Mutations in *ACY1,* the Gene Encoding Aminoacylase 1, Cause a Novel Inborn Error of Metabolism

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N-terminal acetylation of proteins is a widespread and highly conserved process. Aminoacylase 1 (ACY1; EC 3.5.14) is the most abundant of the aminoacylases, a class of enzymes involved in hydrolysis of *N*-acetylated proteins. Here, we present four children with genetic deficiency of ACY1. They were identified through organic acid analyses using gas chromatography–mass spectrometry, revealing increased urinary excretion of several *N*-acetylated amino acids, including the derivatives of methionine, glutamic acid, alanine, leucine, glycine, valine, and isoleucine. Nuclear magnetic resonance spectroscopy analysis of urine samples detected a distinct pattern of *N*-acetylated metabolites, consistent with ACY1 dysfunction. Functional analyses of patients' lymphoblasts demonstrated ACY1 deficiency. Mutation analysis uncovered recessive loss-of-function or missense *ACY1* mutations in all four individuals affected. We conclude that *ACY1* mutations in these children led to functional ACY1 deficiency and excretion of *N*-acetylated amino acids. Questions remain, however, as to the clinical significance of ACY1 deficiency. The ACY1-deficient individuals were ascertained through urine metabolic screening because of unspecific psychomotor delay (one subject), psychomotor delay with atrophy of the vermis and syringomyelia (one subject), marked muscular hypotonia (one subject), and follow-up for early treated biotinidase deficiency and normal clinical findings (one subject). Because *ACY1* is evolutionarily conserved in fish, frog, mouse, and human and is expressed in the central nervous system (CNS) in human, a role in CNS function or development is conceivable but has yet to be demonstrated. Thus, at this point, we cannot state whether ACY1 deficiency has pathogenic significance with pleiotropic clinical expression or is simply a biochemical variant. Awareness of this new genetic entity may help both in delineating its clinical significance and in avoiding erroneous diagnoses.

N-terminal acetylation of proteins is a widespread and highly conserved process that is involved in protection and stability of proteins (Polevoda and Sherman 2000). Although the biosynthetic pathways leading to acetylated amino acids are well studied, less attention has been paid to the catabolism of *N*-acetylated peptides. *N*-acetylated amino acids can be released by an *N*-acylpeptide hydrolase from peptides generated by proteolytic degradation (Perrier et al. 2005). Several types of aminoacylases can be distinguished on the basis of substrate specificity. Aminoacylase 1 (ACY1; EC 3.5.1.14), the most abundant type, is a soluble homodimeric zinc-binding enzyme that catalyzes the formation of free aliphatic amino acids from *N*-acetylated precursors (fig. 1). It is encoded by the aminoacylase 1 gene (*ACY1*) on chromosome 3p21 that comprises 15 exons (Naylor et al. 1979, 1982; Miller et al. 1990; Cook et al. 1993). ACY1 activity was discovered as early as 1881, when Schmiedeberg coined the name "*Histozym*" (tissue enzyme) for an intracellular enzymatic activity catalyzing the hydrolysis of *N*-benzoylglycine (hippuric acid) (Schmiedeberg 1881). In 1922, Smorodinzew reported on ACY1-catalyzed hydrolysis of various acylated amino acids (Smorodinzew 1922). Preferred substrates of ACY1 are aliphatic amino acids with a short-chain acyl moiety, especially *N*-acetyl-methionine (Birnbaum et al. 1952). However, ACY1 can also catalyze the reverse reaction, the synthesis of acetylated amino acids (Lindner et al. 2000).

Although deficiency of aminoacylase 2 (ACY2, aspartoacylase; EC 3.5.1.15), which catalyzes the hydrolysis of *N*-acetyl-aspartate to aspartate and acetate, causes Canavan disease (MIM 271900) (Kaul et al. 1993; Baslow and Resnik 1997), deficiency of ACY1 in humans has not been reported so far. The clinical features of Canavan disease comprise progressive psychomotor regression and muscular hypotonia, enlarged head, spasticity, severe visual impairment, and death in

