Mutations in *ABHD12* Cause the Neurodegenerative Disease PHARC: An Inborn Error of Endocannabinoid Metabolism

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Polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract (PHARC) is a neurodegenerative disease marked by early-onset cataract and hearing loss, retinitis pigmentosa, and involvement of both the central and peripheral nervous systems, including demyelinating sensorimotor polyneuropathy and cerebellar ataxia. Previously, we mapped this Refsum-like disorder to a 16 Mb region on chromosome 20. Here we report that mutations in the *ABHD12* gene cause PHARC disease and we describe the clinical manifestations in a total of 19 patients from four different countries. The ABHD12 enzyme was recently shown to hydrolyze 2-arachidonoyl glycerol (2-AG), the main endocannabinoid lipid transmitter that acts on cannabinoid receptors CB1 and CB2. Our data therefore represent an example of an inherited disorder related to endocannabinoid metabolism. The endocannabinoid system is involved in a wide range of physiological processes including neurotransmission, mood, appetite, pain appreciation, addiction behavior, and inflammation, and several potential drugs targeting these pathways are in development for clinical applications. Our findings show that ABHD12 performs essential functions in both the central and peripheral nervous systems and the eye. Any future drug-mediated interference with this enzyme should consider the potential risk of long-term adverse effects.

Inherited neurodegenerative diseases affecting both the peripheral and central nervous systems and the eye can be caused by a variety of metabolic disturbances. Mitochondrial dysfunction is a potent cause,[1,2] arising either from mutation in the mitochondrial genome—e.g., neuropathy, ataxia, retinitis pigmentosa (NARP, MIM 551500) and Kearns-Sayre syndrome (ophthalmoplegia, retinal pigmentation, ataxia, and frequently peripheral neuropathy, MIM 530000)—or from a mutated nuclear gene. Friedreich ataxia (MIM 229300) and POLG-related diseases (MIM 174763) are examples of the latter. Defects involving peroxisomal metabolism, such as Refsum disease (MIM 266500) and alpha-methylacyl-CoA racemase (AMACR; MIM 604489) deficiency, also give rise to similar phenotypes.[3]

Recently, in a Norwegian family we described a progressive, autosomal-recessive, neurodegenerative disease that we ascertained initially as a phenocopy for Refsum disease (Figures 1A–1E). We named the disorder polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract, or PHARC[4] (MIM 612674). The disease is slowly progressive, with recognition of the first symptoms typically in the late teens. Although the condition has similarities to Refsum disease, patients do not have anosmia and both phytanic acid levels and peroxisomal function are normal. We mapped the disease to a 16 Mb region on chromosome 20.[4] Subsequently, additional affected individuals in four countries were identified, and we used homozygosity mapping to identify candidate regions for the mutated gene, followed by sequencing of candidate genes.

For the present study, DNA was obtained from 19 persons affected with PHARC disease and from healthy siblings and parents. The patients (10 females and 9 males) had a mean age of 32.5 years (range 6–62 years) and originated from Norway (n = 8), Algeria (n = 7), the United Arab Emirates (n = 3), and the USA (n = 1) (Table 1). In the previously published Norwegian family, individuals 1.1 and 1.2 are siblings and 1.3 is their third cousin. There are two affected siblings in families 2, 8, 9, and 10, and three affected in family 6. The adults gave informed consent to the investigation and publication of the results. The healthy individuals were not subject to clinical investigation, whereas the affected individuals have all been examined by neurologists, ophthalmologists, and otologists (Table 1). The study was approved of by the Regional Ethics Committee of Western Norway and by the local ethics committees of the University Hospitals of Bonn, Constantine, and Algiers.

