rabbit IgG Alexa Fluor 488 conjugate (Invitrogen, Molecular Probes) was applied. DNA was stained by DAPI (1/500) and sections were mounted in ProLong Diamond Antifade mounting medium (Thermo Fisher). Images were collected by using an Olympus FV3000 RS with a 20x objective.

RT and RT-qPCR. Total RNA of individual cerebral organoids was extracted using the RNAeasy Mini Kit (Qiagen, 74104). Total RNA (0.5 µg) was reverse transcribed using the Iscript™ cDNA Synthesis Kit (Bio-Rad, 1708891). Real-time quantitative PCRs were performed using the Power SYBR green master mix (Applied Biosystems). qPCR primers were as previously described:59 5′-CGCAATCGAAGCACTTCG3′ and 5′-GAATGATTGCGCCCTCCG3′. Neurons on day 21 were dissociated using the Iscript cDNA Synthesis Kit (Bio-Rad, 1708891). Real-time quantitative PCRs were performed using the Power SYBR green master mix (Applied Biosystems, 4309155) on the 7900HT Fast real-time PCR system (Applied Biosystems). qPCR primers were as follows: c-fos 5′-AAGCTCCAGGGGATCCTC-TGTAGGTCACAGGTTTTTGACA-3′ and 5′-CGCTGAAGAGATGTCCAAAGGC3′. Whole dataset from Family F3 was already published66 and deposited in the European Genome-phenome Archive, accession numbers EGAS00001000048. Whole datasets from Families F5 and F15 are available upon request from the corresponding authors. Consent restrictions preclude deposition of c-fos qPCR experiments. 7-dpf larvae were first incubated with increasing concentrations (up to 15 mM) of PTZ (Sigma, P6500) for 45 min and were then decapitated. The bodies were used for genotyping and 20 larval heads of the same genotype were pooled together for total RNA extraction using the RNAeasy Mini Kit (Qiagen, 74104). Total RNA (0.5 µg) was reverse transcribed using the Iscript™ cDNA Synthesis Kit (Bio-Rad, 1708891). Real-time quantitative PCRs were performed using the Power SYBR green master mix (Applied Biosystems, 4309155) on the 7900HT Fast real-time PCR system (Applied Biosystems). qPCR primers were as follows: c-fos 5′-AAGCTCCAGGGGATCCTC-TGTAGGTCACAGGTTTTTGACA-3′ and 5′-CGCTGAAGAGATGTCCAAAGGC3′. Twenty-four-well locomotion and convulsion test assay. A Basler Ace (acA1300-200um; 1280 × 1024) camera was used to acquire videos of larval zebrafish at 50 fps. A custom hardware setup was designed to acquire full frame videos of 24-Well flat bottom plates using a 25 mm lens attachment to the camera placed at bottom plates using a 25 mm lens attachment to the camera placed 63 cm above the plate. The 24-well plates were backlit using a white light LED lightbox that delivered uniform lighting across the entire field. 7-dpf larval fish were placed individually in each well in 500 µl of egg water and acclimated to the setup for 10 min. Then, 500 µl of either egg water (negative control) or PTZ 30 mM (15 mM final concentration, Sigma, P6500) was added to the well. Three videos of 2 min each were acquired for a duration of 10 min for each condition tested. Fish locomotion was tracked online during video recording on a custom written software in LabView (www.critta.org). Analysis of locomotion was automated and performed blind offline after each experiment using custom written Python scripts (available on Github: https://github.com/mechunderlyingbehavior/24-Well-Larval-Locomotion). In this assay, low and high speed were defined as average speeds of 0 to 8 mm/s and >8 mm/s−1, respectively, based on a recent publication measuring convulsive-induced speed changes in zebrafish larvae67.

Data availability

The data that support the findings of this study are available within the paper and its supplementary information files. All identified variants have been deposited in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) under the name “UGDH001”. Whole dataset from Family F3 was already published68 and deposited in the European Genome-phenome Archive, accession numbers EGAS00001000048, EGAS00001000048. Whole datasets from Families F5 and F15 are available upon request from the corresponding authors. Consent restrictions preclude deposition of sequencing data of the other families, however, specific information (e.g., secondary variants) can be obtained upon request from the corresponding authors. Lists of primers and antibodies are in Supplementary Tables 4 and 5, respectively. The source data underlying Fig. 3a–f, Fig. 4a, and Suppl. Fig. 3a–d, Suppl. Fig. 4a, b, and d are provided as a Source Data file 1 (for gels) and 2 (for graphs).

Code availability

The custom written Python scripts used to track fish locomotion is available following this link: https://github.com/mechunderlyingbehavior/24-Well-Larval-Locomotion.git.
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Competing interests

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Additional information

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