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Alteration of ornithine metabolism leads to dominant and recessive hereditary spastic paraplegia

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Hereditary spastic paraplegias are heterogeneous neurological disorders characterized by a pyramidal syndrome with symptoms predominantly affecting the lower limbs. Some limited pyramidal involvement also occurs in patients with an autosomal recessive neurocutaneous syndrome due to *ALDH18A1* mutations. *ALDH18A1* encodes delta-1-pyrroline-5-carboxylate synthase (P5CS), an enzyme that catalyses the first and common step of proline and ornithine biosynthesis from glutamate. Through exome sequencing and candidate gene screening, we report two families with autosomal recessive transmission of *ALDH18A1* mutations, and predominant complex hereditary spastic paraplegia with marked cognitive impairment, without any cutaneous abnormality. More interestingly, we also identified monoallelic *ALDH18A1* mutations segregating in three independent families with autosomal dominant pure or complex hereditary spastic paraplegia, as well as in two sporadic patients. Low levels of plasma ornithine, citrulline, arginine and proline in four individuals from two families suggested P5CS deficiency. Glutamine loading tests in two fibroblast cultures from two related affected subjects confirmed a metabolic block at the level of P5CS *in vivo*. Besides expanding the clinical spectrum of *ALDH18A1*-related pathology, we describe mutations segregating in an autosomal dominant pattern. The latter are associated with a potential trait biomarker; we therefore suggest including amino acid chromatography in the clinico-genetic work-up of hereditary spastic paraplegia, particularly in dominant cases, as the associated phenotype is not distinct from other causative genes.

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Abbreviations: HSP = hereditary spastic paraplegia; P5CS = delta-1-pyrroline-5-carboxylate synthase

Introduction

Hereditary spastic paraplegias (HSPs) are a family of neurodegenerative disorders mainly affecting the corticospinal tract. Clinically, they are characterized by a pyramidal syndrome with paraparesis and spasticity, primarily detectable in the lower limbs and in the gait, and classified as either pure or complex, according to the absence or presence of accompanying neurological or extra-neurological signs (Blackstone, 2012; Finsterer *et al.*, 2012). Genetically, they can follow all modes of inheritance: autosomal dominant, autosomal recessive, X-linked and mitochondrial. Many genes have already been described, and more are regularly identified (Novarino *et al.*, 2014); in this respect, next generation sequencing techniques are powerful tools to elucidate the genetic basis of HSPs, either for known gene screening or for new gene identification.

In the last decade, mutations in *ALDH18A1* have been implicated in an autosomal recessive neurocutaneous syndrome, characterized by severe developmental delay with marked cognitive impairment, associated with progeroid features, cutis laxa, joint hyperlaxity, short stature, cataract and frequent microcephaly (Baumgartner *et al.*, 2000, 2005; Bicknell *et al.*, 2008; Skidmore *et al.*, 2011; Martinelli *et al.*, 2012; Zampatti *et al.*, 2012; Fischer *et al.*, 2014; Gardeitchik *et al.*, 2014; Handley *et al.*, 2014; Wolthuis *et al.*, 2014). Pyramidal signs were reported in 12 of 15 patients identified so far, but were often limited to brisk deep tendon reflexes. *ALDH18A1* encodes delta-1-pyrroline-5-carboxylate synthase (P5CS), an enzyme that catalyses the first common steps of proline and ornithine biosynthesis from glutamate (Hu *et al.*, 1999). Plasma amino acid levels were abnormal in three reported families (Baumgartner *et al.*, 2000, 2005; Martinelli *et al.*, 2012; Fischer *et al.*, 2014), with low proline, ornithine (2/3), citrulline and arginine (3/3) levels associated with mild fasting hyperammonaemia. In this paper, we report seven pedigrees with *ALDH18A1* mutations segregating in a recessive or, for the first time, a dominant inheritance mode associated with abnormal plasma amino acid levels.

Materials and methods

Patient recruitment and clinical evaluation

Families FSP410, FSP429, FSP470, FSP856 and SR45 were of French ($n = 2$), Spanish ($n = 1$), Italian ($n = 1$) or Portuguese ($n = 1$) ancestry and had been identified as part of the SPATAX (https://spatax.wordpress.com/) cohort of patients with HSP. Two sporadic cases were Caucasian, from Australia/UK and USA. All patients were examined by at least one of the co-authors. Blood samples were obtained after informed and signed consent according to local ethics regulations. DNA was extracted using a standard protocol.

Whole genome mapping and exome sequencing in Family FSP410

Whole genome linkage analysis was performed using Illumina LINKAGE_12 SNP microarrays. Genotypes were determined

using Beadstudio (Illumina) and analysed with MERLIN 1.0 (Abecasis *et al.*, 2002) (Supplementary Fig. 1). Exome sequencing was performed on four affected subjects (Patients 6, 8, 25 and 29; Fig. 1). Targeted capture was done with the Agilent SureSelect Human All Exon Capture V4 XT 51MB kit. Paired-end 100 bp sequencing was performed on an Illumina HiSeq2000 system. Sequence reads were trimmed using FastX and duplicate reads were removed by Picard v1.59 (http://picard.sourceforge.net). Sequence reads were aligned to the human genome reference (hg19) using the Burroughs-Wheeler algorithm v0.6.2, followed by a local realignment around indels (insertions/deletions) using the Broad Institute Genome Analysis Tool Kit (GATK v1.4; DePristo *et al.*, 2011). Variants (SNP/INDEL) were called using GATK's Unified Genotyper module and annotated with Annovar (Wang *et al.*, 2010). They were sorted according to their localization in putatively linked or not excluded loci, expected transmission mode (heterozygous in all four individuals), frequency lower than 0.1% in public databases (CompleteGenomics, http://www.completegenomics.com/public-data/69-Genomes/; EVS, http://evs.gs.washington.edu/EVS; 1000genomes, http://www.1000genomes.org/; Exome Aggregation Consortium, http://exac.broadinstitute.org/), absence in internal controls, frequency in internal database (<5%), effect on the coding sequence (non-synonymous, splice site, frameshift or nonsense), coverage above 10×, and amino acid conservation score (GERP + + > 0, (http://mendel.stanford.edu/SidowLab/downloads/gerp/). Segregation in all affected individuals was checked through Sanger sequencing according to standard protocols.

ALDH18A1 analysis in 435 exomes of index patients with hereditary spastic paraplegia

To assess the frequency of *ALDH18A1* mutations in autosomal dominant HSP, exome sequencing data of 160 index patients with autosomal dominant HSP were examined. Because of the previous description of *ALDH18A1* mutations transmitted in an autosomal recessive mode of inheritance, the search was extended to 275 index patients with autosomal recessive or sporadic HSP. Exome data were shared and are available (GEM.app database, https://genomics.med.miami.edu/) as part of an international collaborative effort (Gonzalez *et al.*, 2013).

Panel sequencing in 95 index patients with hereditary spastic paraplegia

An amplicon-based panel including all exons of *ALDH18A1* was designed in-house with Primer3Plus (Supplementary Table 1). For 95 patients with autosomal dominant and recessive HSP selected from the SPATAX cohort, mostly with either European (32/45 with available origin) or North African (12/45) ancestry, all amplicons were amplified using the Fluidigm Access Array technology (IFC Controller AX, FC1 Cycler, 48.48 Access Arrays), and sequenced on the MiSeq Illumina sequencer as paired-end 2 × 250 bp reads. The Burrows-Wheeler algorithm v0.7.8 was applied to align sequence reads to the UCSC Genome Browser hg19 version of the human genome and variants were called via the GATK software package v3.1-1 after realignment and recalibration. Variants meeting the aforementioned criteria were confirmed using Sanger sequencing, and segregation in other affected members in the family was verified, when possible. In all *ALDH18A1*-mutated index cases, mutations in 74 previously described HSP genes were excluded with a Roche/Nimblegen capture in-house panel followed by MiSeq sequencing (unpublished data).