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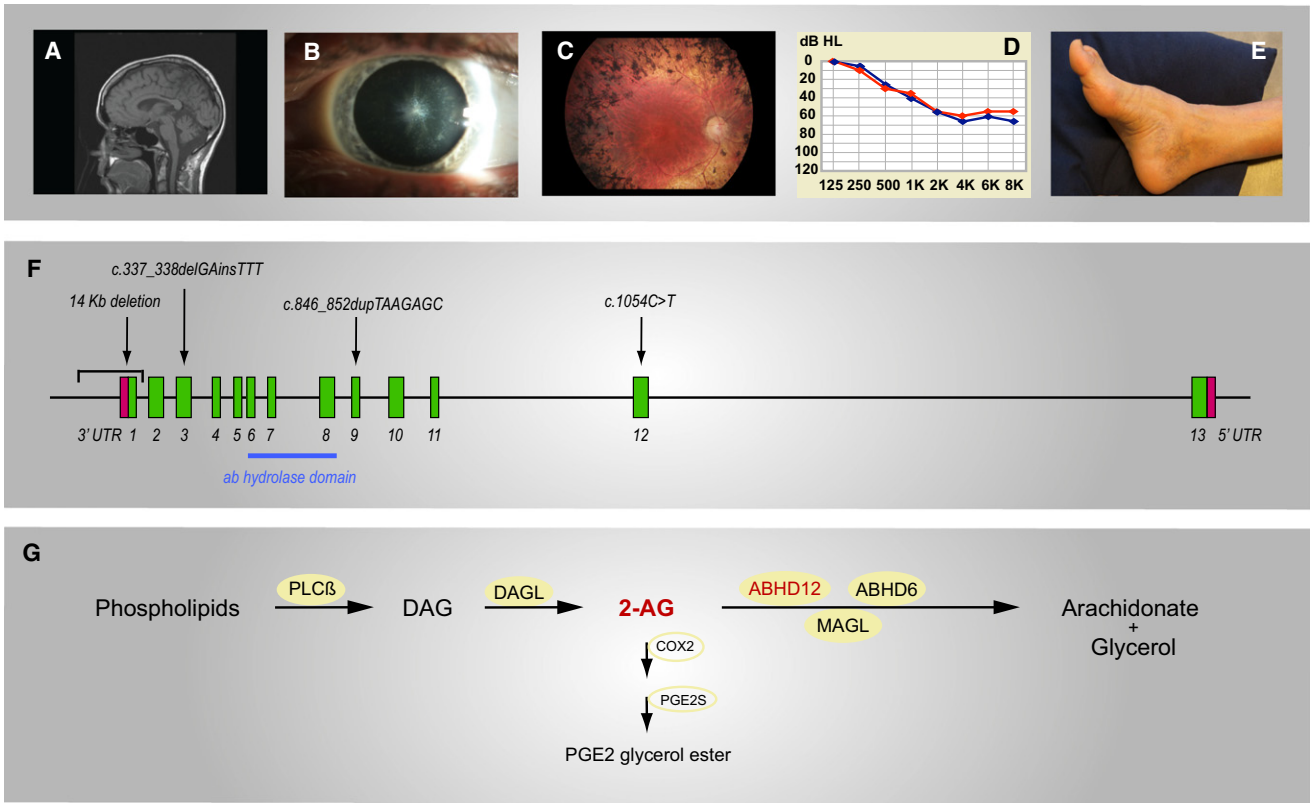

Figure 1. Phenotype and Genotype of PHARC Patients with Genetic Disruption of the 2-AG-Hydrolyzing Enzyme ABHD12
(A–E) Main symptoms in PHARC patients are shown. A summary of the symptoms and findings in each case is given in Table 1.
(A) MRI scan of an American female aged 50 (case 7.1) showing cerebellar atrophy.
(B) Star-shaped cataract of the posterior pole of the lens in a Norwegian male aged 24 (case 4.1).
(C) Fundus of a Norwegian male aged 56 (case 1.2) showing bone-spicule-shaped pigment deposits in the mid-periphery, pallor of the optic disc, attenuation of retinal vessels, and maculopathy.
(D) Audiogram of a Norwegian male aged 16 (case 5.1) showing sensorineural hearing loss of both right (red curve) and left (blue curve) ear, around 60 dB in the higher frequencies.
(E) Signs of peripheral neuropathy with pes cavus and hammertoes in a Norwegian male aged 56 (case 1.2).
(F) The *ABHD12* gene is located 25,223,379–25,319,477 bp from pter on the reverse strand of chromosome 20 (NCBI build 36.3). Two isoforms containing the α/β-hydrolase domain have been identified, differing only in the last exon. The positions of the homozygous mutations found in the families from the Emiraties (14 Kb deletion), Norway (c.337_338 delGAinsTTT), Algeria (c.846_852 dupTAA GAGC), and USA (c.1054C>T) are indicated.
(G) 2-AG is formed nearly exclusively by the hydrolysis of diacylglycerol (DAG), catalyzed by DAG lipases (α or β). The main pathway for formation of DAG from phospholipids is catalyzed by phospholipase Cß (PLCß). Several enzymes are responsible for the breakdown of 2-AG to arachidonate and glycerol. Although MAGL is responsible for 85% of the 2-AG hydrolysis in the mouse brain, ABHD12 and ABHD6 may be important for hydrolysis in specific cell types and/or cellular compartments. 2-AG is also a substrate for the inducible enzyme cyclooxygenase-2 (COX2), which is involved in neuroinflammation. COX2 converts 2-AG to the corresponding hydroperoxy derivative, which is further metabolized to prostaglandin E2 glycerol ester by prostaglandin E2 glycerol ester synthase (PGE2S).

