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Supplemental information

Bi-allelic variants in *OGDHL* cause a neurodevelopmental spectrum disease featuring epilepsy, hearing loss, visual impairment, and ataxia

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Table S3. Primers used for mutagenesis PCR of pUASTattB-dOgdh-Flag

Primer name	primer sequence
dOgdh (P867A)-F	cccaaatcgctgctgcgtcacGccgaggctaagagtcctttcagc
dOgdh(P867A)-R	gctgaaaggactcttagcctcggCgtgacgcagcagcgatttggg
dOgdh(R688Q)-F	gtcgagcgtggtaccttctcgcatCAAcaccatgtgctgcaccaccagctg
dOgdh(R688Q)-R	cagctggtggtgcagcacatggtgTTGatgcgagaaggtaccacgctcgac
dOgdh(F749S)-F	ctggtgctgtgggaggctcagtCcggagacttcagcaacacggcc
dOgdh(F749S)-R	ggccgtgttgctgaagtctccgGactgagcctcccacagcaccag
dOgdh(A343V)-F	cagttcgctggactggaggctgTtgatgatggctctggtgatgtc
dOgdh(A343V)-R	gacatcaccagagccatcatcaAcagcctccagtccagcgaactg
dOgdh(W237C)-F	aactctctggagcaatgcaacTGCatccgcaagcgttttgagacc
dOgdh(W237C)-R	ggtctcaaaacgcttgcggatGCAgttgcattgctccagagagtt
dOgdh(D507V)-F	ttccacaaggattgtgttattGTTttggtcggataccgccgcaat
dOgdh(D507V)-R	attgcggcggtatccgaccaaAACaataacacaatccttgtggaa
dOgdh(R261W)-F	gagaagcgtctgatcctggccTGGttgacccgtgccaccggcttt
dOgdh (R261W)-R	aaagccggtggcacgggtcaaCCAggccaggatcagacgcttctc
dOgdh(R316G)-F	gtcatcatgggcatgccccatGGTggacgtcttaacaccttggcc
dOgdh(R316G)-R	ggccaaggtgttaagacgtccACCatggggcatgcccatgatgac

Clinical case reports

Individuals 1 and 2 are two siblings born to consanguineous parents (first cousins) of Iranian origin. Individual1 is a 7-year-old boy and individual 2 is a 16-year-old girl initially diagnosed with profound bilateral hearing impairment. The affected girl was diagnosed at the age of 1 year and her younger brother via neonatal screening. In addition to hearing loss, both affected individuals also presented with mild developmental delay, myopia, a mild gait ataxia and learning difficulties in school. The boy achieved normal speech after a cochlear implant while the girl still presented with speech delay and learning problems due to a later cochlear implant. The affected boy experienced a recent seizure at the age of 7 years. No dysmorphic features were detected in either case. The parents and another sibling were unaffected and carriers of the p.(Pro852Ala) variant.

Individual 3 is a 27-year-old boy born after uncomplicated pregnancy and delivery from healthy non-consanguineous parents of Italian origin. No dysmorphisms were present at birth. Early psychomotor development was normal. The first symptoms started at 8 years of age when a severe neurosensory hyopoacusia was found. At 9 years, he developed gait difficulty with stiffness. At the same age, he started to present a dorsal right-convex scoliosis and a retinopathy was detected. Neurological examinations at 16 years showed muscle weakness and diffuse muscle hypotrophy, more severe in limbs and distally. Pyramidal signs such as high tendon reflexes (Babinski and Achilles tendon clonus) were detected and muscle tone was high in limbs. Pes cavus was observed bilaterally, although more evident in the right foot. Achilles tendon retraction was present. The individual presented with distal tremor. Normal head circumference and no alterations in eyes motility were reported; neither cognitive nor behavioral changes were observed. He could stand up without support, and walk with support. During time, the spasticity at limbs worsened and started to be present in arms leading to impossibility to walk and the need of wheelchair. At last evaluation, at 23 years of age, he showed mild dysphagia for both solid and liquids, dysarthria and mild dysmetria in upper limbs associated with slow rigidity and distal weakness. A severe spastic paraparesis in lower limbs was present and the individual could not stand up without support and could not walk. The brain MRI done at 16 yeas showed symmetrical lesions at the pontine tegmentum and mesencephalon, and in temporal-capsular junction that were a little more evident in a second brain MRI made at 18 years. The EEG was normal. No heart involvement was present at the Echocardiogram (with Ejection Fraction 60%). The EMG showed a sensory-motor neuropathy. At that time, he performed a muscle biopsy that was morphologically, as well as histochemically, normal. The mitochondrial respiratory chain activity was normal. Lactate in plasma was normal. Urinary organic acids and plasma amino acids were both normal, as well as vitamin E and vitamin A levels.