Biochemical analyses

Plasma amino acid profiles were obtained by ion-exchange chromatography with standard ninhydrin detection (JEOL Aminotac JLC 500/V) as part of routine diagnostic work-up for Patients FSP410-13, -29, -32, FSP429-21, and FSP856-18 on blood and urine samples. The results were compared with those obtained from the first family published with *ALDH18A1* mutations (Baumgartner *et al.*, 2005) and from the full cohort of patients from the Necker Hospital (Paris) between 1995 and 2013 ($n > 53\,000$), after exclusion of patients <15 years or with a known metabolic disorder, leading to a final set of 5023 patients. Age normalization was performed by locally weighted regression (loess) on a reference hospital population of 5043 individuals (data not shown).

Dermal fibroblasts from Patients FSP410-29 and FSP410-32 were grown in Dulbecco's modified Eagle's medium with 10% foetal bovine serum and 1% penicillin-streptomycin in a 37°C incubator with 5% CO_2. After removing the culture medium, they were incubated with 2.5 mM glucose and 1 mM $^{13}C_5$-stable isotope labelled glutamine in phosphate buffer for 18 h. Incubation was quenched by methanol, samples were silylated [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane] and analysed by gas chromatography coupled to a triple quadrupole mass spectrometer (Scion, Brüker). Quantification of labelled versus natural isotope ions was performed in selected reaction monitoring mode.

Results

Identification of a mutation in *ALDH18A1* in Family FSP410

Under an autosomal dominant inheritance model, whole genome linkage analysis in Family FSP410 identified five putatively linked loci with multipoint logarithm of odds (LOD) scores reaching the maximal expected values for this pedigree (ranging from +1.64 to +1.95) as well as various uninformative regions with LOD scores varying from −1.90 to +0.70 (Supplementary Fig. 1). Whole exome sequencing performed in four patients provided 117 to 130 million reads per sample, 98% of which could be aligned to the targeted sequence. Mean depth of the targeted sequence was 125- to 132-fold. From 114 285 to 119 137 SNPs (single nucleotide polymorphisms) and from 10 546 to 11 159 indels were identified. Two missense variants respecting the abovementioned criteria segregated with the disease in the entire family: a c.359T > C/p.V120A

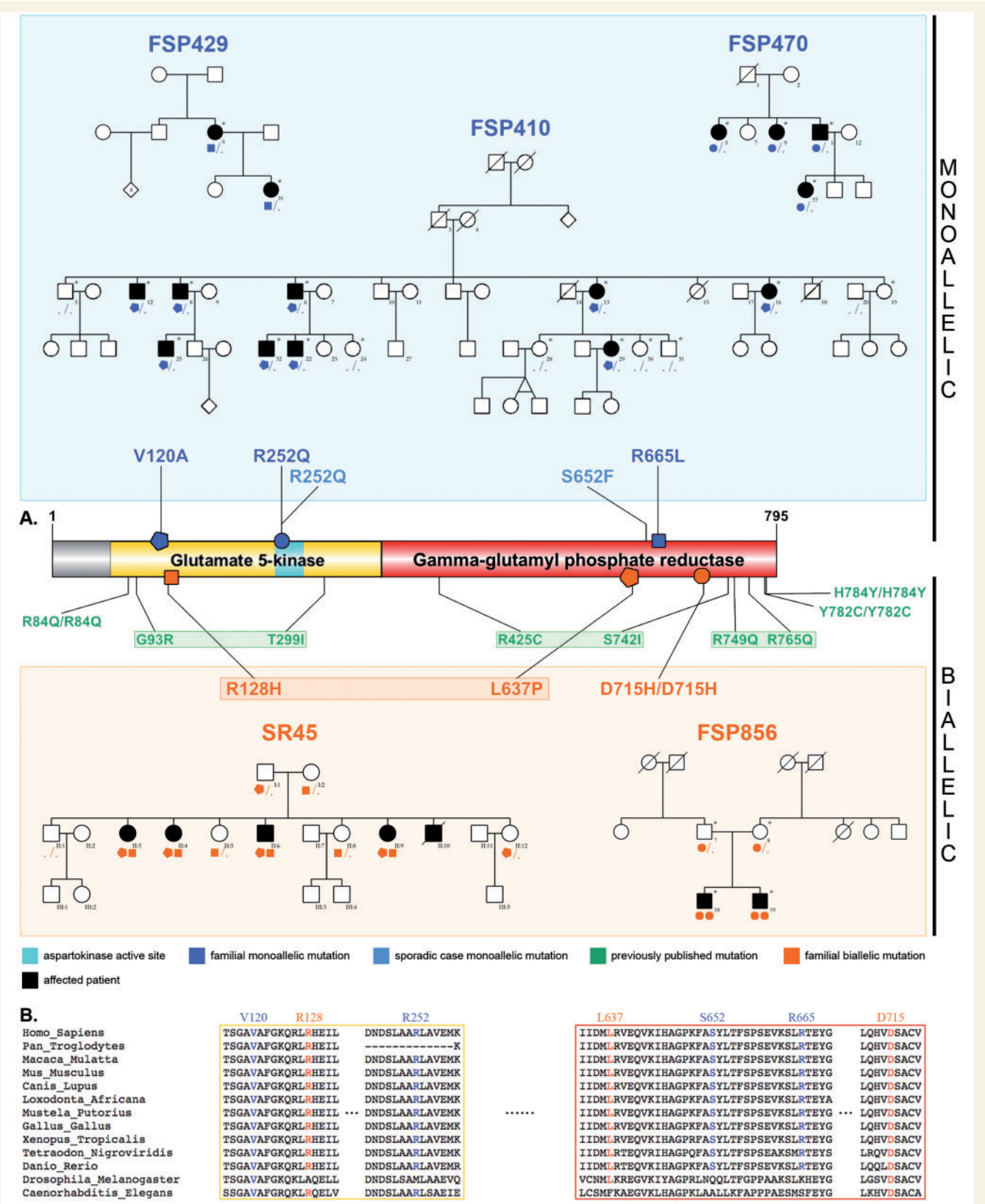

Figure 1 Pedigrees, *ALDH18A1* mutations and conservation across species. (**A**) Schematics of P5CS and its gamma-glutamyl kinase and gamma-glutamyl phosphate reductase domains. Newly described monoallelic mutations (blue), newly described biallelic mutations (orange), and previously reported biallelic mutations (green) are represented, along with pedigrees of the families of this study, showing the segregation. Sampled individuals are indicated with an asterisk. In autosomal dominant families, all affected patients carry one *ALDH18A1* mutation; the penetrance seems to be complete as no tested unaffected family member has any mutation. (**B**) Alignment of P5CS paralogues, showing the high conservation of all affected residues reported.

(NM_002860.3) change in *ALDH18A1* (OMIM 138250), at chr10:g.97 397 138; and a c.2250G > T/p.W750C (NM_001195263) change in *PDZD7* (OMIM 612971), at chr10:g.102 770 396. Only the *ALDH18A1* variant was predicted to be deleterious by all four prediction software used (Table 1) and was located in a functional domain of the protein. It affected a highly conserved amino acid across species (Fig. 1). *ALDH18A1* was previously implicated in an autosomal recessive syndrome that associates cutaneous, ocular, metabolic and neurological findings, with pyramidal signs (Baumgartner *et al.*, 2005), often limited to brisk reflexes; *PDZD7* contributes to Usher syndrome type II (OMIM 605472), either as a modifier or in a digenic pattern of transmission (Ebermann *et al.*, 2010). We found no additional causative *PDZD7* variants in 95 HSP index cases and had less *in silico* elements in favour of its pathogenicity; we therefore focused on *ALDH18A1*.