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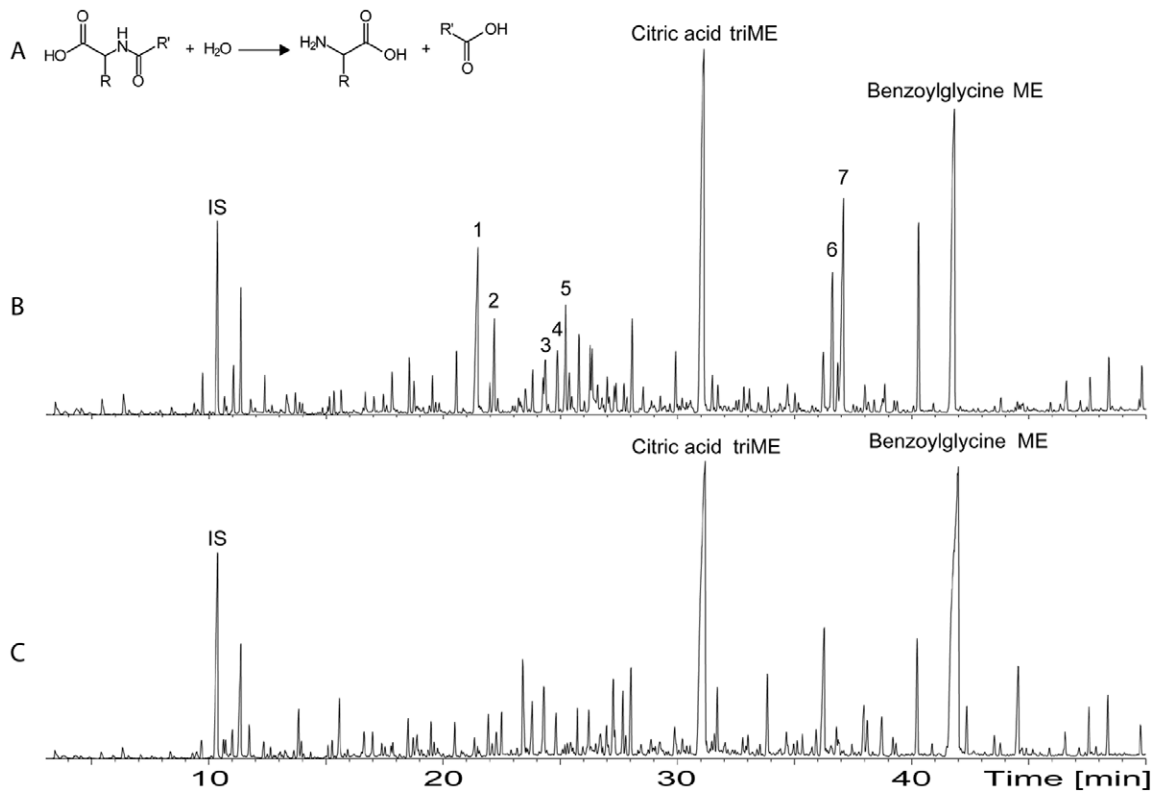

Figure 1 *A,* Deacetylation of *N*-acetylamino acids that is catalyzed by ACY1. For R = $(CH_2)_2SCH_3$ and R′ = CH_3, the *N*-acetylamino acid is *N*-acetylmethionine, a preferred substrate of ACY1. *B* and *C,* Organic acids detected by GC-MS in the urine from (*B*) a patient (OS-104 II-1) with ACY1 deficiency and (*C*) a parent as a control. Abbreviations used for peak labels are as follows: ME = methyl ester; IS = internal standard (isopropylmalonic acid diME); 1 = *N*-acetylalanine ME; 2 = *N*-acetylvaline ME; 3 = *N*-acetylisoleucine ME; 4 = *N*-acetylglycine ME; 5 = *N*-acetylleucine ME; 6 = *N*-acetylmethionine ME, 7 = *N*-acetylglutamic acid diME. Consistent with lack of enzymatic deacetylation, the urinary concentrations of *N*-acetylamino acids are increased in the ACY1-deficient individual.

early childhood (Canavan 1931; Gordon 2001). Diagnosis is based on abnormally high excretion of *N*-acetylaspartic acid in urine and on enzyme and mutation analyses.

Here, we report on four children who were found to excrete significant amounts of distinct *N*-acetylated amino acids, including the derivatives of methionine, glutamine, alanine, leucine, glycine, valine, and isoleucine, as determined by gas chromatography–mass spectrometry (GC-MS) analysis for organic acids. The children had ACY1 deficiency in their cultured cells, providing evidence of a "new" inborn error of metabolism. Nuclear magnetic resonance (NMR) spectroscopy performed on the urine of the patients confirmed a pattern of *N*-acetylated metabolites consistent with a deficiency in aminoacylase 1, which was demonstrated by an enzyme activity test in Epstein-Barr virus (EBV)–transformed lymphoblasts. In all four families, mutation analysis identified recessive *ACY1* mutations that segregated with the disease status.

Methods

Patients

Clinical histories and findings, as well as family histories, of patients with increased excretion of urine metabolites suggestive of defective aminoacylase 1 function were obtained from medical records.

Metabolite Identification and Quantitation

Organic acids, including *N*-acetylated amino acids, were determined by GC-MS following liquid extraction from urine and derivatization with diazomethane, as is done on a daily basis for the identification of individuals with inborn errors of metabolism (Lehnert 1994). Amino acid analysis was performed on Biochrom and Biotronik amino acid analyzers by use of derivatization with ninhydrine, which reacts with primary (nonacetylated) amino groups. NMR spectroscopy of body fluids was performed as described elsewhere (Engelke et al. 2004). *N*-acetylated amino acid compounds were either purchased from Sigma or from the University of Nijmegen or

were prepared at the Freiburg University Children's Hospital Laboratory of Metabolism.

Enzyme Assays

Enzymatic activity of ACY1 was determined in 13,000 *g* supernatant obtained from homogenized EBV-transformed lymphoblasts. Saline-washed cell pellets were homogenized by ultrasound in 50 mM Tris-HCl buffer (pH 8.0) containing 5 μM $ZnCl_2$ (Giardina et al. 1997) and 0.1% (w/v) Triton X-100 after they had undergone one freeze-thaw cycle. ACY1 activity was assessed by incubating the 13,000 *g* supernatant prepared from the homogenate with ACY1's high-affinity substrate *N*-acetylmethionine (dissolved in 0.1 M HEPES buffer, pH 8.0, according to the recommendation of Giardina et al. [1997]) at a final concentration of 20 mM at 37°C. Aliquots were removed from the incubation mixture at multiple time points between 0 and 120 min. They were spotted onto filter-paper cards (Schleicher & Schuell 903 paper) that were immediately frozen at −80°C to stop the reaction. Methionine concentrations were subsequently determined by tandem mass spectrometry, and their increase over the time was used to calculate ACY1 activity. Patient samples were analyzed at least in duplicate, and the data presented here are the respective mean values. Protein concentrations were determined by use of the Lowry method (with BSA as the standard) (Lowry et al. 1951).