Individual 4 is a 15-year-old boy born after uncomplicated pregnancy and delivery from healthy non-consanguineous parents of German origin. In the first years of live he developed normally. At 6 years

he developed a severe epileptic encephalopathy with absences, myoclonic-atonic seizures, bilateral tonic-clonic seizures and a severe obtundation status. He was treated on the intensive care-unit with propofol, adrenocorticotropin, a ketogenic diet and various antiepileptic drugs. At that time, he appeared to have developmental regression and severe deficits in cognitive function. The epilepsy stabilized afterwards on valproate and lamotrigine. Since 8 years the individual is seizure free and cognitive functions recovered.

Individual 5 is a 5-year-old girl born at 37.1 weeks gestational age to healthy non-consanguineous parents of Ukrainian origin. She initially presented at 6 months of age with developmental regression in the setting of presumed viral encephalitis and appeared to never fully recover. She subsequently developed infantile spasms and was readmitted to the hospital at 12 months of age. At that time, she appeared to have persistent seizures, global developmental delay, hypotonia, and central visual impairment with nystagmus. She now manifests as severe chronic static encephalopathy and intractable epilepsy, with EEG demonstrating electro-clinical spasms and transition to Lennox Gastaut Syndrome. She was on clonazepam, which caused some regression, but she did respond well to high dose prednisone for a while. She relapsed and has been advised to follow a ketogenic diet. On neurological examination, she presented with significant truncal hypotonia with head lag, spasticity of extremities (worse in lower extremities), clonus with extension of right foot, brisk reflexes (brachioradialis-symmetric) and bilateral hip dysplasia. She requires support to sit, stand, and support her head. She does smile and show facial expressions but is overall nonverbal. She has not had loss of skills or regression since the last visit. She receives multiple therapies (PT, OT, speech) at home and as an outpatient.

Individual 6 is a 6-year-old boy born after uncomplicated pregnancy and delivery to healthy consanguineous parents of Pakistani origin. The first examination at 5 months of age showed that he was unable to hold his neck neither fix his gaze. He presented with global developmental delay, where he started to partially hold his neck around 4 years of age, but he is still unable to sit or crawl. He is unable to grasp things, he only coos and he is only able to respond to sensory stimuli like touch. Seizures started at 2 years of age, presented as multifocal clonic episodes with grunting sounds, and then evolved to generalized tonic clonic. Each episode lasted for about 2-3 minutes, and there were about 3-4 episodes per day. He was started on clonazepam for a year, with subsequent addition of valproic acid. Currently, seizures have been controlled for the last 1.5 years. On the last examination, he was diagnosed with microcephaly (43cm-below 3rd centile) and swallowing difficulties. He has a highly arched palate. He has no visual interaction but makes roving eye movements. Eye examination showed bilateral optic atrophy. He is unable to walk and has a spastic quadriplegic gait with hip dysplasia and scoliosis. The brain MRI done at 6 years old shows extensive bilateral periventricular leukomalacia, corpus callosum hypoplasia and the thalami and both cerebelli are spared.

Individual 7 is a 6-year-old boy born after uncomplicated pregnancy and delivery to healthy consanguineous Syrian parents at 41 weeks of gestation with a birth weight of 3400 gram (-0.39SDS) and a length of 50 cm (-0.38SDS). He initially presented at 3 months of age with craniosynostosis (sagital suture, scaphocephaly) for which he was operated at the age of 5 months. Subsequently, a mild developmental delay with mainly language delay was noticed and a growth retardation (-2.9 SDS) due to partial GH deficiency was diagnosed. Metabolic screening and SNP-array were unremarkable. Hearing was normal. An MRI cerebrum at the age of 2 years and 3 months was performed to investigate the cause of the GH deficiency, and showed as an incidental finding a low-grade glioma brain tumor, confirmed by sterotactic biopsy, in his right frontal cortex, with growth into the corpus callosum for which he was subsequently treated with vincristine and carboplatine. At the age of 2 years and 7 months, he was operated to remove a keloid scar at the left ear helix. He achieved walking at 15 months; at the age of 3 years, he was using 3-4 word sentences. He presents with mild dysmorphic features, which include scaphocephaly, arched eye brows, narrow palpebral fissures and a thin upper and full lower lip. No cutaneous abnormalities were observed. At the age of 6 years, he has language delay. At the last investigation, he has a head circumference of 54.5 cm (+1.61 SD), length of 96.2 cm (-1.73SD) and weight of 14.9 kg (-1.08 SD).