Additional families with *ALDH18A1* mutations

To confirm the implication of *ALDH18A1* in autosomal dominant HSP, and further assess its potential role in autosomal recessive HSP, we screened 530 patients through whole exome sequencing ($n = 435$) or panel sequencing ($n = 95$). Segregating heterozygous variants were found in two French families compatible with an autosomal dominant inheritance mode: a c.1994G > T/p.R665L change at g.97 371 129 in Family FSP429, and a c.755G > A/p.R252Q change at g.97 392 769 in Family FSP470. The same p.R252Q change was also identified in Patient 25014 with pure HSP living in the USA. Reference haplotypes for two SNPs flanking the *ALDH18A1* mutation (rs 9787589, rs 10882640) allowed excluding the existence of a common ancestor with Family FSP470, that presents homozygous variants at both positions. A heterozygous c.1949G > T/p.S652F change at g.97 371 198 was identified in a sporadic patient from Australia/UK (Patient GSHSP44). In two families with complex autosomal recessive HSP, biallelic variants were identified: compound heterozygosity for a c.1910T > C/p.L637P change at g.97 373 512 and a c.383G > A/p.R128H change at g.97 397 114 in Family SR45 from Portugal, and a homozygous c.2143G > C/p.D715H change at g.97 370 017 in Spanish Family FSP856. All mutations affected conserved amino acids and were absent or rare in exome databases (Fig. 1 and Table 1).

Clinical characteristics of patients with *ALDH18A1* mutations

In Family FSP410, clinical assessment was available for seven of nine patients (Table 2). The common clinical picture was a complex, slowly progressive but finally severe HSP, with onset in adolescence or adulthood (ranging from 14 to 59 years) and predominant gait signs, motor

Table 1 Characteristics of novel *ALDH18A1* mutations

Family	Mutation characteristics		Database frequency			Pathogenicity prediction					Conservation	
	hg19 (chr10)	cDNA and protein changes	dbSNP 138	EVS	ExAC	SIFT score	PolyPhen-2 HDIV	PolyPhen-2 HVAR	LRT score	MutationTaster score	GERP + + score	PhyloP score
FSP410	97397138	NM_002860.3: c.359T > C; p.Val120Ala	0	0	0/122000 +	0 (D)	1 (D)	0.995 (D)	D	D (0.999993)	5.6	2.134
FSP429	97371129	NM_002860.3: c.1994G > T; p.Arg665Leu	0	0	0/122000 +	0.06	0.949 (P)	0.78 (P)	D	D (0.999969)	5.6	2.639
FSP470	97392769	NM_002860.3: c.755G > A; p.Arg252Gln	0	0	0/122000 +	0.23	0.997 (D)	0.941 (D)	D	D (0.999779)	5.97	2.832
GSHSP44	97371168	NM_002860.3: c.1955C > T; p.Ser652Phe	0	0	0/122000 +	0.02 (D)	0.892 (P)	0.694 (P)	D	D (0.999952)	5.6	2.639
25014	97392769	NM_002860.3: c.755G > A; p.Arg252Gln	0	0	0/122000 +	0.23	0.997 (D)	0.941 (D)	D	D (0.999779)	5.97	2.832
FSP856	97370017	NM_002860.3: c.2143G > C; p.Asp715His	0	0	1/122892 - 0 hmz	0 (D)	1 (D)	1 (D)	D	D (0.999999)	5.43	2.722
SR45 – mutation 1	97397114	NM_002860.3: c.383G > A; p.Arg128His	0	0	4/122890 - 1 hmz	0.01 (D)	0.999 (D)	0.978 (D)	D	D (0.999981)	5.6	2.642
SR45 – mutation 2	97373512	NM_002860.3: c.1910T > C; p.Leu637Pro	0	0	0/122000 +	0 (D)	1 (D)	0.999 (D)	D	D (0.999999)	6.07	2.326

Summary of characteristics for all variants reported. The database frequencies were looked for in dbSNP138 (http://www.ncbi.nlm.nih.gov/SNP/snp_summary.cgi), Exome Variant Server (EVS, http://evs.gs.washington.edu/EVS) and Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/). Pathogenicity scores are evaluated as follows: SIFT (http://sift.jcvi.org/) predicts deleteriousness (D) under 0.05; PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) classifies SNPs as probably damaging (D; HDIV ⩾ 0.957, HVAR ⩾ 0.909), possibly damaging (P; 0.453 ⩽ HDIV ⩽ 0.956, 0.447 ⩽ pp2_hdiv ⩽ 0.908), or benign (B; HDIV ⩽ 0.452, HVAR ⩽ 0.446); LRT (Chun and Fay, 2009) differentiates variants with deleterious (D), neutral (N) or unknown (U) effect; MutationTaster (http://www.mutationtaster.org/) classifies them as 'disease_causing_automatic' (A), 'disease_causing' (D), 'polymorphism' (N) or 'polymorphism_automatic' (P) with a given probability value, 1 being the most probable. For both GERP + + (http://mendel.stanford.edu/SidowLab/downloads/gerp/) and PhyloP (http://compgen.bscb.cornell.edu/phast/help-pages/phyloP.txt), higher scores indicate better residue conservation.
Hmz = homozygous.

Table 2 Clinical characteristics of patients with *ALDH18A1* mutations: autosomal dominant families

Family N°/origin	FSP410/Italy							FSP429/France		FSP470/France				Spo/USA	Spo/Australia and UK
ALDH18A1 variant/ inheritance	p.Val120Ala/Autosomal dominant							p.Arg665Leu/Dominant		p.Arg252Gln/Dominant				p.Arg252Gln/ Sporadic	p.Ser652Phe/ Sporadic
Individual N° (sex)	29 (F)	13 (F)	6 (M)	32 (M)	8 (M)	16 (F)	22 (M)	6 (F)	21 (F)	5 (F)	9 (F)	11 (M)	22 (F)	25014 (F)	GSHSP44 (F)
Age at exam (years)	45	70	71	43	61	50	28	66	37	52	50	48	20	76	46
Age at onset (years)	18	22	49	14	59	42	?	24	13	19	21	43	1	31	44
Symptoms at onset	Muscle cramps	Weakness	Stiff legs	Stiff legs	Pain and weakness in LL	Stiff legs, mild instability	Reflex pyramidal syndrome	Stiff legs	Unsteadiness	Falls	Stiff legs	Stiff legs	Stiff legs	Falls	Toe walking, tripping
Disease duration (years)	27	48	22	29	2	25	?	34	24	33	29	5	19	45	12
Disability score (max 7)	4/7	5/7	7/7	3/7	NA	3/7	0/7	6/7	4/7	3/7	4/7	4/7	3/7	5/7	4/7
Spasticity at gait	Severe	Severe	Severe	Moderate	NA	Moderate	None	Severe	Severe	Moderate	Severe	Moderate	Moderate	Moderate	Mild
Spasticity at rest	Mild Ashworth 1/4	Mild	Severe	Mild	NA	Mild	None	None Ashworth 0/4	Moderate	Moderate Ashworth 2/4	Mild Ashworth 2/4	Mild	Mild	Severe	Mild
Weakness	Distal > proximal	Diffuse (3/5 MRC grading 4 limbs)	Proximal, distal	Distal > proximal (4 limbs)	L > R	Distal (3/5) > proximal	None	Distal	Distal > proximal	Proximal, distal	Proximal, distal	Proximal, distal	Proximal, distal	Distal > proximal	Distal > proximal
Increased reflexes LL	Yes (ankles abolished)	Yes (ankles abolished)	Yes (ankles abolished)	Yes (ankles abolished)	Yes	Yes	Yes	Yes (ankles decreased)	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Increased reflexes UL	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Normal	Yes	Yes	Yes	Yes	Yes	Yes
Extensor plantar response	Indifferent	Yes	Yes	Indifferent	Yes (R)	Yes	R indifferent	Yes	Indifferent	Yes	Yes	Yes	Yes	Yes	Yes
Hoffman sign	NA	NA	NA	Yes	Yes	Yes	Yes	NA	Yes	Yes	Yes	Yes	No	NA	NA
Decreased vibration sense at ankles	ND	ND	Yes	Mild	NA	No	No	No	Mild	No	No	No	No	Moderate	No
Urinary symptoms	Pollakiury	No	Yes	Pollakiury	NA	Yes	No	No	Urinary incontinence	Urinary urgency	Urinary urgency	No	No	Urinary urgency	Urinary urgency
Cerebellar gait	No	No	Mild	Mild	NA	Mild	No	Yes (CCFS 0.939)	No	No	No	No	No	No	No
Cerebellar signs UL	No	No	Mild	No	NA	No	No	Yes	No	No	No	No	No	No	No
Dysarthria	Spastic	No	Anarthria (age 70)	Spastic	NA	Spastic	No	No	Yes	No	No	No	No	No	No
Cognitive impairment	No	No	Dementia (age 69)	No	Mild memory impairment	Mild memory impairment	No	No	No	No	No	No	No	No	No
Cutaneous findings	No	No	No	No	NA	No	No	No	No	No	No	No	No	Chronic cellulitis changes	No
Ocular findings	NA	NA	NA	NA	NA	NA	Congenital cataract	NA	NA	NA	NA	NA	Cataract (age 8 months)	Bilateral senile cataracts (69)	NA
Other clinical features	Pes cavus, neuropathic pain	Neuropathic pain	Low back pain, neuropathic pain (LL)	Pes cavus, infantile psychosis, mild horizontal nystagmus		Neuropathic pain (LL), gaze nystagmus, neurosensory deafness, temporal epilepsy	Mild pes cavus	Pes cavus	Pain (knee and back)	Pes cavus	Pes cavus, tremor (left UL)	Mitral leak	Gastrointestinal reflux	Resting tremor, uterine cancer (69)	Pes cavus, hip replacement, groin pain
MRI	Normal brain MRI (2002)	ND	ND	Mild CC atrophy	Normal brain MRI	Large cisterna magna on MRI	NA	White matter anomalies, pons hypersignal, thin dorsal cord	Thin dorsal cord	NA	Left arachnoidien cyst	NA	NA	Cortical atrophy (frontoparietal predominance), chronic microvascular disease (69)	Spinal cord atrophy
Other paraclinical tests	ENMG: motor neuronopathy (2009)	NA	NA	ENMG: motor neuronopathy (2014)	Normal ENMG	ENMG: motor neuronopathy (LL, L>R) (2004)	NA	NA	NA	NA	NA	NA	NA	NA	NA