Mutation Analysis

DNA from the four individuals excreting *N*-acetylamino acids was screened for the presence of mutations in *ACY1*. To this purpose, blood samples were obtained after informed consent, and genomic DNA was isolated either directly from blood samples or after EBV transformation of peripheral blood lymphocytes by use of the QiaAmp Blood sample kit (Qiagen). The intron-exon structure of *ACY1* was obtained by alignment of cDNA (GenBank accession number BC014112) and genomic (GenBank accession number NT_022517.17) sequences. Exons and the corresponding intron-exon boundaries of *ACY1* were amplified by PCR with the following primers: Ex1_f, ACCTCGCTGGACCCTAAGTC; Ex1_r, AGCCCCAGTCCCTCTATCC; Ex2_f, CACGGTATCCTACCCCTGTG; Ex2_r, TACTTGGGGAATGGCTGGAG; Ex3+4_f, CTGGGTATGCTCCACTCTCC; Ex3+4_r, GGACCATGAGCAACTTGAGG; Ex5_f, ACCACTCCACCTGTCACTCC; Ex5_r, TCCTTGGCCTTGAGTTTCTC; Ex6–8_f, GGGTAAAGTCCAGGACACAGG; Ex6–8_r, CTCAACTTTGCTGTGCAACC; Ex9+10_f, AGAGGAGCCTGGAATGAGG; Ex9+10_r, GCGGCAGCAACAGATAAAAG; Ex11+12_f, GGCGGTACCACAGAGGATAG; Ex11+12_r, AATGCCCAGACATATGCAGAC; Ex13+14_f, TGTACTAGGCACAGCCCACTC; Ex13+14_r, AAGAGCCGTTAGGGAAAAGC; Ex15_f, ATATAGTGCCTGGGCAGTGG; Ex15_r, GGCTGGATGGTACTGAATGG. Product sizes were as follows: exon 1, 117 bp; exon 2, 373 bp; exons 3+4, 437 bp; exon 5, 312 bp; exons 6–8, 603 bp; exons 9+10, 401 bp; exons 11+12, 494 bp; exons 13+14, 458 bp; and exon 15, 541 bp. PCR was performed in a total volume of 50 μl containing 1 × PCR buffer (including 1.5 mM $MgCl_2$), 30 pmol primers, 2 mM dNTPs, 30 ng template DNA, and 1.5 U *Thermus aquaticus* DNA polymerase (Qiagen). Amplification was performed with denaturation at 94°C for 4 min followed by 33 cycles of 30 s at 94°C, 30 s of annealing (exons 1, 3+4, 5, 6–8, 9+10, and 15 at 63°C; exon 2 at 65°C; exons 11+12 at 59.5°C; and exons 13+14 at 63.5°C), and 60 s at 72°C. Final extension was performed at 72°C for 10 min. Q-Solution was added to all PCR reactions. Products were bidirectionally sequenced using the Big-Dye Terminator Kit (PE Applied Biosystems [ABI]) on an ABI capillary sequencer. Data were evaluated with the CodonCode Aligner sequence analysis software (CodonCode). By use of allele-specific restriction analysis and denaturing high-performance liquid chromatography, 210 control chromosomes were screened for missense mutations.

Northern Blot Analysis

Tissue specificity of expression of the human *ACY1* gene was analyzed by northern blot hybridization, by use of a 657-bp *Bsm*I/*Sca*I fragment of the human cDNA (IMAGp958G107Q [RZPD Web site]) as a probe. Human multiple-tissue northern blots (BioChain Institute and BD Biosciences) were hybridized overnight at 63.5°C in Church buffer (500 mM phosphate buffer pH 7.2, 7% SDS [w/v], and 1 mM EDTA supplemented with 100 μg/ml salmon sperm DNA).

The *ACY1*-specific probe was radiolabeled with α-^{32}P-dCTP (Amersham) by use of a Megaprime DNA labeling kit (Amersham). Filters were rinsed twice at room temperature with 2 × saline sodium citrate (SSC)/0.1% SDS and were washed once for 10 min with 0.2 × SSC/0.1% SDS. Membranes were exposed to BioMax MS films (Kodak) in the presence of an intensifying screen.

Results

Metabolite Analyses

GC-MS analysis identified four individuals with a pattern of urinary organic acids dominated by high signals of several *N*-acetylated amino acids, including derivatives of methionine, glutamic acid, alanine, valine, glycine, leucine, and isoleucine (fig. 1). In all patients, repeated urine samples were tested, and the abnormal pattern was consistently observed. Quantitation of amino acids with a free α-amino group (reacting with ninhydrine) in serum/plasma and urine revealed no major abnormality. Only limited family studies were possible; metabolite patterns of urine of the parents from family OS-127 and of the unaffected brother OS-104 II-2 were unremarkable. In family OS-120, only the mother could be analyzed, and her urine showed a normal metabolite pattern.

The characteristic pattern of *N*-acetylated amino acids initially found by GC-MS was corroborated by NMR spectroscopy of all four patients' urine samples (table 1). In addition, increased levels of the metabolites *N*-acetylglutamine, *N*-acetylasparagine, *N*-acetylserine, and *N*-acetylthreonine were found by NMR spectroscopy. These metabolites were not detected by GC-MS

following our standard derivatization procedure with diazomethane. In plasma/serum samples of all three individuals studied in this regard (OS-104 II-1, OS-124 II-1, and OS-127 II-1), NMR spectroscopy yielded no detectable *N*-acetylated amino acids.