Individual 8 is a 2.5-year-old boy from a consanguineous family of Iranian origin. He initially presented at 3-4 months of age with upper and lower limb muscles weakness, facial muscle hypotonia (with no facial expression), ptosis, nystagmus, unclear speech and drooling. He presents with moderate developmental delay and severe mental retardation. EMG findings are compatible with myopathic process of proximal and distal muscles- suspected to have CMS.

Individual 9 is a 12-year-old girl born to consanguineous healthy parents of Iraqi origin. She presented at 2 months of age with failure to thrive and excessive crying. She is macrocephalic (54 cm + 2SD). She presented with global developmental delay (control head was achieved at 1 year old, sitting at 3 years old and she is still unable walk steadily). She has intellectual disability and language delay (can only speak a few understandable words). She experiences sleep apnea and a sleep movement disorder in the form of laugh and cry and abnormal body movements. She developed generalized tonic-clonic seizures at 12 months of age which were controlled on levitracetam. She currently presents with mild dysmorphic features, which include bilateral ptosis, highly arched palate and down slanting of the eyes. Upon neurological and ophthalmologic investigation, she was found to be visually impaired and also bilaterally hearing deficit needing hearing aids. She is hypotonic and has an abnormal gait needing support to walk. She experienced hip dysplasia which was treated by a hip spica cast. EEG showed generalized and multifocal slowing inter-mixed with multifocal sharp discharges.

Additional candidate variants from WES

Diverse clinical research centers from Europe, Asia and the US independently achieved and subsequently shared results leading to *OGDHL* as a new disease-causing gene.

In family 1, WES analysis and variant filtration identified two rare homozygous variants that could fit the disorder in this family: *DCHS1*:NM_003737:exon14:c.G6191A:p.R2064H and *OGDHL*:NM_018245:exon20: c.C2554G:p.P852A. However, after testing more family members (both unaffected parents and an unaffected sibling of family 1) via Sanger sequencing, only the *OGDHL* variant segregated with disease in the family, and the *DCHS1* variant was excluded.

The Ghezzi lab (for family 2) performed WES and variant filtering and prioritization as described in ref. 14. After filtering steps and assuming a recessive trait, we identified 5 genes with two heterozygous rare (<1%) variants, but the only gene encoding a mitochondria-targeted protein was *OGDHL* (another gene encoding a protein associated with mitochondria, *MYO19*, was ruled out because the two variants were in cis, on the paternal allele).

The Houlden lab (for family 5, 6, 7, and 8) performed WES and variant filtering by discharging firstly all synonymous and in-silico predicted benign changes. The raw list of single nucleotide variants (SNVs) and indels was then filtered. Only exonic and donor/acceptor splicing variants were considered. In accordance with the pedigree and phenotype, priority was given to rare variants [<1% in public databases, including 1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Exome Aggregation Consortium (ExAC v0.2)] that fit a recessive model. The Weber lab (for family 3, and 4) have followed similar strategies as described in ref 16.

