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Clinical, Neuroimaging, and Metabolic Footprint of the Neurodevelopmental Disorder Caused by Monoallelic *HK1* Variants

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Abstract

Background and Objectives

Hexokinase 1 (encoded by *HK1*) catalyzes the first step of glycolysis, the adenosine triphosphate–dependent phosphorylation of glucose to glucose-6-phosphate. Monoallelic *HK1* variants causing a neurodevelopmental disorder (NDD) have been reported in 12 individuals.

Methods

We investigated clinical phenotypes, brain MRIs, and the CSF of 15 previously unpublished individuals with monoallelic *HK1* variants and an NDD phenotype.

Results

All individuals had recurrent variants likely causing gain-of-function, representing mutational hot spots. Eight individuals (c.1370C>T) had a developmental and epileptic encephalopathy with infantile onset and virtually no development. Of the other 7 individuals (n = 6: c.1334C>T; n = 1: c.1240G>A), 3 adults showed a biphasic course of disease with a mild static encephalopathy since early childhood and an unanticipated progressive deterioration with, e.g., movement disorder, psychiatric disease, and stroke-like episodes, epilepsy, starting in adulthood. Individuals who clinically presented in the first months of life had (near)-normal initial neuroimaging and severe cerebral atrophy during follow-up. In older children and adults, we noted progressive involvement of basal ganglia including Leigh-like MRI patterns and cerebellar atrophy, with remarkable intraindividual variability. The CSF glucose and the CSF/blood glucose ratio were below the 5th percentile of normal in almost all CSF samples, while blood

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Glossary

DEE = developmental and epileptic encephalopathy; **G6P** = glucose-6-phosphate; **HK** = hexokinase; **NDD** = neurodevelopmental disorder.

glucose was unremarkable. This biomarker profile resembles glucose transporter type 1 deficiency syndrome; however, in HK1-related NDD, CSF lactate was significantly increased in all patients resulting in a substantially different biomarker profile.

Discussion

Genotype-phenotype correlations appear to exist for *HK1* variants and can aid in counseling. A CSF biomarker profile with low glucose, low CSF/blood glucose, and high CSF lactate may point toward monoallelic *HK1* variants causing an NDD. This can help in variant interpretation and may aid in understanding the pathomechanism. We hypothesize that progressive intoxication and/or ongoing energy deficiency lead to the clinical phenotypes and progressive neuroimaging findings.

Introduction

Developments in high-throughput sequencing technologies in recent years, such as exome or genome sequencing, enable us to comprehensively and timely investigate the genetic causes of neurodevelopmental disorders (NDDs) with or without epilepsy.[1] Often the clinical spectrum of NDDs is broad, with large interindividual differences; therefore, the clinical phenotypes are nondistinctive. While the identification of new disease genes is progressing rapidly, our understanding of the cellular pathways leading to pathology lags far behind. This hampers variant interpretation, which, in the absence of a biomarker or functional testing, solely relies on reverse clinical phenotyping. Moreover, a full understanding of the pathomechanism is essential for therapy development.[1-3]

The gene *HK1* encodes hexokinase (HK)1, one of the 2 isoforms of hexokinases that are associated with mitochondria, following their interaction with the outer membrane mitochondrial porin, the voltage-dependent anion channel. Hexokinases catalyze the ATP-dependent phosphorylation of glucose to glucose-6-phosphate, the first step and rate-limiting reaction in glycolysis. By phosphorylating glucose, hexokinases effectively prevent glucose from leaving the cell and thus commits glucose to intracellular metabolism, e.g., energy metabolism. Of note, HK1 is the sole hexokinase isoform found in the cells and tissues, which rely most heavily on glucose metabolism for their function, including neurons and astrocytes, retinal cells, erythrocytes, platelets, and fibroblasts.[4]

Pathogenic variants in *HK1* can cause different disorders with different types of inheritance. One specific homozygous variant in the promotor region has been attributed to hereditary motor and sensory neuropathy Russe type (HMSNR; MIM #605285),[5] while different biallelic variants (in all protein domains with exception of the interdomain helix) that result in *HK1* variants with reduced stability[6] explain hereditary nonspherocytic to hemolytic anemia (MIM #235700).[7] In rare cases, however, *HK1* variants in hemolytic anemia were also reported to be associated with multiple malformations including NDD[8,9] or intrauterine fetal death.[10] One specific monoallelic variant, which is located outside the catalytic pocket in the HK1 C-terminal subdomain, is known to underly retinitis pigmentosa 79 (RP79; MIM #617460).[11] In addition, monoallelic variants, which affect a tissue-specific regulatory element have been reported in congenital hyperinsulinism.[12] Furthermore, monoallelic *HK1* variants causing NDD with visual defects and brain anomalies have recently been reported in 12 individuals (NEDVIBA; MIM# 618547).[13-15] The variants causing the latter phenotype are found in the regulatory HK1 N-terminal subdomain and at the beginning of the interdomain helix.