Enzyme Assays

ACY1 activity was assessed in EBV-transformed lymphoblasts of patients from families OS-104, OS-124, and OS-127 and of 19 controls. The results clearly demonstrate decreased enzyme activity of the patients' cells (0.08, 0.07, and 0.04 nmol min^{-1} mg^{-1}) compared with the control values (mean 1.24 nmol min^{-1} mg^{-1}; SD 0.53 nmol min^{-1} mg^{-1}) (fig. 2).

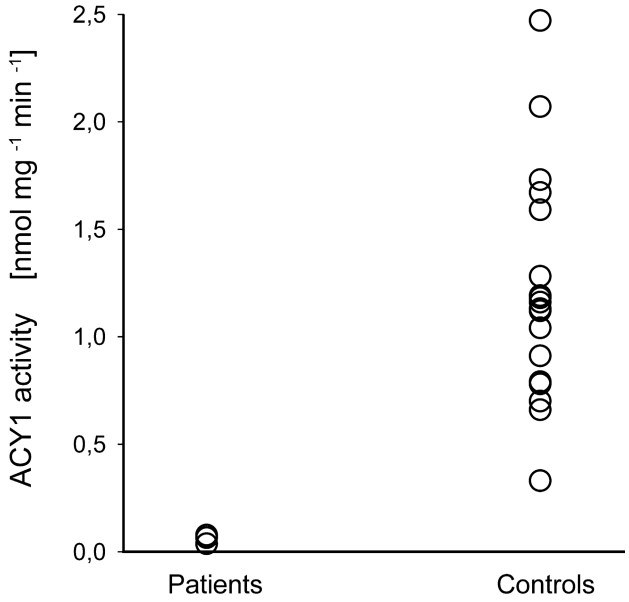

Figure 2 Assessment of ACY1 activity in lymphoblasts of 3 patients (OS 104 II-1, OS 124 II-1, and OS 127 II-1) and of 19 controls revealed much lower enzyme activity in the patients' cells ($P = .001$ in Student's t test).

Patients

Three of the four ACY1-deficient individuals had been ascertained by the presence of *N*-acetylamino acids in their urine in the course of selective screening for inborn errors of metabolism. The fourth patient had biotinidase deficiency detected by newborn screening, and the *N*-acetylamino aciduria was detected in the course of organic acids analysis to follow up his first diagnosis.

OS-104 II-1 is the eldest child of consanguineous Turkish parents residing in Germany. Two younger brothers are unaffected. The boy's cognitive development appears normal. At present, he attends high school. He started walking at age 1 year. At age 3 years, the parents first observed muscle weakness that prompted a thorough investigation at age 11 years. During that examination, the boy presented with low-normal muscle tone and a shambling gait. Ultrasound, electromyography, and NMR tomography of the musculature from the lower extremities showed unremarkable results. In addition, routine histological, histochemical, enzyme histochemical, and immunohistochemical studies of a biopsied specimen from the left quadriceps muscle did

Table 1

Concentrations of *N*-Acetylamino Acids in Urine, as Quantified by ^{1}H-NMR Spectroscopy

	Concentration in Urine of Individual[a] (mmol/mol creatinine)					
	OS-104 II-1					
Metabolite	Sample 1	Sample 2	Sample 3	OS-127 II-1	OS-124 II-1	OS-120 II-1
N-acetylalanine	93	115	91	69	312	156
N-acetylasparagine	89	79	66	155	356	133
N-acetylglutamic acid	193	186	169	281	741	284
N-acetylglutamine	111	98	86	148	526	143
N-acetylisoleucine	18	23	<10	58	40	30
N-acetylglycine	108	125	92	175	429	515
N-acetylvaline	<10	<10	<10	30	ND	ND
N-acetylserine	104	111	99	<10	393	208
N-acetylthreonine	18	23	17	<10	41	28
N-acetylmethionine	41	45	46	80	75	50

Note.—In addition to the listed metabolites, *N*-acetylleucine was always detected but—because of interferences—was difficult to quantify. Futhermore, an unidentified *N*-acetyl compound, with the chemical shift 2.108 ppm for $HN-CO-CH_3$, was found. In control urine samples, concentrations of *N*-acetylated amino acids are <40 mmol/mol creatinine.

[a] ND = not detected.