From the same region as the original Norwegian family (family 1, Table 1),[4] we ascertained a further five, apparently unrelated, patients (including a brother and sister, family 2) with suspected PHARC disease (family 2-5, Table 1). Homozygosity mapping was performed with GeneChip 250K NspI arrays (GEO accession number GSE23151). The data were exported and treated for further analysis by the programs GTYPE and Progeny Lab. Regions of homozygosity were identified with the PLINK program[5]. All eight Norwegian patients from five families were homozygous for overlapping parts of the previously published 16 Mb region on chromosome 20 (Figure S1, available online), indicating distant relationship. The inclusion of these five additional patients enabled us to refine the candidate region to approximately 6.4 Mb (23,553,833–29,936,849 bp from pter, NCBI build 36.3). Twenty-three of approximately 60 genes in this region were sequenced, and a homozygous indel mutation in exon 3 in the *ABHD12* gene (c.337_338 delGAinsTTT; Figure 1F, Figure S2) was identified in all eight patients. The reference sequence for *ABHD12* was NM_001042472.1. This frameshift mutation predicts the replacement of an asparagine at codon 113 with phenylalanine leading to a downstream premature stop codon (p.Asp113PhefsX15). The mutation segregated fully with the disease in these families. We screened 190 local healthy blood donors and found two heterozygous carriers of this mutation, corresponding to a disease incidence of approximately 1/36,000 in this

Table 1. Clinical Findings and Results of Investigations in the 19 Patients with PHARC Disease

Family/ Case	Age (yr) and Sex	Sensory and Motor Neuropathy	Neurography and EMG	Sensorineural Hearing Loss	Ataxia	MR/CT of Brain	Pyramidal Tract Signs	Retinitis Pigmentosa	ERG	Cataract
Norway mutation: c.337_338delGAinsTTT [p.Asp113PhefsX15]										
1.1	62 F	38 years; pes cavus; sensory loss; absent ankle reflexes	Demyelinating polyneuropathy	Twenties	No	Normal	No	38 years	Rod-cone dystrophy	28 years
1.2	56 M	37 years; pes cavus from childhood	Demyelinating polyneuropathy	Thirties	37 years; gait ataxia	Normal	Extensor plantar response at lower limbs; spasticity; hyperreflexia	37 years	Rod-cone dystrophy	37 years
1.3	46 M	38 years; no pes cavus; sensory loss distally	Demyelinating polyneuropathy	From childhood	43 years; gait ataxia; upper limb intention tremor	Cerebellar atrophy	Extensor plantar response at lower limbs; spasticity; hyperreflexia	46 years	Rod-cone dystrophy	25 years
2.1	58 M	51 years; pes cavus; sensory loss; reduced tendon reflexes	Demyelinating/axonal polyneuropathy	Twenties	No	Cerebellar atrophy	Extensor plantar response at lower limbs	35 years	Rod-cone dystrophy	26 years
2.2	54 F	53 years; pes cavus; normal sensibility; reduced tendon reflexes	ND	Twenties	No	ND	No	25 years	Flat	25 years
3.1	36 F	Pes cavus; normal sensibility; reduced tendon reflexes in lower limbs	Demyelinating polyneuropathy	Deaf by the age of 10	Yes	Atrophy of vermis and medulla oblongata	Extensor plantar response at right side; spasticity	36 years	Rod-cone dystrophy	32 years
4.1	24 M	Pec cavus; hammertoes; reduced tendon reflexes in upper and lower limbs	Demyelinating polyneuropathy	Late in teens	No	Slight ventricular assymmetry. No cerebellar atrophy	Indifferent plantar response	No	Normal	15 years
5.1	16 M	Pes cavus; reduced sensibility; reduced tendon reflexes in upper limbs, absent in lower limbs	Demyelinating polyneuropathy	13 years	No	Normal	No	No	Normal	16 years (slight)
The Emirates mutation: 14 Kb deletion removing exon 1										
6.1	24 M	Pec cavus from childhood; absent tendon reflexes	Abnormal	Deaf by the age of 14	Mild	Normal	Indifferent plantar response	Twenties	ND	15 years
6.2	20 M	Pes cavus from age 4; absent tendon reflexes	Demyelinating polyneuropathy	6 years	2 years; gait, limb, and speech ataxia; wheelchair-bound from age 10	Cerebellar atrophy (age 3)	Extensor plantar response	Yes	ND	Yes
6.3	6 F	Absent tendon reflexes	ND	Yes	Speech and limb	Cerebellar atrophy	Indifferent plantar response	No	ND	Yes