The structural analysis suggests that the missense variants within the N-terminal regulatory domain and interdomain alpha helix may disrupt the regulatory glucose-6-phosphate binding site. Because this binding site is responsible for product inhibition of HK1, disruption of glucose-6-phosphate binding could result in an inability to autoregulate, leading to gain of function and constitutive glucose phosphorylation. This is underlined by the presence of >20 individuals with heterozygous *HK1* truncating alleles in the ExAC database, suggesting that HK1-associated dominant disease phenotypes probably results from either a gain-of-function or dominant-negative mechanism instead of haploinsufficiency.[14]

We investigate the clinical phenotypes, MRIs, and the CSF of 15 previously unpublished individuals with monoallelic *HK1* variants and an NDD phenotype to add distinctive radiologic and laboratory features (biomarkers), contributing to precise definition and recognition of the phenotype, and to improve our understanding of the underlying disease mechanism.

Methods

Eligible individuals with monoallelic disease causing variants in *HK1* and an NDD phenotype were identified through

Table 1 Clinical Features and Genotypes of 15 Individuals With Monoallelic *HK1* Variants

Individual	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
HK1 variant[a]	c.1370C>T; p.Thr457Met								c.1334C>T; p.(Ser445Leu)						c.1240G>A; p.(Gly414Arg)
Age range follow-up/ (death)[b]	Toddler[b]	Infantile	Preschool	Toddler	School[b]	School	(Pre) adolescent	Infantile[b]	Young adult	School	Young adult	(pre) adolescent	Young adult	Young adult	Adult
Intellectual delay/ development	Profound/VA	Profound/VA	Profound/ VA	Profound/VA	Profound/VA	Profound/VA	Profound/ GDD	Profound/VA	Moderate/ GDD	Mild/GDD	No (IQ 78–91)/GDD	Moderate/ GDD	Moderate/ GDD	Mild/GDD	Mild/GDD
Static encephalopathy/ progression of disease	Yes/yes	Yes/yes	Yes/yes	Yes/no	Yes/no	Yes/yes	Yes/no	Yes/yes	Yes/yes (progressive hypokinetic rigid syndrome from age 23)	Yes/no (regression of speech as infant)	Yes/no	Yes/yes (motor impairment, secondary paraplegia, progressive scoliosis, at age 15	Yes/no	Yes/yes (progressive cognitive decline, psychiatric problems, epilepsy as adult)	Yes/yes
Epilepsy, EEG findings (age)	DEE, slow and discontinuous background, multifocal polyspikes and slow waves (5 m, 8 m)	DEE, hypsarrythmia (6 m)	DEE, not available	DEE, hypsarrythmia (3 m), multifocal sharp slow waves especially over the right occipital area, single generalized sharp slow wave (16 m)	DEE, slow background, multifocal sharp waves and sharp slow waves (4.5 y)	DEE, continuously occurring, generalized sharp slow waves and spike waves, sometimes interrupted by short suppression phases (7 y)	DEE, almost continuous, generalized spike-wave complexes (10 y)	DEE, burst suppression (6 d, 11 d); discontinuous background and at times a burst-suppression appearance (6 m)	No, not performed	No, unremarkable (5.5 y)	No, not performed	No, not performed	No, not performed	Yes, mildly abnormal (slow background) (26 y)	Yes, right tempero-occipital dysfunction, without signs of epileptic activity (46 y, after stroke-like episode)
Movement disorder	No	No	No	No	No	No	Yes (focal dystonia arms)	No	Yes	No	No	Yes (focal dystonia hands)	No	No	No
Other	KD	KD with no effect		KD with no effect	Germ cell mosaic; KD with no effect	Germ cell mosaic		KD with no effect	ASD, RP	No (especially no RP)	Nonspecific cognitive difficulties; ASD		RP	RP; congenital night blindness, generalized hypotonia	RP; 2 x stroke-like episodes, broad-based gait; alopecia totalis; microcytic erythrocytes, no anemia

Abbreviations: ASD = autism spectrum disorder; DEE = developmental and epileptic encephalopathy; GDD = global developmental delay, KD = ketogenic diet with no effect; RP = retinitis pigmentosa; VA = virtual absent.
Age ranges: neonate 0–4 wk, early infant 1–2 mo, infant 2–6 mo, late infant 6–12 mo, toddler 1–3 y, preschool 3–4 y, school 4–10 y, (pre)adolescent 10–18 y, young adult 18–30 y, and adult 30–50 y.
[a] NM_000188.3; NP_000179.2.
[b] Individuals died.