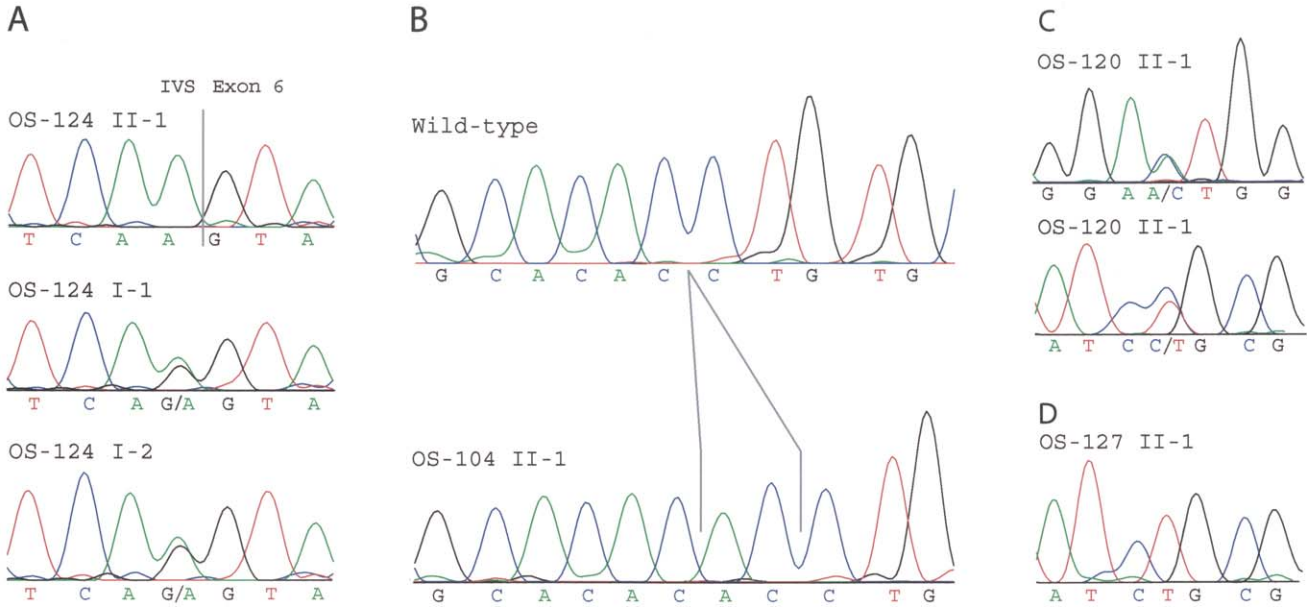

Figure 3 Results of the mutational analysis of *ACY1* in four patients with ACY1 deficiency. *A,* Sequence chromatograph from the affected individual OS-124 II-1 and his consanguineous parents (father, OS-124 I-1; mother, OS-124 I-2), showing a homozygous G→A transversion at position −1 of the obligatory exon 6 splice-acceptor site [IVS5−1G→A] in the patient and heterozygous mutations in both parents, consistent with homozygosity by descent. *B,* Sequence chromatograph from a control individual and the affected individual OS-104 II-1, depicting a homozygous 2-bp insertion in exon 15 resulting in a frame shift [1105^1106insAC] and a postmature stop codon [369PfsX46]. *C,* Two sequence chromatographs of the affected individual OS-120 II-1 depicting her compound heterozygous missense mutations 699A→C in exon 10 (E233D) and 1057C→T in exon 14 (R353C), which predicts the substitutions of evolutionary conserved amino acid residues E233D and R353C, respectively. *D,* Sequence chromatograph from the affected individual OS-127 II-1, showing the homozygous missense mutation 1057C→T in exon 14 (R353C).

not reveal any significant myopathological abnormalities (H. H. Goebel, University of Mainz, Germany; see Acknowledgements).

OS-127 II-1 is the first child of healthy, nonconsanguineous German parents. The boy was identified in a general neonatal screening as being biotinidase deficient, for which he is currently being given treatment with 5 mg biotin/d. Under this medication, his cognitive and motor skills have developed completely normally up to his current age of 17 mo.

OS-124 II-1 is the second child of consanguineous Turkish parents. He has a healthy older sister. Because of early impaired psychomotor development characterized by markedly disturbed central coordination and low muscle tone, magnetic resonance imaging of the head and spine was performed at age 3 mo, after cerebellar hypoplasia had been suggested prenatally by sonography. Cranial imaging demonstrated hypoplasia of the corpus callosum and the vermis cerebelli, as well as delayed myelination of the supratentorial white matter. The lumbar spinal cord exhibited a syringomyelia with a length of 3.5 cm.

OS-120 II-1 is the daughter of healthy parents of German (mother) and Italian (father) origin. Her younger brother is healthy. At age 2 years, a neuropediatric evaluation was initiated because of moderate retarded cognitive and motor development.

Mutation Analysis

Molecular analysis detected *ACY1* mutations in all four individuals with an increased urinary excretion of *N*-acetylated amino acids suggestive of ACY1 dysfunction (fig. 3). Consistent with parental consanguinity, two patients had homozygous mutations. In individual OS-124 II-1 we detected a homozygous obligatory splice-site mutation (IVS5−1G→A) predicting malfunction of the acceptor splice-site from exon 6. This mutation (IVS5−1G→A) predicts the skipping of exon 6 (amino acid residues 121 to 146) and a premature stop codon (fig. 2*A*). Segregation analysis showed that both parents (OS-124 I-1 and I-2) were heterozygous for this mutation. Individual OS-104 II-1 was homozygous for a 2-bp insertion (1105^1106insAC) predicting a frame shift beginning with amino acid residue 369. Notably, the mutation does not lead to premature termination of translation but predicts a C-terminal mutated protein (369PfsX46) that is longer than the wild-type protein

(408 amino acid residues) and affects the C-terminal peptidase domain (fig. 3*B*). Segregation analysis confirmed heterozygous carrier status in both parents (OS-104 I-1 and I-2). Child OS-120 II-1 was compound heterozygous for two missense mutations, 699A→C (E233D) and 1057C→T (R353C) (fig. 3*C*). Mutation 1057C→T (R353C) was transmitted from the mother; DNA from the father was not available. Both missense mutations (E233D and R353C) predict the substitution of amino acid residues located in the large peptidase domain (fig. 4) that has been evolutionary conserved in *Mus musculus* (mouse), *Rattus norvegicus* (rat), *Xenopus laevis* (frog), and *Danio rerio* (fish). Proband OS-127 II-1 was homozygous for missense mutation 1057C→T (R353C). The parents are not known to be consanguinous. Sequence analyses revealed that they are both heterozygous carriers of the 1057C→T mutation (fig. 3*D*). The E233D missense mutation was absent from 210 control chromosomes. The R353C sequence variant was identified in 1 of the 210 chromosomes of the control cohort.