Table 1. *Continued*

Family/ Case	Age (yr) and Sex	Sensory and Motor Neuropathy	Neurography and EMG	Sensorineural Hearing Loss	Ataxia	MR/CT of Brain	Pyramidal Tract Signs	Retinitis Pigmentosa	ERG	Cataract
USA mutation: c.1054C>T [p.Arg352X]										
7.1	50 F	34 years; pes cavus; hammertoes; sensibility slightly reduced	Abnormal	17 years	18 years; dysarthria; gait ataxia; jerky eye movements; tremor in hands	Cerebellar atrophy Increased signal in periventricular white matter.	Flexor plantar response; spasticity; preserved reflexes	Twenties	ND	22 years
Algeria mutation: c.846_852dupTAAGAGC [p.His285fsX1]										
8.1	11 M	Absent tendon reflexes and moderate muscle weakness of lower limbs; normal sensibility	ND	No	3-4 years; limb and gait ataxia; horizontal nystagmus; dysarthria; dysmetria upper and lower limbs; delayed walking at 15 month; action and intention tremor	Cerebellar atrophy	Extensor plantar response at lower limbs	No	ND	No
8.2	10 F	Absent tendon reflexes of lower limbs; normal sensibility	ND	No	4–5 years; gait ataxia	Vermian atrophy	Extensor plantar response at lower limbs	No	ND	No
9.1	44 M	Pes cavus; sensory loss; absent tendon reflexes at lower limbs; scoliosis	Demyelinating polyneuropathy	Yes	7–10 years; gait and limb ataxia; cerebellar dysarthria; dysmetria at upper limbs with adiadocokinesia; head titubation	Vermian atrophy	Extensor plantar response at lower limbs; macroglossia	amblyopia	ND	
9.2	26 F	Pes cavus; sensory loss; reduced tendon reflexes at upper limbs, and absent at lower limbs	Severe demyelinating polyneuropathy	Deaf	4–9 years; gait and limb ataxia; horizontal nystagmus; moderate dysarthria; dysmetria at upper and lower limbs	Vermian atrophy	Extensor plantar response at lower limbs; tongue fasciculations	Yes	ND	Yes
10.1	26 F	Pes cavus; sensory loss; absent tendon reflexes	Severe demyelinating polyneuropathy on nerve biopsy	6 years	6–12 years; gait and limb ataxia	Normal	Indifferent plantar response	No	ND	No
10.2	19 F	12 years; pes cavus; sensory loss; absent tendon reflexes at upper and lower limbs	ND		No	ND			ND	
11.1	32 F	Pes cavus; sensory loss and absent tendon reflexes at lower limbs	Axonal polyneuropathy	Yes	16–20 years; gait ataxia; dysarthria; dysmetria at upper limbs	Cerebellar atrophy	Extensor plantar response at lower limbs	Decreased visual acuity and amblyopia	ND	No

Data on patients from four different countries (11 families) are shown. All individuals in one family are siblings, except for 1.3, who is the third cousin of 1.1 and 1.2. All adult patients have polyneuropathy of demyelinating type and sensorineural hearing loss (three patients are deaf), and nearly all adult patients have developed cataracts. Retinitis pigmentosa is typically recognized in the twenties or thirties. Ataxia is present in about half of the patients, with cerebellar atrophy and pyramidal tract signs like spasticity and extensor plantar response. The onset of ataxia is highly variable, starting particularly early in the families from the Emirates and Algeria.

population. This indicates that the frequency of PHARC in Western Norway is comparable to, or may be even higher than, relevant differential diagnoses like Friedreich ataxia and Refsum disease.