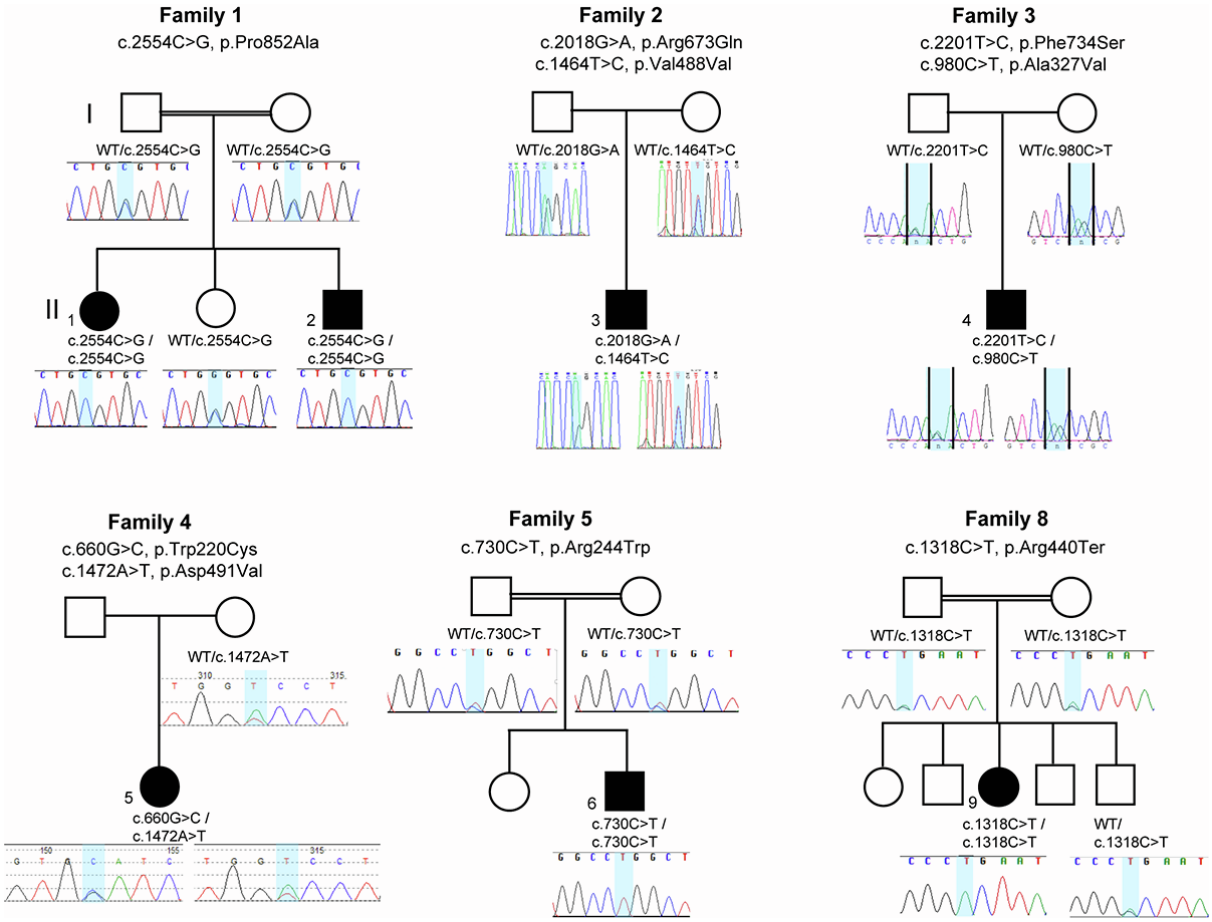

Figure S1. Segregations of *OGDHL* variants for families 1, 2, 3, 4, 5, and 8 were confirmed by Sanger sequencing. In family 1, 2, and 3, Sanger sequencings were performed using reverse primers, therefore, the results show reverse complementary sequences of *OGDHL*.