CC = corpus callosum; CCFS = composite cerebellar functional severity score; ENMG = electro-neuro-myogram; HC = head circumference; ID = intellectual delay; L = left; LL = lower limbs; MD = motor delay; MRC = medical research council; NA = not available; ND = not done; PMD = psychomotor delay; R = right; UL = upper limbs.

neuropathy (3/4 tested) and spastic dysarthria (4/6). Mild cerebellar signs in gait (3/6) and upper limbs (1/6) were described; one patient presented with dementia at age 69. One carrier (Patient FSP410-22) presented with congenital cataract but also showed, on last examination at age 28, an isolated mild pyramidal syndrome of the four limbs with enhanced upper limbs reflexes, brisk patellar reflexes, absent ankle reflexes, bilateral Hoffman signs and unilaterally indifferent plantar cutaneous reflex with no spasticity. Families FSP429 and FSP470 presented with moderate to severe spasticity in gait with few or no associated signs. Cerebellar signs were present in Family FSP429 (1/2), cataracts and gastrointestinal reflux in Patient FSP470-22. Brain MRI was abnormal in Patient FSP429-6 with mild FLAIR hyperintensity of the corticospinal tract posterior of the ventricles, a localized and heterogeneous T_2 hyperintensity in the pons, and moderate spinal cord atrophy (Supplementary Fig. 2). Both additional heterozygous cases presented with pure HSP, with spinal cord atrophy on MRI in Patient GSHSP44. Pes cavus was reported in 7 of 15 patients with monoallelic *ALDH18A1* variant.

In patients from autosomal recessive HSP Families FSP856 and SR45 (Table 3), cognitive impairment was primary, together with moderate to severe early onset HSP. Three of four patients in Family SR45 had a continuum of presentations from tetraparesis to tetraplegia, and two showed pseudobulbar palsy. Both brothers from Family FSP856 had upper limb postural tremor suggesting cerebellar involvement. They also presented microcephaly and facial dysmorphism (2/2), while patients in Family SR45 had short stature (4/4), cataract (1/4) and facial dysmorphism (3/4). Brain MRI showed a thin corpus callosum in patients from Family SR45 but was normal in one patient from Family FSP856 (Supplementary Fig. 2). Of note, both the father and the mother in Family FSP856 carry the heterozygous mutation, while none of them show clinical signs on examination.

Biochemical analyses in blood and dermal fibroblasts

Blood amino acid chromatography (Table 4) was performed in four patients from two unrelated autosomal dominant families. Three family members of Family FSP410 had low citrulline levels, associated with low proline (2/3), ornithine (1/3, 1/3 borderline), and borderline levels of arginine (3/3). A profound decrease of plasma citrulline was also observed in Patient FSP429-21, associated with low arginine and borderline ornithine levels. By contrast, amino acid levels were normal in Patient FSP856-18 with autosomal recessive inheritance (data not shown). In all tested patients, levels of other amino acids in blood and all amino acids in urine were within normal range. The amino acids profiles from the patients in this study, along with the two first patients reported with *ALDH18A1* mutations (Baumgartner *et al.*, 2005), were compared to 5023 profiles from Necker Hospital full cohort of patients aged over 15 years that had no known metabolic disorders. The comparison showed that low levels of ornithine, citrulline, arginine and proline (Fig. 3A) were significantly predictive of *ALDH18A1* mutations (area under the receiver operating characteristic curve 97%, with 95% confidence interval: 96–99%, Supplementary Fig. 3A). Adding elevated glutamine as a covariate may increase prediction (Supplementary Fig. 3B).

In cultured dermal fibroblasts from two patients of Family FSP410, proline biosynthesis was estimated by the stable isotope label enrichment in proline after loading for $^{13}C_5$-labelled glutamine (Baumgartner *et al.*, 2005). Assuming a stationary phase at 18-h incubation, the two patients harbouring *ALDH18A1* mutations had 42% residual flux (stable isotope ratios 0.68 + 0.08 and 0.95 + 0.06, average: 0.81) compared to age-matched healthy controls (stable isotope ratio 1.83 ± 0.05 and 2.01 ± 0.14, average: 1.92) (Fig. 3B). This confirms a reduction of proline biosynthesis consistent with an enzymatic deficiency of P5CS.

Discussion

Here we report seven pedigrees with *ALDH18A1* mutations. Two families with biallelic mutations presented complex autosomal recessive HSP with marked cognitive impairment and no cutaneous involvement. Remarkably, we also identified, in three independent families, monoallelic *ALDH18A1* mutations segregating with autosomal dominant HSP, together with low plasma levels of specific amino acids, especially citrulline, and *ex vivo* demonstration of a metabolic block at the level of P5CS. Two additional sporadic cases harboured monoallelic mutations in *ALDH18A1*. Therefore, this study broadens the clinical spectrum of autosomal recessive *ALDH18A1*-linked pathology, and describes a new mode of inheritance associated with a potential trait biomarker in plasma. Expanding phenotype and describing new modes of transmission becomes more and more frequent in HSP (Esteves *et al.*, 2014; Tesson *et al.*, 2015); such bimodal transmission has also been described long ago, for example in human myotonia, caused by either dominant or recessive mutations of *CLCN1* (Koch *et al.*, 1992).

ALDH18A1 encodes P5CS, a bifunctional enzyme that catalyses the first common step in proline and ornithine biosynthesis (Hu *et al.*, 1999, 2008*b*). This enzyme, located in the mitochondrial inner membrane, catalyses the conversion of glutamate to gamma-glutamyl semi-aldehyde (Fig. 3), which spontaneously converts to pyrroline-5-carboxylate (P5C). P5C can thereafter be metabolized to proline by P5C reductase (PYCR1), or to ornithine, citrulline and finally arginine through the urea cycle (Fig. 3). P5CS comprises two domains, with different enzymatic activities: an N-terminal ATP-dependant gamma-glutamyl kinase domain, responsible for the

Table 3 Clinical characteristics of patients with *ALDH18A1* mutations: autosomal recessive families