Concurrent mapping studies in one family from the United Arab Emirates and four families from Algeria were performed with Genechip 10K XbaI arrays followed by analysis on selected individuals with the GeneChip 6.0 array (Affymetrix, Santa Clara, USA). Regions of homozygosity were identified with the HomoSNP software (Figure S3). These patients, initially diagnosed with recessive ataxia, defined a 5.5 Mb linkage interval in the 20p11.21-q12 region on chromosome 20 (24,393,550–29,940,293 bp from pter, NCBI build 36.3, Figure S1). Twelve of the 29 genes of this region were sequenced, and a 14 Kb deletion (g.25,312,257_25,326,263 del14007insGG, NCBI Ref.Seq: NC_000020.10) in *ABHD12*, encompassing the promoter region and exon 1 of the gene (Figure 1F, Figures S4A–S4C), was identified in the family from the Emirates. No copy-number variations in this region have been reported to the Database of Genomic Variants (hg 18). The seven patients in the four Algerian families were homozygous for a 7 bp duplication in exon 9 (c.846_852 dupTAAGAGC) in *ABHD12* (Figure S2), which directly replaces the histidine residue at codon 285 with a stop codon (p.His285fsX1). Also in these families the mutation segregated fully with the disease. Finally, a patient from the USA of French-Canadian heritage with suspected PHARC disease was found to be homozygous for a nonsense mutation (c.1054C>T) in exon 12 in *ABHD12* (Figure 1F, Figures S1 and S2), leading to a predicted stop codon in position 352 in the protein (p.Arg352X). The finding of four different deleterious *ABHD12* mutations in a total of 19 patients with PHARC disease from four countries clearly supports a causal genotype-phenotype relationship.

The addition of several new families requires refinement of our earlier clinical description.[4] The essential clinical features are summarized in Figures 1A–1E and Table 1. PHARC in the Norwegian patients, and in the single American patient, appears to be a slowly progressive disease with recognition of the first symptoms typically in the teens. Cataracts, hearing loss, and a predominantly demyelinating peripheral neuropathy are present in all adult patients (Table 1), whereas the presence and extent of ataxia is variable. Retinitis pigmentosa typically presents in young adult life (twenties or thirties), and electroretinograms in most patients show a rod-cone dysfunction. The disorder in families from Algeria and the Emirates shows an earlier onset of ataxia that has both central and peripheral characteristics (Table 1). No evidence of behavioral disturbances or abnormalities related to appetite was detected in our adult patients. Cerebral cortical function appears to be spared, with only one patient having mental retardation (case 9.1) and another epilepsy (case 7.1, myoclonic seizures). Adult heterozygous carriers of *ABHD12* mutations do not have an obvious phenotype, implying that their residual enzyme activity is sufficient to avoid clinical symptoms.

Each of the four different *ABHD12* mutations is interpreted as a null mutation that would either abolish or severely reduce the activity of the encoding enzyme, α/β-hydrolase 12 (ABHD12). PHARC may, therefore, be considered a human *ABHD12* knockout model. The question also arises whether less detrimental mutations may cause various incomplete phenotypes. The serious and progressive disease seen in our patients suggests that ABHD12 performs an essential function in the peripheral and central nervous systems and in the eye. This is supported by the high expression of *ABHD12* in the brain, with a striking enrichment in microglia (Figure 2), as shown by our replotting of data from GNF Mouse Gene Atlas V3. Expression is also high in macrophages. Currently, the only known substrate for ABHD12 is the main endocannabinoid 2-arachidonoyl glycerol (2-AG) (Figure 1G). This compound has important functions in synaptic plasticity[6,7] and neuroinflammation.[8,9] In acute ischemia and/or excitotoxicity, 2-AG appears to have neuroprotective properties,[9–11] but the effects of long-term increased levels of this metabolite have not been investigated.