Northern Blot Analysis

The expression pattern of the human *ACY1* gene was determined using human multiple-tissue northern blots (fig. 5). The *ACY1* probe detected a single band of the expected size of ~1.6 kb, consistent with the previously reported size of the human cDNA (Cook et al. 1993). The highest expression level was detected in kidney. *ACY1* expression was moderately high in brain and was weaker in placenta, spleen, uterus, and lung, as reported elsewhere (Cook et al. 1993). In addition, we detected expression in prostate, testis, small intestine, and colon, tissues that have not been previously studied (data not shown). We found no indication for alternative transcripts.

Discussion

Enzyme assays confirmed ACY1 deficiency in cells of individuals with *N*-acetylamino aciduria. Therefore, we searched for mutations in the *ACY1* gene in four affected individuals belonging to families from Turkey, Germany, and Italy. On the basis of the pedigrees with unaffected parents and consanguinity in two families (OS-104 and OS-124), autosomal recessive inheritance was assumed. Sequence analysis in affected children of these families identified homozygous loss-of-function mutations predicting abnormal splicing in OS-124 II-1 (IVS5−1G→A) and aberrant translation in OS-104 II-1 (1105^1106insAC), compatible with homozygosity by descent. In the other two families, homozygous (OS-127 II-1 and R353C) and compound heterozygous (OS-120 II-1, R353C, and E233D) missense mutations were identified, predicting the substitution of evolutionary conserved amino acid residues. To exclude rare polymorphisms, 210 control chromosomes were checked for the presence of the missense mutations and yielded negative results for E233D. Interestingly, we found one R353C allele in the control population, which is consistent with a rare polymorphism or a more common mutation. This is in line with the identification of this sequence variant in two of four individuals with ACY1 deficiency. The enzyme activity test has shown the functional relevance of harboring this mutation in both of them. Altogether, we clearly demonstrated that recessive *ACY1* mutations are responsible for the distinct urinary metabolic profile consistent with ACY1 dysfunction. Interestingly, mutations could be identified in all individuals with elevated concentrations of several *N*-acetylamino acids. Thus, we have no indication for genetic heterogeneity. Segregation analyses revealed that all parents studied were heterozygous carriers of the mutations. Urine analyses in parents yielded normal results, confirming recessive transmission of ACY1 deficiency.

Next, we asked whether ACY1 deficiency is related to the observed clinical phenotypes in the reported patients or merely represents a biochemical abnormality. The organic acids analysis that led to the detection of the ACY1-deficient individuals is part of selective screening for inborn errors of metabolism, which is not performed routinely in healthy children but only in individuals in whom a metabolic disease is considered. This results in a strong bias. To further clarify this, studies of family members would be advantageous. However, this was not possible in the family cohort we studied. The clinical presentation of the four patients is heterogeneous: (i) mild psychomotor retardation, (ii) moderate psychomotor retardation and brain and spinal cord malformations, (iii) muscle weakness but normal cognitive development, and (iv) the unremarkable clinical course of a biotinidase-deficient and early treated boy. This phenotypic variability does not support ACY1 deficiency being a disease.

However, the protein sequence of ACY1 is conserved between fish, frog, mouse, rat, and human, supporting an evolutionary conserved role in physiology. Strong expression of the human gene and its mouse ortholog *Acy1* in brain, liver, and kidney suggest a role of the enzyme in amino acid metabolism of these organs.

Our finding of *ACY1* expression in the kidney, which is consistent with previous observations (Cook et al. 1993; Lindner et al. 2000), is also in line with a possible functional role of ACY1 in recycling of *N*-acetylated amino acids in the kidney, which can explain the highly elevated concentrations of *N*-acetylated amino acids in the urine of the four children harboring *ACY1* mutations. Thus, in the case of severe dietary protein restriction, ACY1 dysfunction might contribute to reduced