Family N°/Origin	FSP856/Spain		SR45/Portugal			
ALDH18A1 variant/ Inheritance	p.Asp715His, p.Asp715His/Recessive		p.Arg128His, p.Leu637Pro/Recessive			
Individual N° (sex)	18 (M)	19 (M)	II:3 (F)	II:4 (F)	II:6 (M)	II:9 (F)
Age at exam (years)	42	39	44	49	46	43
Age at onset (Years)	7	7	<1	<1	<1	<1
Symptoms at onset	Toe walking, ID, speech delay	ID, gait difficulties	MD, growth retardation	MD, growth retardation	PMD	Severe MD, growth retardation
Disease duration (years)	35	32	44	49	46	43
Disability score (max 7)	6/7	3/7	7/7	7/7	6/7	7/7
Spasticity at gait	Severe	Moderate	Severe, ambulation lost at 30	Severe	Severe, ambulation lost at 15	Severe, no ambulation
Spasticity at rest	Severe	Moderate	Severe Ashworth 4/4	Severe Ashworth 3/4 UL; 4/4 LL	Severe Ashworth 4/4 LL; 2/4 UL	Severe Ashworth 4/4
Weakness	Proximal, distal	No	Tetraplegia, drop-feet	Tetraparesia, drop-feet	MRC grading LL 0/5, UL 4/5	Tetraplegia
Increased reflexes LL	Yes	Yes	Yes (ankles abolished)	Yes (ankles abolished)	Yes (ankles abolished)	Not awakened
Increased reflexes UL	Yes	Yes	Yes	Yes	Yes	Not awakened
Extensor plantar response	Yes	Yes	Yes	Yes	Yes	Indifferent
Hoffman sign	NA	NA	NA	NA	NA	NA
Decreased vibration sense at ankles	Mild	Mild	NA	NA	No	NA
Urinary symptoms	Urinary retention, pollakiuria	No	Incontinence	Incontinence	Incontinence	Incontinence
Cerebellar gait	NA	No	No	No	No	NA
Cerebellar signs UL	Postural tremor	Postural tremor	No	No	No	NA
Dysarthria	No	No	Pseudobulbar, loss of speech (age 30)	Pseudobulbar	Spastic	No speech ever
Cognitive impairment	Intellectual deficit IQ 49	Intellectual deficit IQ 35-50	ID, progressive deterioration	ID, progressive deterioration	ID, progressive deterioration	Profound
Cutaneous findings	No	No	No	No	No	No
Ocular findings	NA	NA	NA	NA	Probable cataract (on neurological examination)	NA
Other clinical features	Microcephaly (HC 52 cm, −2 SD), facial dysmorphy	Microcephaly (HC 52 cm, −2 SD), facial dysmorphy	Microcephaly, growth retardation, facial dysmorphy; generalized muscle atrophy	Microcephaly, short stature, generalized muscle atrophy	Mild facial dysmorphy, distal amyotrophy (UL, LL)	Severe growth retardation, cyphoscoliosis, archaic reflexes, extreme muscle atrophy
MRI	Normal brain MRI	NA	NA	NA	CC atrophy, periventricular white matter anomalies, mild cortical atrophy	NA

CC = corpus callosum; CCFS = composite cerebellar functional severity score; ENMG = electro-neuro-myogram; HC = head circumference; ID = intellectual delay; L = left; LL = lower limbs; MD = motor delay; MRC = medical research council; NA = not available; PMD = psychomotor delay; R = right; UL = upper limbs.

Table 4 Amino acid levels on plasma chromatography

Patient	Age	Proline	Ornithine	Citrulline	Arginine	Valine	Leucine	Isoleucine	Threonine	Glutamine	Alanine	Methionine	Lysine
FSP410-032	43	163	**35**	**12**	*58*	*190*	116	*51*	*69*	517	*237*	22	*146*
FSP410-029	45	**117**	83	**8**	*63*	215	110	61	208	740	400	23	201
FSP410-013	70	**73**	*46*	**8**	*54*	*178*	*108*	*51*	*91*	632	*277*	*18*	178
FSP429-021	40	209	*54*	**1**	**28**	267	119	*52*	114	470	304	*19*	175
Normal ranges		150–220	50–100	20–35	60–100	210–280	100–150	50–80	95–195	430–670	285–415	20–35	155–230

Values are given in μmol/l. Bold indicates low values, italics indicates borderline values.

glutamate phosphorylation to gamma-glutamyl phosphate, and a C-terminal NADPH-dependant gamma-glutamyl phosphate reductase domain, which catalyses the reduction and conversion to gamma-glutamyl semi-aldehyde (Fichman *et al.*, 2014). Although P5CS expression in the brain is not strong (Hu *et al.*, 1999), it has a measurable activity (Hu *et al.*, 2008*b*).

ALDH18A1 mutations account for a phenotypic spectrum ranging from pure autosomal dominant to complex autosomal recessive HSP

In humans, mutations in two genes coding for enzymes involved in proline biosynthesis have been described in overlapping neurocutaneous syndromes; *ALDH18A1* and *PYCR1*. *PYCR1* mutations are implicated in an autosomal recessive neurocutaneous syndrome with mental retardation, cutis laxa, joint hyperlaxity, progeroid dysmorphy, growth retardation, microcephaly, and corpus callosum dysgenesis (Reversade *et al.*, 2009; Dimopoulou *et al.*, 2013; Gardeitchik *et al.*, 2014). These patients present normal amino acid levels (Reversade *et al.*, 2009).

As for *ALDH18A1*, the first two patients with demonstrated P5CS deficiency carried a homozygous missense mutation in the glutamate-5-kinase domain of the protein (Baumgartner *et al.*, 2000, 2005), and presented a syndrome associating cutis laxa, joint hyperlaxity, hypotonia, developmental delay, cataract, pyramidal signs and plasma amino acids anomalies—low proline, ornithine, citrulline and arginine. Subsequently, Bicknell *et al.* (2008) reported four siblings with a homozygous missense mutation in the gamma-glutamyl phosphate reductase domain of P5CS; the clinical picture was very similar with the exception of plasma amino acids levels, which showed no abnormalities. Until now, 15 patients with *ALDH18A1* biallelic mutations have been reported (Baumgartner *et al.*, 2000, 2005; Bicknell *et al.*, 2008; Skidmore *et al.*, 2011; Martinelli *et al.*, 2012; Zampatti *et al.*, 2012; Fischer *et al.*, 2014; Gardeitchik *et al.*, 2014; Handley *et al.*, 2014; Wolthuis *et al.*, 2014). Developmental delay, failure to thrive, hypotonia, cognitive impairment, cutis laxa, joint hyperlaxity, growth retardation and cataract are cardinal signs (Table 5). Dystonia, seizures in infancy, thin corpus callosum, pes planus, hip dislocation, bone malformations, gastro-oesophageal reflux and inguinal hernia are frequent signs orientating towards the molecular diagnosis. Pyramidal signs are often reported (12/15), but not cardinal; most of the patients presented brisk reflexes and pyramidal syndrome was judged severe in three only.

The patients that we report with biallelic *ALDH18A1* mutations (Families FSP856 and SR45) share common features with those previously reported suffering from neurocutaneous syndromes: developmental delay (4/6), intellectual deficiency (6/6), short stature (4/6), facial dysmorphism (6/6), cataract (1/6) and thin corpus callosum (1/3). Of interest is the severity of pyramidal signs, with moderate to severe spasticity (6/6), to tetraplegia (1/6) and pseudobulbar palsy (2/6). Although severe HSP was previously reported in *ALDH18A1* patients, its frequency was low (3/15). Moreover, though cutis laxa has been reported to improve or disappear with age in some patients (Bicknell *et al.*, 2008), it was never noted in any of our patients, while it was previously considered as an obligate sign. We therefore are expanding the clinical spectrum of biallelic *ALDH18A1* mutations, with emphasis on the pyramidal component alteration; and are establishing that the cutaneous abnormalities may be lacking.