The endocannabinoid signaling system is the focus of increasing scientific interest, in part because of the potential for developing novel therapeutic agents.[11–13] The system is tightly regulated and appears to be important for many physiological processes including neurotransmission, pain appreciation, appetite, mood, addiction behavior, body temperature, and inflammation.[11] Key players in these pathways are the G protein-coupled cannabinoid receptors CB1 and CB2 and their endogenous ligands, endocannabinoids, as well as enzymes that synthesize or hydrolyze these ligands.[14] The most abundant endocannabinoid, 2-AG, (Figure 1G) is formed on demand from the membrane lipid diacylglycerol (by diacylglycerol lipase α or β).[14] Endocannabinoids act locally as lipid transmitters and are rapidly cleared by hydrolysis. Interestingly, our patients did not show overt cannabinomimetic effects.

Several enzymes are involved in 2-AG hydrolysis[15,16] (Figure 1G), and there is evidence that these enzymes are differentially expressed in various cell types[17] and cellular compartments.[7,16,17] In the mouse brain, monoacylglycerol lipase (MAGL) accounts for 85% of the hydrolase activity,[11,17] with additional contributions from ABHD12 and α/β-hydrolase 6 (ABHD6).[16] The apparent paradox of a purported minor role of ABHD12 in 2-AG hydrolysis versus the serious PHARC phenotype in the brain and eye suggests either that ABHD12 is of crucial importance only in certain cell types[12] or that it is also acting on a hitherto unknown substrate other than 2-AG. The finding that microglial cells have a particularly high expression of *ABHD12*, but very low levels of *MGLL* (encoding MAGL) and *ABHD6* (Figure 2), indicates that the former alternative of differential cellular expression exists. Moreover, microglia dysfunction is known to be involved in