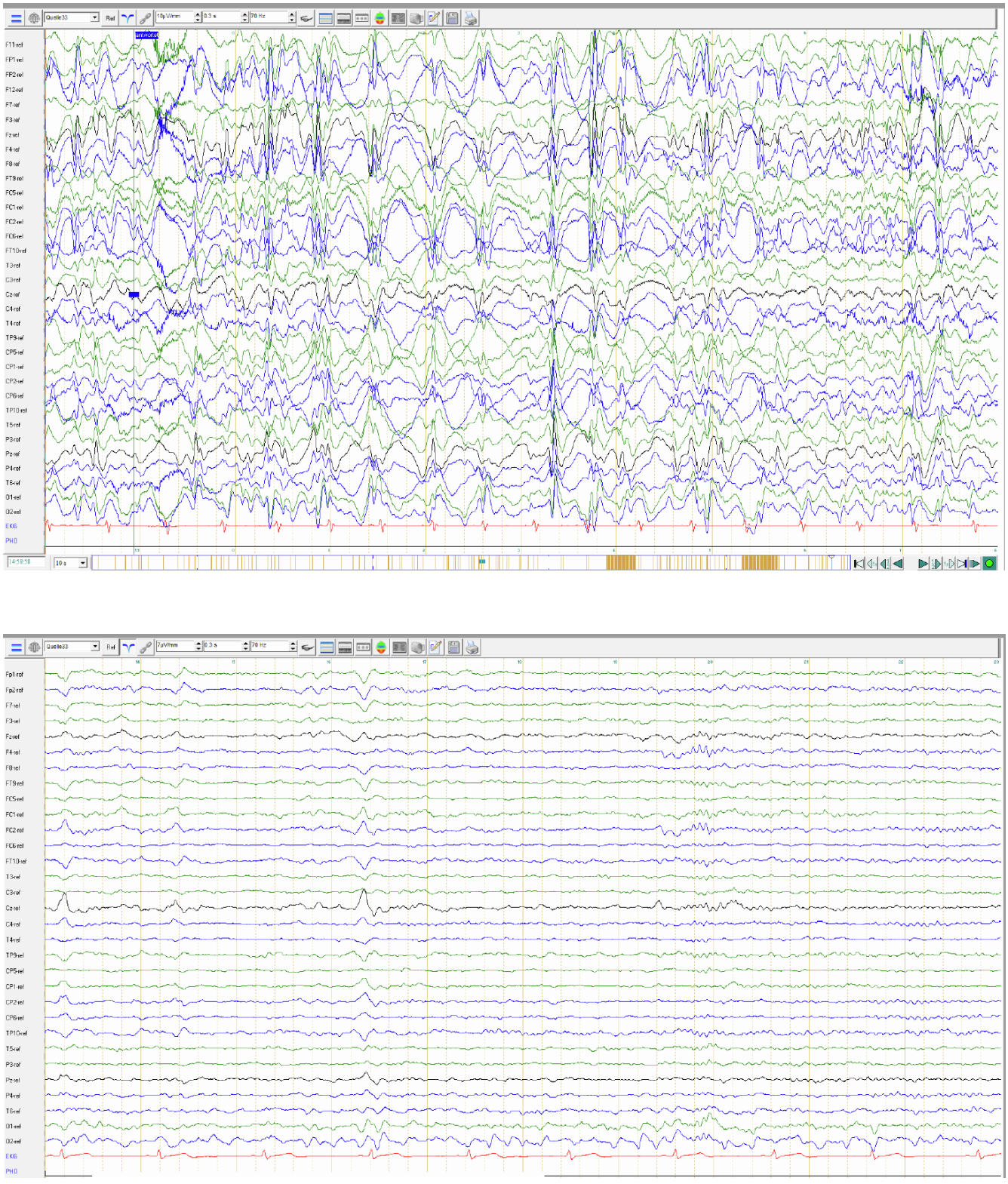

Figure S2. Interictal EEGs from individual 5 (family 4) during phase 2 nREM sleep showing high-amplitude and irregular, bilateral slow wave/sharp wave complexes with loss of normal sleep structure and physiological spindles (age 7 years; treatment with bromides, lamotrigine, fenobarbital, ketogenic diet). Her follow-up sleep EEG at age 15 years showed was nearly normal (no treatment).