More importantly, we also report the first three families with autosomal dominant HSP segregating *ALDH18A1* mutations. The following arguments are in favour of the pathogenicity of the variants we report: (i) the absence of reported mutations in all examined public databases (~63 000 exomes); (ii) amino acid conservation through phylogenesis; (iii) *in silico* pathogenicity predictions (Table 1); (iv) location within a putatively linked locus in Family FSP410 (Supplementary Fig. 1); (v) absence of pathogenic variants in all known HSP genes; (vi) multiplicity of the mutated families and multiplicity of patients with the mutations amongst the families; (vii) location within the aspartokinase active site for p.R252Q; (viii) a second occurrence of the p.R252Q mutation with a different haplotype; (ix) abnormal plasma amino acids levels in carriers from two unrelated families (Families FSP410 and FSP429); and (x) reduced glutamate-to-proline metabolic flux in dermal fibroblasts of two patients of Family FSP410 (42% of levels measured in control fibroblasts). However, the clinical picture of these patients largely differs from biallelic mutation carriers. Spasticity is the cardinal

Table 5 Summary of clinical characteristics of published families with *ALDH18A1* mutations

Reference	Baumgartner *et al.*, 2000, 2005	Bicknell *et al.*, 2008	Skidmore *et al.*, 2011	Martinelli *et al.*, 2012	Zampatti *et al.*, 2012; Gardeitchik *et al.*, 2014	Wolthuis *et al.*, 2014	Fischer *et al.*, 2014	Fischer *et al.*, 2014	Handley *et al.*, 2014	This study (FSP856)	This study (SR45)	This study (FSP410)	This study (FSP429)	This study (FSP470)	This study (GSHSP44)	This study (25014)
Mutations (protein level)	R84Q/R84Q	H784Y/H784Y	c.1923 + 1 G > A/c. 1923 + 1 G > A	G93R/T299I	S742I/R425C	Y782C/Y782C	V601Gfs*/ V601Gfs*	L711Cfs*/ L711Cfs*	R749Q/R765Q	D715H/ D715H	R128H/L637P	V120A	R665L	R252Q	S652F	R252Q
Functional effect	Reduced P5CS activity	Normal PRO and ORN synthesis	Reduced fibroblasts proliferation and P5CS expression	Decreased P5CS activity, dissociation to dimers or monomers; no mitochondrial alterations in cultured fibroblasts			Abolished P5CS expression, swollen mitochondria in fibroblasts (electron microscopy)		Reduced P5CS expression, lipid droplet enlargement	NA	NA	Lowered PRO synthesis in fibroblasts	NA	NA	NA	NA
Low plasma amino acids levels (other findings)	ORN, CITRU, ARG, PRO (paradoxal hyperammonaemia)	Normal (mild post-prandial hypoornithinaemia in 2/3)	Normal	ORN, CITRU, ARG, PRO (paradoxal hyperammonaemia)	Normal	Normal	CITRU, ARG	NA	Normal	NA	NA	CITRU (3/3), PRO (2/3), low or borderline ORN(2/3), and ARG (3/3)	CITRU, ARG, (borderline ORN)	NA	NA	NA
Neurological signs at onset	DD, hypotonia	DD, hypotonia	DD, hypotonia	DD, hypotonia	DD, hypotonia	DD, hypotonia	DD, hypotonia		DD, hypotonia	Intellectual deficiency	Global delay, growth retardation	Pyramidal signs	Pyramidal signs	Pyramidal signs	Pyramidal signs	Pyramidal signs
Pyramidal signs	Yes (2/2), severe in 1	Hypertonicity LL (4/4), UL (2/4)	Yes	NA	Yes	Brisk reflexes	NA	NA	Brisk reflexes	Moderate to severe	Severe, tetraplegia (2/4) or tetraparesis (1/4)	Moderate to severe	Severe	Moderate to severe	Mild	Moderate to severe
Other neurological findings	Peripheral axonal neuropathy, seizures in infancy (1/2), distal dystonia (1/2)	Seizures in infancy (2/4), absent speech	Flexion contractures of elbows, wrists (32w gestation)	Seizures in infancy	Seizures, dystonia, absent speech		Disseminated tremors, finger contractures	Seizures	Joint contractures	No	No	Mild cerebellar signs (2/6), motor neuropathy (2/6), dementia (1/6)	Cerebellar signs (1/2)	No	No	No
Cutis laxa (other cutaneous findings)	Yes	Yes	Yes (sparse hair)	Yes	Yes (sparse hair)	Yes	Yes	Yes	Yes	No	No	No	No	No	No	Chronic cellulitis changes
Skeletal findings	Joint hyperlaxity, pes planus	Joint hyperlaxity, hip dislocation (3/4), kyphoscoliosis	NA	Joint hyperlaxity, pes planus, coxa valga	Joint hypermobility, osteopenia, rib anomalies	Joint laxity, bilateral hip dislocation and dysplasia	Unilateral hip dislocation, kyphosis	Joint hypermobility, congenital luxation of left hip	Bilateral coxa valga			Pes cavus (3/6)	Pes cavus (1/2)	Pes cavus (2/4)	Pes cavus, hip replacement	
Facial dysmorphy	Yes	NA	Yes	Yes	Yes	NA	Yes	NA	Yes	Yes	Yes	No	No	No	No	No
Growth retardation/ microcephaly	Yes/yes	Yes/yes	Yes/yes	Yes/macrocephaly	Yes/no	Yes/yes	Yes/yes	Yes/yes	Yes/yes	No/yes	Yes/yes	No/no	No/no	No/no	No/no	No
Cataracts (other ocular findings)	Yes	Yes 1/4	(Corneal clouding)	Yes	Yes	(Retinitis pigmentosa)	Yes	(Corneal clouding)	Yes 1/2	NA	Yes 1/4	Yes 1/6	NA	Yes 1/4	NA	No
Gastro-oesophagal reflux	2/2	NA	NA	NA	Yes	NA	NA	NA	NA	NA	NA	NA	NA	1/4	NA	NA

(continued)

Table 5 Continued

Reference	Baumgartner *et al.*, 2000, 2005	Bicknell *et al.*, 2008	Skidmore *et al.*, 2011	Martinelli *et al.*, 2012	Zampatti *et al.*, 2012; Gardeitchik *et al.*, 2014	Wolthuis *et al.*, 2014	Fischer *et al.*, 2014	Fischer *et al.*, 2014	Handley *et al.*, 2014	This study (FSP856)	This study (SR45)	This study (FSP410)	This study (FSP429)	This study (FSP470)	This study (GSHSP44)	This study (2501 4)
Inguinal hernia	NA	1/4	Bilateral	Bilateral	1/2	Yes	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Vascular findings	NA	NA	Arterial tortuosities	NA	NA	NA	Cardiovascular malformations	Narrow aortic arch, probable aneurysm	NA	NA	NA	NA	NA	Mitral leak (1/4)	NA	NA
Other						Abnormal fat distribution, undescended testis	Bilateral hearing loss		Undescended testis			Infandle psychosis (1/6)				
MRI	NA	Paucity of WM, thin CC	NA	Mild cortical atrophy, thin CC, tortuosity of brain vessels	Cerebellar vermis hypoplasia, thin CC	Thin CC	Normal	Agenesis of CC	Thin CC, prominent lateral and 3rd ventricles, cerebellar hypoplasia, mildly delayed myelination	Normal	Thin CC, mild cerebellar atrophy, WM anomalies	Mild CC atrophy (1/3)	WM anomalies, thin dorsal cord, pons hypersignal (1/2)	Left arachnoidien cyst (1/4)	Spinal cord atrophy	Cortical atrophy (frontoparietal predominance)

ARG = arginine; CC = corpus callosum; CITRU = citrulline; DD = developmental delay; LL = lower limbs; NA = not available; ORN = ornithine; PRO = proline; UL = upper limbs; WM = white matter.

and first presentation, and is often pure with few or no accompanying signs. Seven of 15 patients had pes cavus that could be a consequence of the pyramidal syndrome, while pes planus was classically described in autosomal recessive pathology. Interestingly, Patients FSP410-22 and FSP470-22 presented with congenital cataract, which is a distinctive sign, when present, in patients with biallelic *ALDH18A1* mutations (Baumgartner *et al.*, 2000, 2005; Zampatti *et al.*, 2012). A peripheral neuropathy was demonstrated in three of four patients that had electromyography in Family FSP410, but was clinically present in 9 of 21 patients, as in the first reported patients with biallelic *ALDH18A1* mutations (Baumgartner *et al.*, 2005). Cerebellar signs were noted in 4 of 14 autosomal dominant patients, as in two of six patients with biallelic mutations. It has never been reported before, but cerebellar atrophy was evidenced in several reports (Zampatti *et al.*, 2012; Handley *et al.*, 2014). These elements are again in favour of the implication of *ALDH18A1* mutations in these cases. Of note, the clinical presentation was not homogeneous among the respective families, especially for Family FSP410. Age, disease duration, lifestyle, and dietary factors are all potential modifiers underlying this variability.