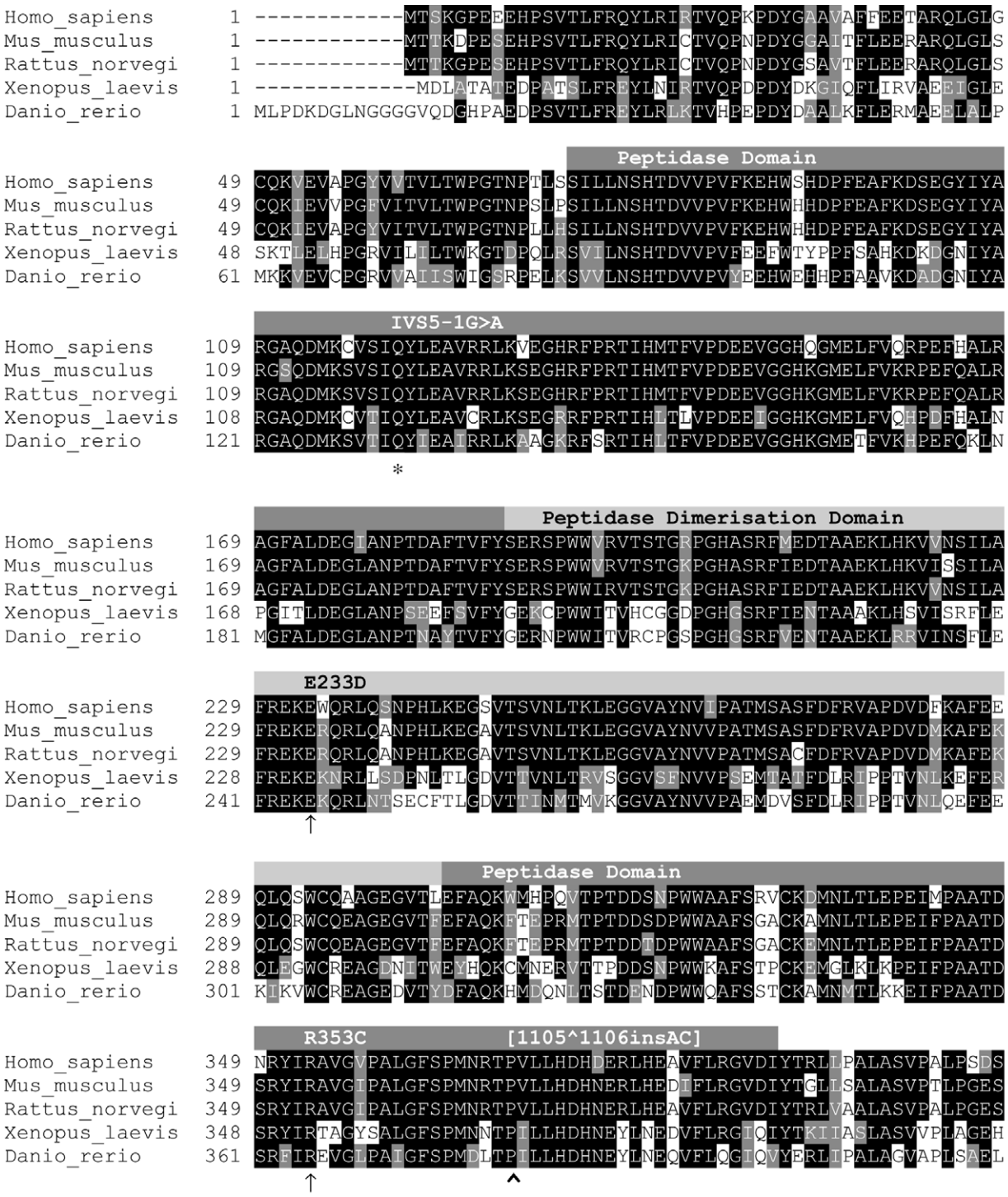

Figure 4 Sequence alignment of human ACY1 and corresponding orthologs from various species. Highlighted letters and letters on a gray background represent identical and conserved amino acid residues, respectively. Most parts of the orthologous proteins are highly conserved between species and predict a large peptidase domain, which includes a central peptidase dimerization region. Human ACY1 shares amino acid sequence identity and similarity, respectively, with *M. musculus* (85%/91%), *R. norvegicus* (87%/93%), *X. laevis* (60%/76%), and *D. rerio* (61%/77%). The amino acids affected by the missense mutations (E233D and R353C) are marked with arrows. The obligatory acceptor splice-site mutation [IVS5−1G→A] is marked with an asterisk (*) and predicts the skipping of exon 6 (aa 121 to aa 146) and a premature stop codon [120QfsX1]. The 2-bp insertional mutation [1105^1106insAC] is marked with an arrowhead and predicts a frame shift leading to a severely altered C-terminus [369PfsX46]. Ortholog proteins were identified by BLAST search. The multiple-protein alignment was constructed using the MAP program. Functional domains were predicted with the Scansite program. The corresponding accession numbers of the protein sequences are: Homo_sapiens AAH14112, Mus_musculus AAH05631, Rattus_norvegicus AAH78930, Xenopus_laevis AAH77639, and Danio_rerio NP_957289.

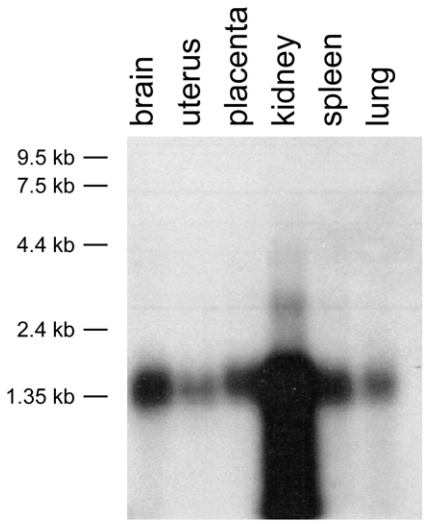

Figure 5 Human adult tissue northern blot of the *ACY1* gene. The *ACY1* probe detects a single band of ~1.6 kb, which corresponds to the predicted size of the human cDNA. Expression is highest in kidney, strong in brain, and weaker in placenta and spleen. *ACY1* mRNA is also expressed in uterus and lung. RNA size markers are indicated on the left side.

availability of amino acids due to impaired amino acid salvage. This could be a disadvantage during periods of profound catabolism or starvation. In addition, ACY1 deficiency could possibly result in accumulation of acetylated amino acids in analogy to *N*-acetylaspartic acid in aminoacylase 2 deficiency (Canavan disease).