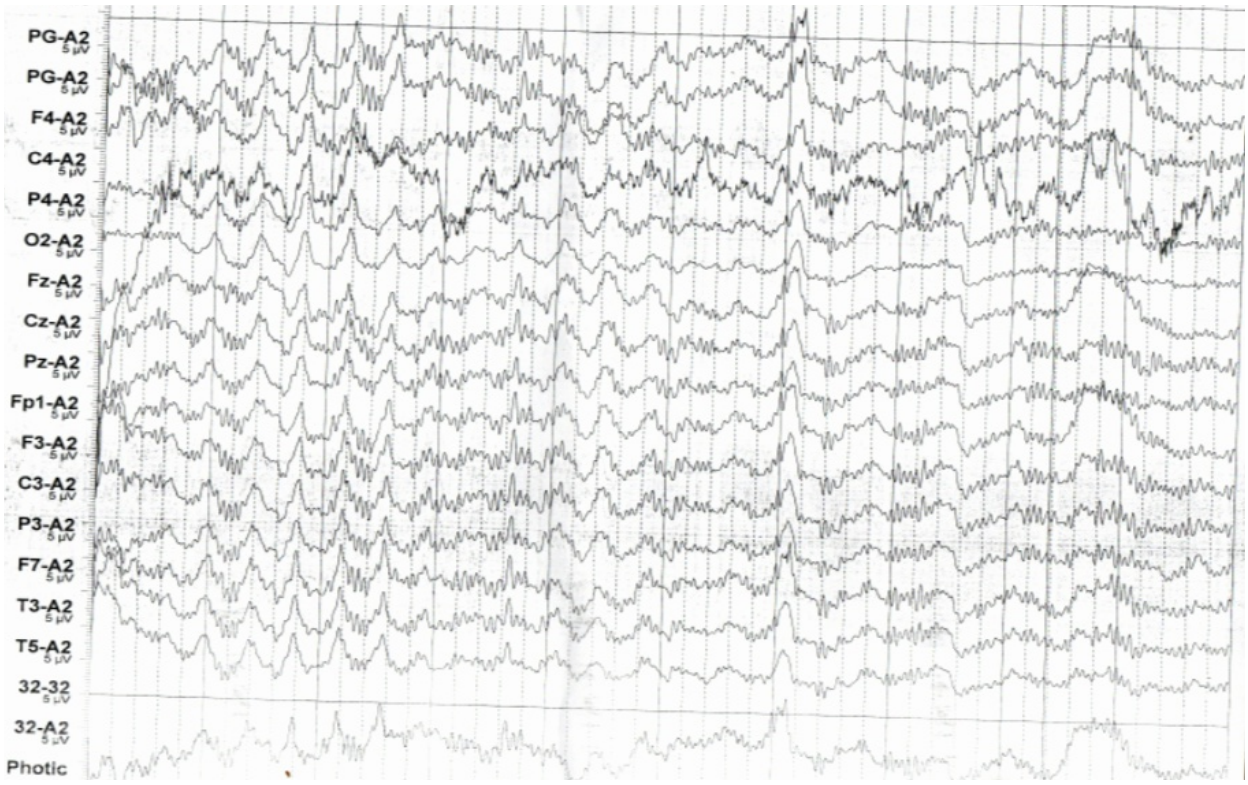

Figure S3. Interictal EEG from individual 6 (family 5) at 6 years and 11 months of age showing diffuse sharp wave/slow wave complexes within a globally slowed background activity in the theta range.