What is the pathogenic mechanism linked to *ALDH18A1* mutations?

In autosomal recessive families, all previously reported patients had either homozygous variants or compound heterozygous mutations affecting the same functional domain and, therefore, a presumably complete enzymatic block of one of the P5CS-catalysed reactions (Fig. 3). In addition, abnormal metabolic profiles were only reported in patients with mutations affecting the glutamate-5-kinase domain (Baumgartner *et al.*, 2000; Martinelli *et al.*, 2012) or with a complete abolition of protein expression (Fischer *et al.*, 2014). Consistently, the amino acid profile is normal in Patient FSP856-18 that harbours mutations that do not affect the glutamate-5-kinase domain. Of note, Family SR45 is the first reported with compound heterozygous missense mutations involving both domains of P5CS; here, a complete enzymatic block is not expected.

In autosomal dominant families, the most striking anomalies are the low to very low citrulline levels in the plasma of all patients tested, regardless of the protein domain affected by the mutation, together with a less consistent, decreased ornithine, arginine and proline levels. The sum of age-normalized values of these four amino acids is obviously reduced in our patients, as well as in the first family in which the disorder was identified (Fig. 2A), and is significantly, though incompletely, predictive of *ALDH18A1* mutations (Supplementary Fig. 3A). Dermal fibroblasts from Family FSP410 showed a decrease in proline biosynthesis by a stable isotope label substrate-loading test (Fig. 2B). The degree of observed residual flux as measured by the accumulation of labelled proline relative to the

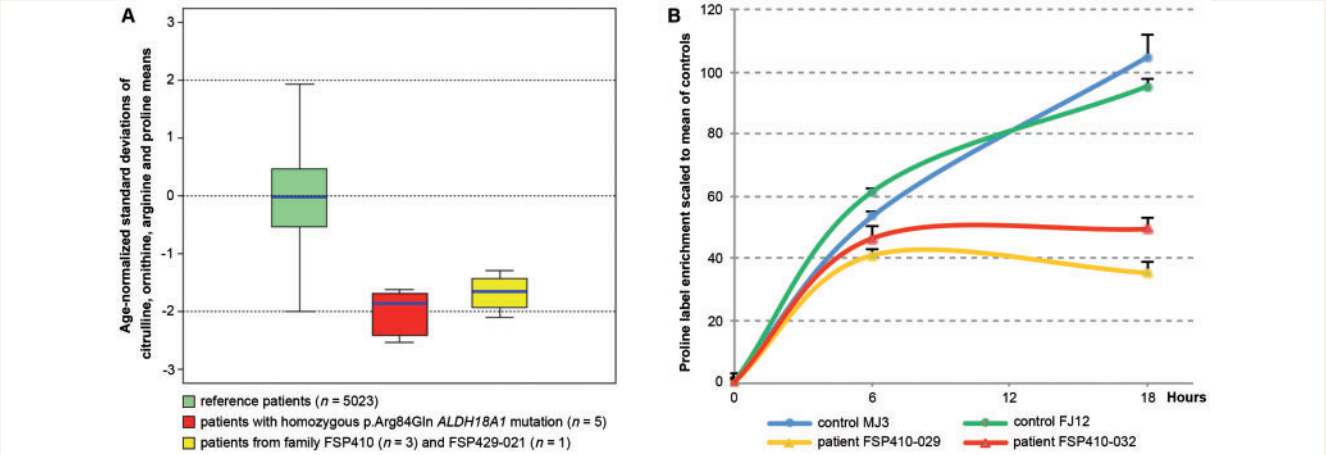

Figure 2 Plasma amino acid profiles and proline biosynthesis flux analysis. (**A**) Boxplots show the mean of four plasma amino acid levels (citrulline, ornithine, proline and arginine) expressed as their age-normalized standard deviations (*y*-axis; see 'Materials and methods' section) compared among three groups of patients: the full Necker hospital cohort of patients with no known metabolic disorders, aged >15 years ($n = 5023$; green); patients from the first family in which *ALDH18A1* mutations were reported ($n = 5$; red); and the patients from Families FSP410 and FSP429 (this study) for which plasma amino acids were available ($n = 4$; yellow). The average of the four amino acid levels in *ALDH18A1* mutated patients maps at approximately −2 SD. (**B**) Time course of stable isotope ratio (labelled versus natural) enrichment of proline reflecting the rate of proline biosynthesis from $^{13}C_5$-labelled glutamine in fibroblasts. Two *ALDH18A1*-mutated patients (red and orange lines) are compared to two controls, i.e. members of the same family with no mutations (green and blue lines). *Y*-axis: label enrichment scaled to the mean of the controls at 18-h incubation. *X*-axis: hours of incubation in the presence of the tracer. Assuming a stationary phase at 18-h incubation time, proline biosynthesis in two patients harbouring *ALDH18A1* p.Val120Ala mutation was estimated to be 42% of controls.

natural isotope is comparable to that measured in the first two patients reported to have biallelic *ALDH18A1* mutations (40%; Baumgartner *et al.*, 2005). However, the method we used differs from that report, and a direct comparison of the effect of different mutations needs to be performed for final conclusion. Regarding the pathogenic mechanisms in autosomal dominant patients, investigations are needed to directly compare the effects of homozygous and heterozygous mutations, especially to evaluate whether the latter leads to haploinsufficiency or a dominant negative gain-of-function. A previous report on a patient homozygous for a frameshift mutation resulting in no protein expression (Fischer *et al.*, 2014) argues against haploinsufficiency as the pathogenic mechanism underlying dominant mutations.

Furthermore, for autosomal recessive patients for which the amino acid profile was normal, another moonlighting, unknown, function of P5CS might be involved in the pathophysiology. Proline metabolism is a major actor in adaptation to osmotic stress and cellular redox stress (Hu *et al.*, 2008*a*, *b*; Yasuda *et al.*, 2013; Fichman *et al.*, 2014). It is also involved in the apoptosis pathway; of note P5CS was shown to be upregulated by p53 (Hu *et al.*, 2008*b*). Another field of interest is the mitochondria, as defects in mitochondrial structure have been described in *PYCR1*-linked neurocutaneous disease (Reversade *et al.*, 2009). No such abnormalities had previously been identified in P5CS deficiency syndromes (Skidmore *et al.*, 2011). Recently, Fischer *et al.* (2014) described swollen mitochondria in fibroblasts from their P5CS-deficient patient by electron microscopy on skin biopsies, but they could not observe them on cultured fibroblasts. In cultured fibroblasts from Patients FSP410-29 and FSP410-32, we found normal mitochondrial structure or membrane potential (data not shown). Renewed efforts are warranted to elucidate the cellular basis of *ALDH18A1*-related phenotypes.