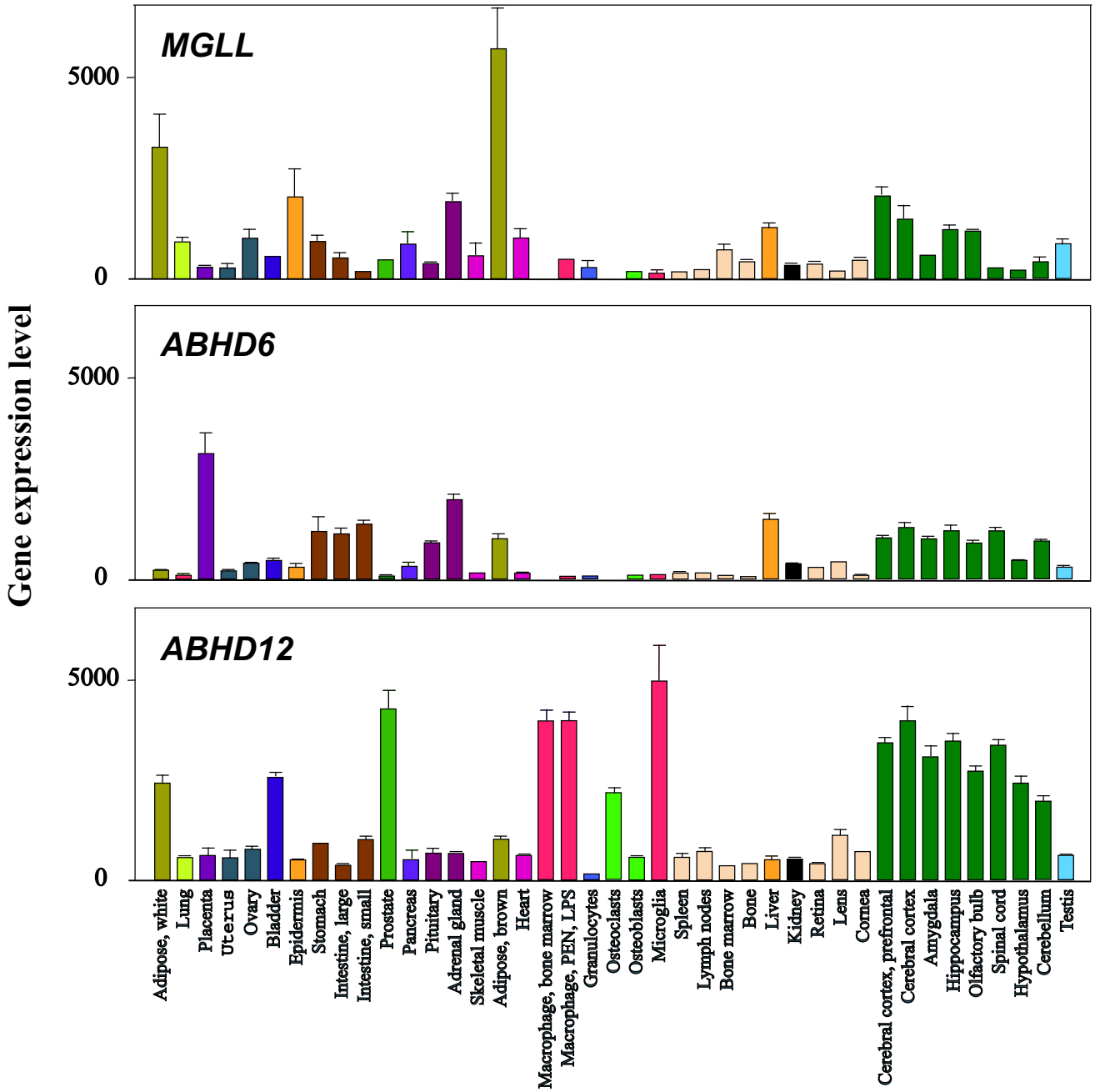

Figure 2. Gene Expression of *ABHD12*, *ABHD6*, and *MGLL* in Mouse Tissues
This is a replot of a subset of the GNF Mouse GeneAtlas V3 data, provided by Lattin et al.[23] The data are published online in the BioGPS database under the alias GeneAtlas MOE430, and the NCBI GEO accession number is GSE10246. There is high expression of *ABHD12*, *ABHD6*, and *MGLL* (encoding MAGL) in different brain tissues (dark green bars). The highest level of *ABHD12* is found in microglia (red bar, lower panel), and the expression is also high in the related cell types macrophages (red bars) and osteoclasts (light green bar). There is scarce expression of both *ABHD6* (mid panel) and *MGLL* (upper panel) in microglia, macrophages, and osteoclasts. Bars represent the mean of two biological replicates (RNA from two separate pools from independent mice), and error bars show standard error of the mean. Regarding eye tissue, however, bars are the mean of two technical replicates (RNA from the same pool was split for two amplifications).

neurodegenerative diseases[18] as well as in retinal dystrophies.[19] Whether ABHD12 acts on more than one substrate is currently unknown, but many hydrolases have overlapping functions, including MAGL, which is involved in lipolysis[20] as well as in hydrolyzing 2-AG.

Despite great interest in manipulating 2-AG hydrolysis in vivo,[8,21] knockout animal models have not yet been developed, and only recently a blocker of MAGL with substantial effect in vivo was reported.[22] Notwithstanding this, inhibition of endocannabinoid hydrolases, including ABHD12, has been suggested as a potential therapy for neurodegenerative diseases such as multiple sclerosis.[21] However, the consequences of irreversible loss of *ABHD12* function, as seen in our patients with PHARC, may serve

as a cautionary reminder that any potential drug inhibiting this enzyme be thoroughly evaluated with respect to the potential risk of severe long-term adverse effects.

In conclusion, mutations in the *ABHD12*-gene causes PHARC, a disease with serious dysfunction of the central and peripheral nervous systems, as well as hearing loss and impaired vision. Our findings have implications for clinicians working with both children and adults and suggest disrupted endocannabinoid metabolism as a cause of neurodegenerative disease.

Supplemental Data

Supplemental Data include four figures and can be found with this article online at http://www.cell.com/AJHG/.

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Web Resources

The URLs for data presented herein are as follows:

BioGPS database, http://biogps.gnf.org
Database of Genomic Variants, http://projects.tcag.ca/variation/?source=hg18
NCBI Build 36.3, http://www.ncbi.nlm.nih.gov/mapview
NCBI Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

Accession Numbers

Microarray data have been deposited in NCBI's Gene Expression Omnibus (GEO) and are accessible through GEO Series accession number GSE23151.

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