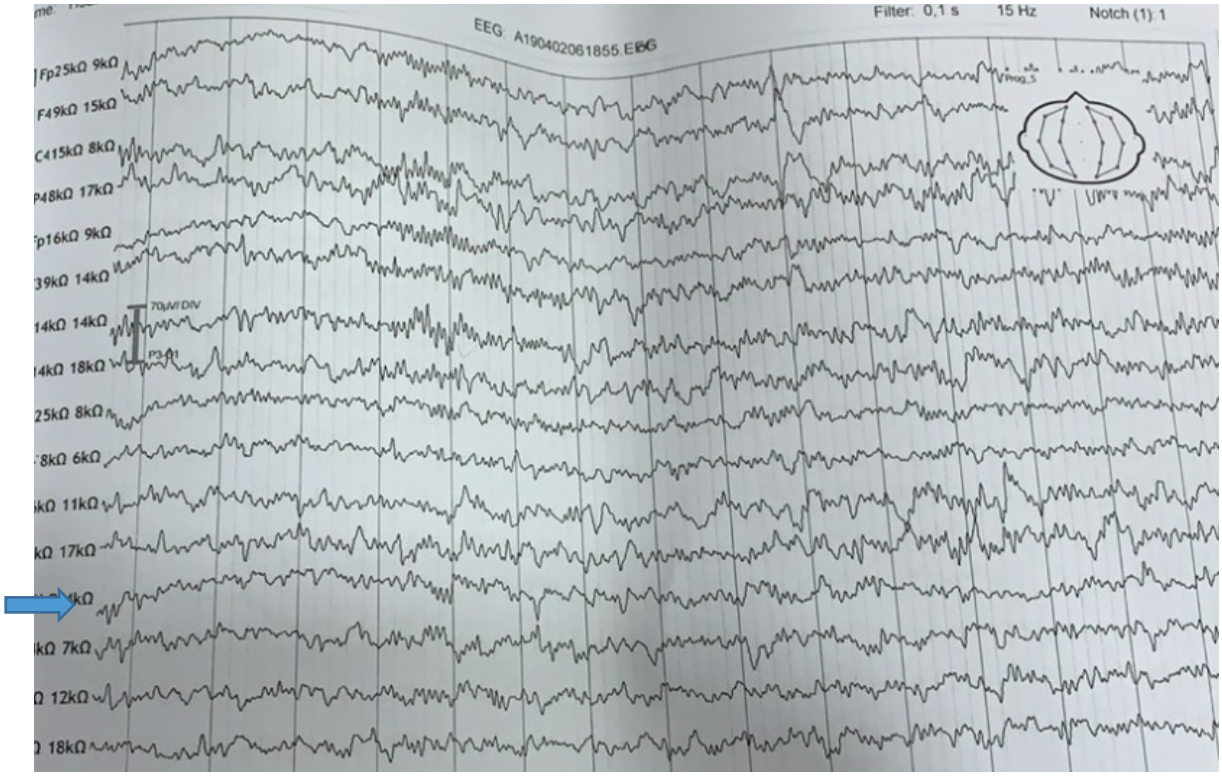

Figure S4. Interictal EEG from individual 9 (family 8) during phase 2 nREM sleep showing high-amplitude and irregular, right temporal slow wave/sharp wave complexes (arrow) with preserved sleep structure and physiological spindles (age 5 years*)*.

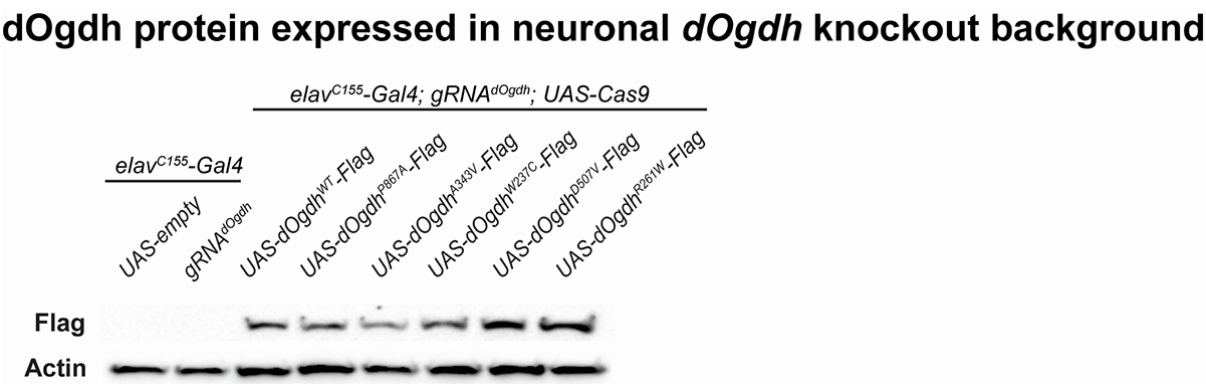

Figure S5. Western blots for *Drosophila* heads expressing wildtype *dOgdh-Flag*, or *dOgdh-Flag* carrying the missense variants (P867A, A343V, W237C, D507V and R261W) in *dOgdh* knockout genomic background.