ACY1 may also have functions beyond the deacylation of amino acids. It has been known since 1881 that ACY1 can also serve as a catalyzer for acylation reactions—for instance, forming hippuric acid from glycine and benzoic acid (Schmiedeberg 1881). However, classic benzoate detoxification is achieved by a different mechanism, involving mitochondrial glycine conjugation. Therefore, Lindner et al. (2000) suggested that benzoylglycine (hippuric acid) synthesis, catalyzed by ACY1, may represent an alternative benzoate detoxification pathway. The main source of benzoic acid originates from plant-derived food rich in aromatic compounds. During human evolution, the proportion of animal-derived food increased at the expense of plant-derived food; in other words, humans changed from herbivores to omnivores. Thus, ACY1-dependent detoxification of benzoic acid may have been an advantage prior to this transition and might still be beneficial in vegetarians. Interestingly, ACY1 also participates in drug metabolism. For example, aminoacylase activity is involved in the metabolism of mercapturates, which are formed from drugs such as paracetamol (Anders and Dekant 1994).

It is possible that ACY1 deficiency has consequences later in life, perhaps as a predisposing factor or modifier. It should be noted that long-term follow-up studies with a greater number of individuals may be required to detect such effects; observations on the basis of four pediatric patients alone may not be sufficient.

Although the identification of "novel" inborn errors of metabolism may reveal pathways and reactions that had gone unnoticed, ACY1 activity was described by the German physician Oswald Schmiedeberg 124 years ago (Schmiedeberg 1881) but was never placed in the context of a genetic disorder. The observation of ACY1-deficient humans offers the opportunity of learning more about the physiological role of ACY1. Awareness of the existence of this genetic entity may lead to the identification of more cases, and their follow-up may clarify whether ACY1 deficiency is a disease or a biochemical variant.

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Web Resources

URLs for resources presented herein are as follows:

BLAST, http://www.ncbi.nlm.nih.gov/blast/ (used to identify ortholog protein sequences and to establish the genomic organization of *ACY1*)

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/ (for cDNA and genomic sequences of *ACY1* [accession numbers BC014112 and NT_022517.17])

MAP, http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html (used to construct multiple-protein alignment)

RZPD Web site, http://www.rzpd.de

Scansite, http://scansite.mit.edu/ (used to predict functional domains)

References

Anders MW, Dekant W (1994) Aminoacylases. Adv Pharmacol 27: 431–448

Baslow MH, Resnik TR (1997) Canavan's disease: analysis of the nature of the metabolic lesions responsible for development of the observed clinical symptoms. J Mol Neurosci 9:109–125

Birnbaum SM, Levintow L, Kingsley RB, Greenstein JP (1952) Specificity of amino acid acylases. J Biol Chem 194:455–470

Canavan M (1931) Schilder's encephalitis periaxialis diffusa: report of a child aged sixteen and one-half months. Arch Neurol Psychiatr 25:299–308

Cook RM, Burke BJ, Buchhagen DL, Minna JD, Miller YE (1993) Human aminoacylase-1: cloning, sequence, and expression analysis of a chromosome 3p21 gene inactivated in small cell lung cancer. J Biol Chem 268:17010–17017

Engelke UF, Liebrand-van Sambeek ML, de Jong JG, Leroy JG, Morava E, Smeitink JA, Wevers RA (2004) *N*-acetylated metabolites in urine: proton nuclear magnetic resonance spectroscopic study on patients with inborn errors of metabolism. Clin Chem 50:58–66

Giardina T, Biagini A, Dalle Ore F, Ferre E, Reynier M, Puigserver A

(1997) The hog intestinal mucosa acylase I: subcellular localization, isolation, kinetic studies and biological function. Biochimie 79:265–273

Gordon N (2001) Canavan disease: a review of recent developments. Eur J Pediatr Neurol 5:65–69

Kaul R, Gao GP, Balamurugan K, Matalon R (1993) Cloning of the human aspartoacylase cDNA and a common missense mutation in Canavan disease. Nat Genet 5:118–123

Lehnert W (1994) Long-term results of selective screening for inborn errors of metabolism. Eur J Pediatr 153:S9–S13

Lindner H, Hopfner S, Tafler-Naumann M, Miko M, Konrad L, Röhm KH (2000) The distribution of aminoacylase I among mammalian species and localization of the enzyme in porcine kidney. Biochimie 82:129–137

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Miller YE, Drabkin H, Jones C, Fisher JH (1990) Human aminoacylase-1: cloning, regional assignment to distal chromosome 3p21.1, and identification of a cross-hybridizing sequence on chromosome 18. Genomics 8:149–154

Naylor SL, Shows TB, Klebe R J (1979) Bioautographic visualization of aminoacylase-1: assignment of the structural gene ACY-1 to chromosome 3 in man. Somat Cell Genet 5:11–21

Naylor SL, Elliott RW, Brown JA, Shows TB (1982) Mapping of aminoacylase-1 and beta-galactosidase-A to homologous regions of human chromosome 3 and mouse chromosome 9 suggests location of additional genes. Am J Hum Genet 34:235–244

Perrier J, Durand A, Giardina T, Puigserver A (2005) Catabolism of intracellular *N*-terminal acetylated proteins: involvement of acylpeptide hydrolase and acylase. Biochimie 87:673–685

Polevoda B, Sherman F (2000) Nα-terminal acetylation of eukaryotic proteins. J Biol Chem 275:36479–36482

Schmiedeberg O (1881) Über Spaltungen und Synthesen im Thierkörper. Arch Exp Pathol Pharmak 14:379–392

Smorodinzew IA (1922) Über die Wirkungen des Histozyms auf die Homologen der Hippursäure. Hoppe-Seyler's Z Physiol Chem 124: 123–139