Mitochondrial ornithine deficiency as a possible common scheme for multiple metabolic disorders

There are few metabolic causes of persistent hypocitrullinaemia after ruling out obvious and usually transient mechanisms of intestinal dysfunction: (i) urea cycle defects—notably, ornithine transcarbamylase (OTC), carbamoylphosphate synthase 1 (CPS1) and *N*-acetylglutamate synthetase (NAGS) deficiencies; (ii) ornithine translocase deficiency (or triple H syndrome); (iii) respiratory chain deficiencies (Rabier *et al.*, 1998); and (iv) P5CS/*ALDH18A1* deficiency (Baumgartner *et al.*, 2000, 2005). Several biochemical hints allow for distinction between the enzymatic defects. Likewise, proline is decreased in P5CS deficiency only, orotic aciduria is present in OTC deficiency only and hyperammonemia is mainly observed in the fed state for urea cycle defects, whereas it manifests during fasting in P5CS deficiency. Decreased plasma citrulline levels could therefore be an excellent trait biomarker for *ALDH18A1*-linked autosomal dominant HSP, particularly as the clinical picture is not specific (relatively pure HSP form). Furthermore, the combination of low ornithine, citrulline, arginine and proline

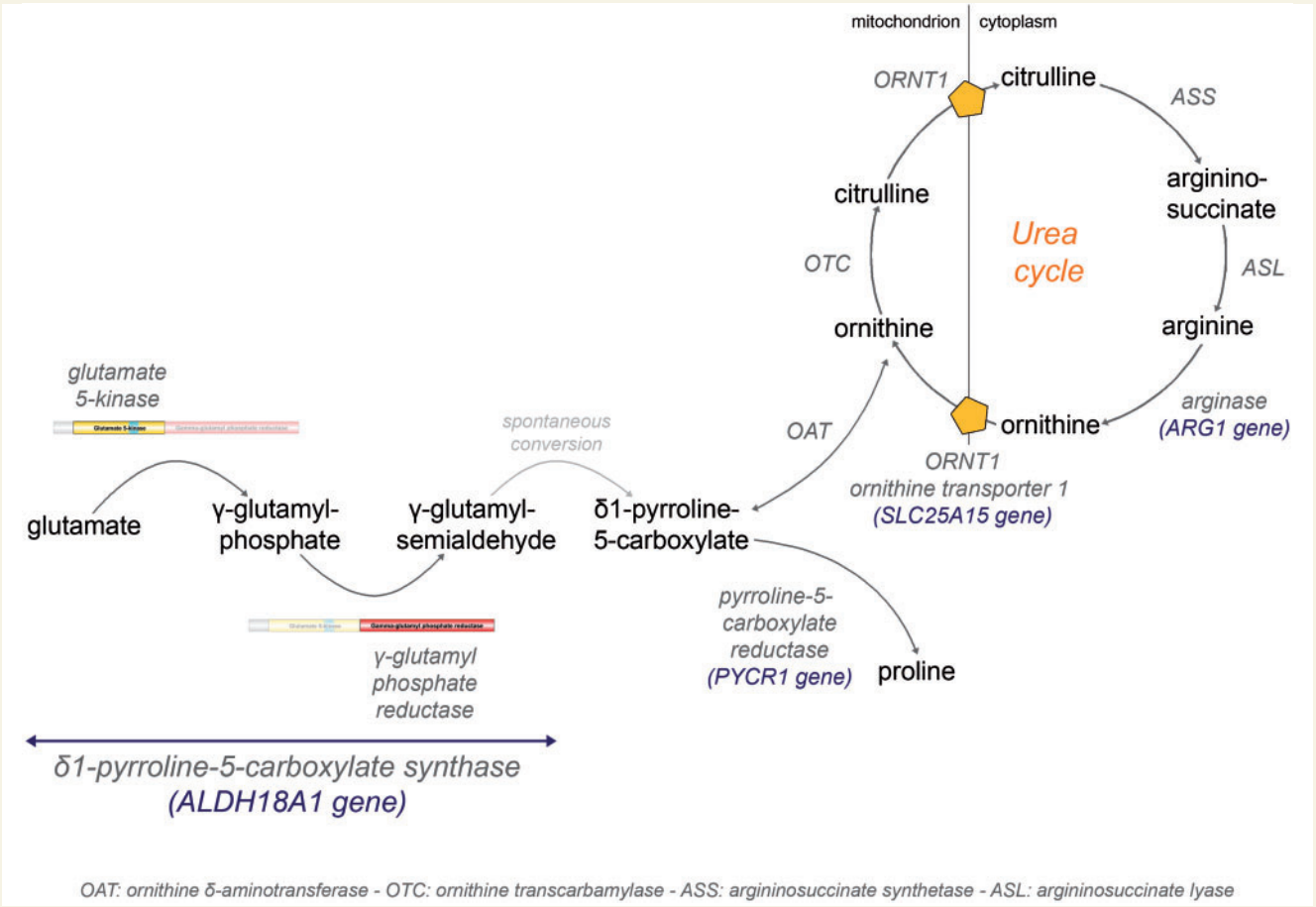

Figure 3 Delta-1-pyrroline-5-carboxylate synthase (P5CS) pathway. Schematic of the P5CS pathway. Enzyme-catalysed steps of proline and ornithine synthesis from glutamate, as well as urea cycle, are in dark grey. Mutations in *ALDH18A1* and *PYCR1* were previously reported in autosomal recessive neurocutaneous syndromes (Baumgartner *et al.*, 2000; Reversade *et al.*, 2009), with some pyramidal involvement. Deficiencies for arginase (*ARG1* mutations) and ornithine transporter (*SLC25A15* mutations, triple H syndrome) also share features of motor neuron degeneration (Sedel *et al.*, 2007). We speculate that a decrease in the mitochondrial ornithine pool may be the common feature of the latter two and *ALDH18A1*-linked autosomal dominant HSP.

in plasma is significantly predictive of *ALDH18A1*-linked autosomal dominant HSP (Supplementary Fig. 3A).

The fact that the urea cycle defects and autosomal recessive HSP share common biomarkers as well as features of motor neuron degeneration is of interest and suggests that common metabolic mechanisms may be involved. Arginase deficiency (*ARG1* mutations), an enzymatic step of the cytosolic part of the urea cycle (Fig. 3), is characterized by very high plasma levels of arginine and presumably decreased ornithine recycling. Triple H syndrome (*SLC25A15* mutations), a defect of the translocase responsible for transporting ornithine from the cytosol into the mitochondria (Fig. 3), leads to high plasma levels of ornithine, thus reflecting low mitochondrial ornithine. Plasma citrulline is normal in arginase deficiency, but decreased in triple H syndrome. Both metabolic diseases are associated with HSP in adults (Sedel *et al.*, 2007). Therefore, while plasma citrulline seems to be the most consistent biomarker of *ALDH18A1*-linked autosomal dominant HSP, a decrease in the mitochondrial pool of ornithine may be the common feature of arginase deficiency, triple H syndrome and P5CS deficiency (Fig. 3). We speculate that mitochondrial ornithine deficiency may be responsible for motor neuron degeneration in these diseases. This may have important therapeutic implications for patients with *ALDH18A1*-linked autosomal dominant HSP, because long-term supplementation in citrulline should be considered by analogy with its successful usage in patients with inborn errors of metabolism that directly or indirectly affect the urea cycle function—i.e. OTC deficiency or lysinuric protein intolerance (Haberle *et al.*, 2012).

In conclusion, we describe seven novel mutations in *ALDH18A1* in seven HSP pedigrees. Six patients from two families with complex autosomal recessive HSP broaden the phenotypic spectrum linked to this gene, with an emphasis on the pyramidal component and the absence of cutaneous signs. More importantly, we report

ALDH18A1 mutations segregating in an autosomal dominant pattern in 17 patients from five pedigrees with pure or slightly complex HSP, thus adding a new gene involved in autosomal dominant HSP. We also describe the first potential plasma biomarker of autosomal dominant HSP that can be implemented in daily practice. Due to the great diversity of HSP, plasma biomarkers are invaluable diagnostic tools for the screening of certain genetic entities among all possible aetiologies of spastic paraplegia or to validate the causality of novel variants found in high throughput sequencing methods in genetic diagnosis. Likewise, plasma very long chain fatty acids and 25-/27-hydroxycholesterols are now established biomarkers of HSP, leading to more effective detection and dedicated care of adrenomyeloneuropathy (*ABCD1* mutations) and SPG5 (*CYP7B1* mutations) (Schule *et al.*, 2010; Kemp *et al.*, 2012). Provided that our biochemical findings are validated in a larger population of *ALDH18A1*-linked autosomal dominant HSP patients, we recommend the use of plasma amino acid chromatography as part of diagnostic work-up in HSP. These results are also illustrative of the notion of 'genetics remodelling' brought by Next Generation Sequencing, as has been demonstrated for other genes (Esteves *et al.*, 2014; Synofzik *et al.*, 2014; Tesson *et al.*, 2015). The questioning of assumed transmission modes and new delineations in phenotype-genotype correlations are hallmarks of that field. Finally, we confirm that the metabolism of amino acids is an important cellular pathway in HSPs, which may help in the understanding of their physiopathology.

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Supplementary material

Supplementary material is available at *Brain* online.

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