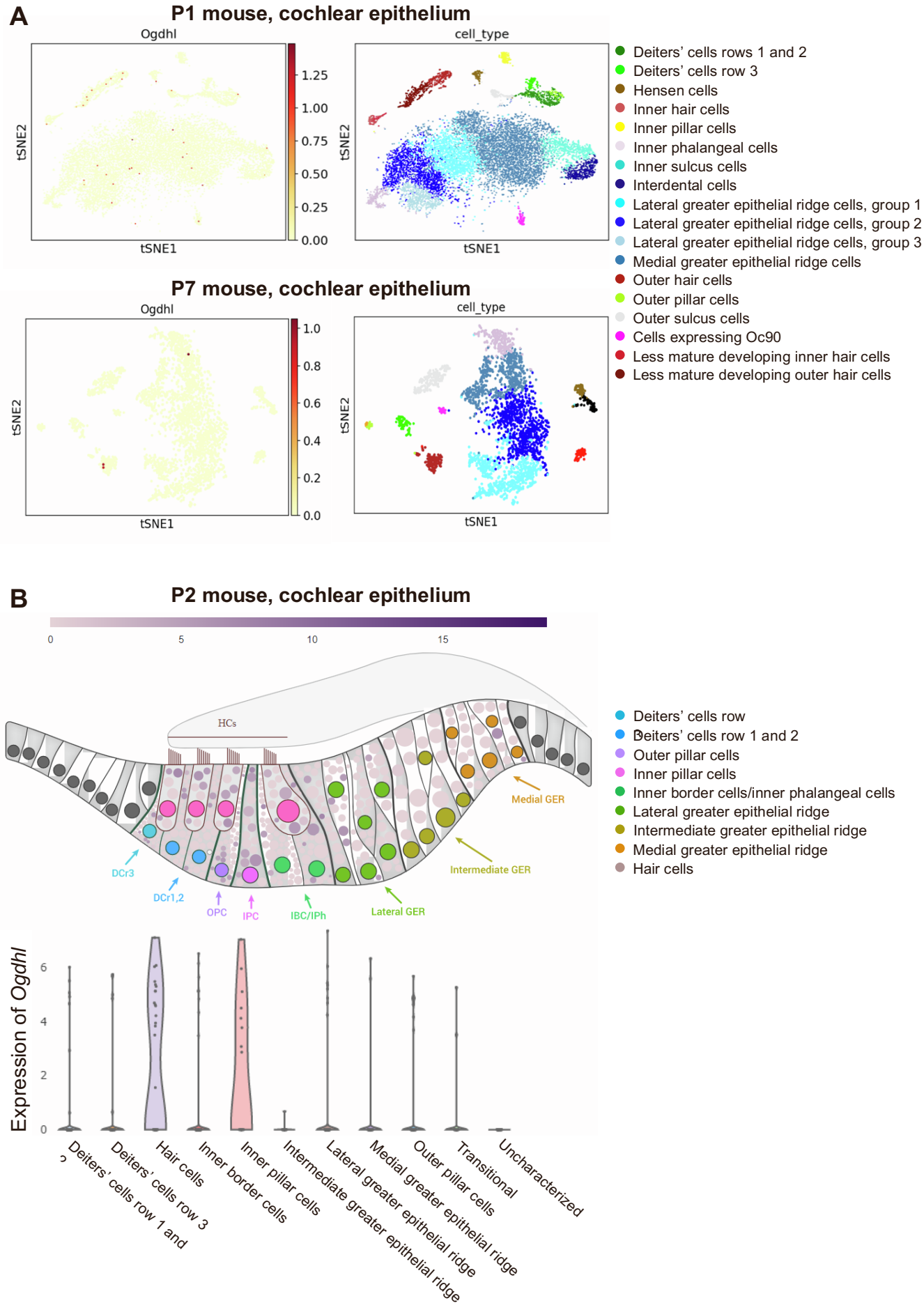
A
P1 mouse, cochlear epithelium
Ogdhl
cell_type
tSNE1
tSNE2
1.25
1.00
0.75
0.50
0.25
0.00
P7 mouse, cochlear epithelium
1.0
0.8
0.6
0.4
0.2
0.0
Deiters' cells rows 1 and 2
Deiters' cells row 3
Hensen cells
Inner hair cells
Inner pillar cells
Inner phalangeal cells
Inner sulcus cells
Interdental cells
Lateral greater epithelial ridge cells, group 1
Lateral greater epithelial ridge cells, group 2
Lateral greater epithelial ridge cells, group 3
Medial greater epithelial ridge cells
Outer hair cells
Outer pillar cells
Outer sulcus cells
Cells expressing Oc90
Less mature developing inner hair cells
Less mature developing outer hair cells
B
P2 mouse, cochlear epithelium
0
5
10
15
HCs
DCr3
DCr1,2
OPC
IPC
IBC/IPh
Lateral GER
Intermediate GER
Medial GER
Deiters' cells row
Deiters' cells row 1 and 2
Outer pillar cells
Inner pillar cells
Inner border cells/inner phalangeal cells
Lateral greater epithelial ridge
Intermediate greater epithelial ridge
Medial greater epithelial ridge
Hair cells
Expression of Ogdhl
6
4
2
0
Deiters' cells row 1 and 2
Deiters' cells row 3
Hair cells
Inner border cells
Inner pillar cells
Intermediate greater epithelial ridge
Lateral greater epithelial ridge
Medial greater epithelial ridge
Outer pillar cells
Transitional
Uncharacterized

Figure S6. Visualization of mouse inner ear single cell RNA-seq data from gEAR portal. To assess *Ogdhl* RNA expression in the postnatal (P) mouse cochlear epithelium, publicly available single cell RNA-seq data from P1, P2 and P7 mice[35;36] were visualized using the gene Expression Analysis Resource (gEAR, https://umgear.org). (A) The upper and lower panel shows tSNE plots of *Ogdhl* expression in the mouse cochlear epithelium at postnatal day (P) 1 and P7 (PMID: 32404924). (B) *Ogdhl* expression in cochlear cell types from P2 mice (upper panel) (PMID: 33472062). Violin plots of *Ogdhl* expression in different cell types (lower panel).