Table 2 Results of Cerebral MRI Studies

Individual, illustrated in	1	2	3	4	5	6, figure 1	7, figure 2	8	9	10	11	12	13	14, figure 3	15, figure 4
Age range at first/last MRI	Neonate/ infant	Infant	Neonate	Toddler	Neonate	Infant, toddler	Infant, school	Neonate, infant	Young adult	School	Young adult	School	Early adult	School/ preadolescent	Adult
Brain atrophy	Yes, in FU	Yes	No	Yes	No	Yes	Yes	Yes, in FU	No	No	Mild	No	No	No	No
Putamen atrophy/Putaminal eye	No/no	No/ no	No/no	No/yes	No/no	No/no	No/ mottled	No/no	Yes/yes	No/no	No/no	Yes/ yes	No/no	Yes/yes	No/no
Globus pallidus T2 hyperintensity	No	No	No	No	No	Yes	No	No	No	No	No	No	No	No	No
Caudate nucleus T2 hyperintensity	No	No	No	No	No	No	'Mottled'	No	No	No	No	Head	No	Head and body	No
Cerebellar atrophy	Mild	No	No	Mild	No	Mild	Mild	Mild	Yes	Yes	Yes	Yes	Yes	No	Yes
Additional findings			PC		CCA	MES									StLE, MES

Abbreviations: CCA = corpus callosum agenesis; FU = follow-up MRI; MES = T2 signal abnormalities in mesencephalon; mo = months; PC = periventricular cysts; StLE = stroke-like episodes; y = years.
Age ranges: neonate 0–4 wk, early infant 1–2 mo, infant 2–6 mo, late infant 6–12 mo, toddler 1–3 y, preschool 3–4 y, school 4–10 y, (pre)adolescent 10–18 y, young adult 18–30 y, and adult 30–50 y.

international collaborations. Clinical data from all individuals as well as CSF data and paired blood glucose and lactate concentrations were included based on the retrospective collection from medical files. Brain MRIs of all individuals were reviewed by the same investigator (E.B.).

Standard Protocol Approvals, Registrations, and Patient Consents: Participants (or their legally authorized representatives) gave written informed consent for genetic investigations and research according to local regulations. Concerning Dutch law, this study does not need ethics approval because it contains solely retrospective data that were already available and had been generated in another context, here clinical care. This case series reports data in aggregate with no case details or vignettes that contain identifying characteristics.

Data not provided in the article because of space limitations may be shared (anonymized) at the request of any qualified investigator for purposes of replicating procedures and results.

Results

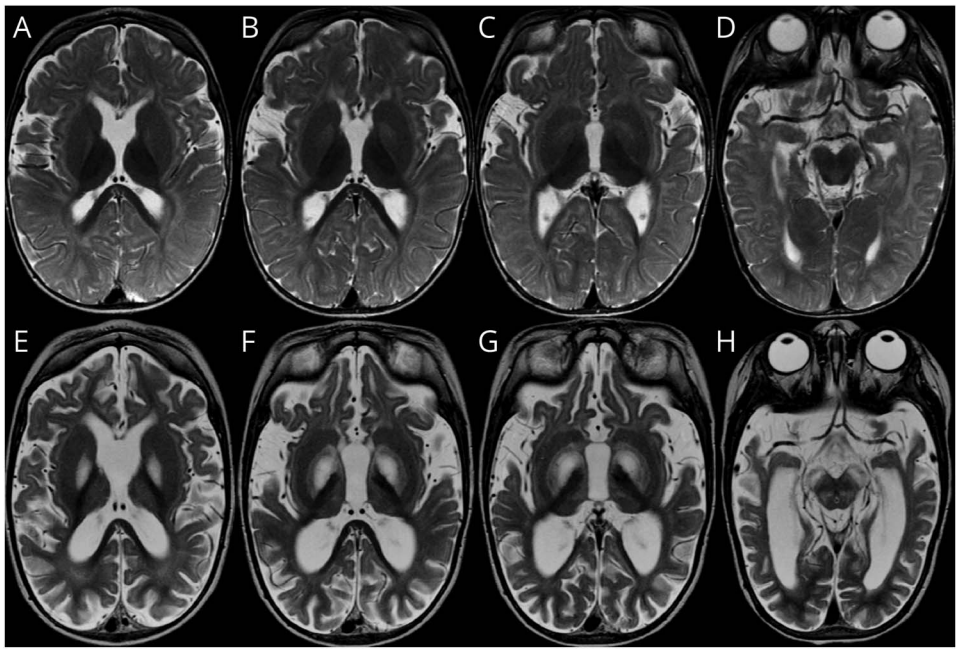
Genotypes and Clinical Phenotypes

As summarized in Table 1, 15 previously unpublished cases (6 male), including 1 pair of siblings were identified, in the age range of infants until adults in the fifth decade at last follow-up. The age at onset ranged from birth/neonatal period to infancy when developmental issues became apparent. In all these individuals, exclusively recurrent monoallelic *HK1* variants (reference sequence GenBank NM_000188.3) were detected in exome sequencing using leukocyte-derived DNA.[13-15] All variants are expected to have a gain-of-function effect. For all but individual 7, segregation analysis by trio exome sequencing or targeted Sanger sequencing was performed and showed absence of the variant in both parents.

While the NDD related to de novo *HK1* variants span a spectrum as common in nearly all NDDs, we observed 2 main phenotypes. The 8 individuals 1–8 with the c.1370C>T (Thr457Met) variant all presented with a developmental and epileptic encephalopathy (DEE) phenotype in the first weeks or months of life with drug-resistant infantile spasms. They showed virtually no development, and 3 of them died at the ages of 8 months, 18 months and 8.5 years, respectively. In none of these cases a movement disorder or retinal changes were reported. Of note, 2 of our cases (Table 1, individuals 5 and 6) carrying this variant were siblings, suggesting parental germline mosaicism.

In 6 individuals with the c.1334C>T (Ser445Leu) and 1 individual with the c.1240G>A (Gly414Arg) variant, we observed a different course of disease. All these individuals showed global developmental difficulties finally resulting in nonspecific cognitive abnormalities with a normal IQ in 1 individual, learning disabilities in one other, and mild-to-

Figure 1 MRI of Individual 6



Axial T2w MRI at infantile (A–D) and at toddler age (E–H) demonstrating extensive volume loss in the course of the disease, with T2 hyperintensity and swelling of bilateral globus pallidus and mesencephalic structures (while caudate nucleus and putamen are not affected) at the last MRI.

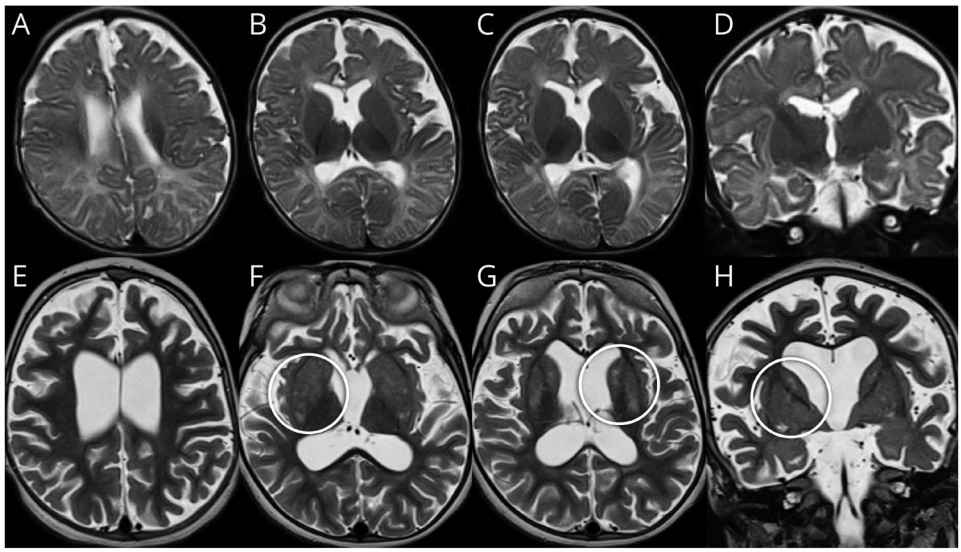
moderate intellectual disability in the other 5. An autism spectrum disorder was observed in 2 individuals. In 4 individuals, retinitis pigmentosa was also found.

In 4 of the 5 individuals who were older than 20 years, the disorder was unexpectedly complicated by a progressive course starting in the early 20s. This encompassed a hypokinetic rigid syndrome in 1 female individual and cognitive decline with neuropsychiatric problems and adult-onset epilepsy in another female individual. Furthermore, a broad-based gait with 2 stroke-like episodes with hemianopsia and post-stroke seizures were seen in the third female individual. In her a partial recovery at the clinic was seen under treatment of antiseizure medication.

Neuroimaging Findings

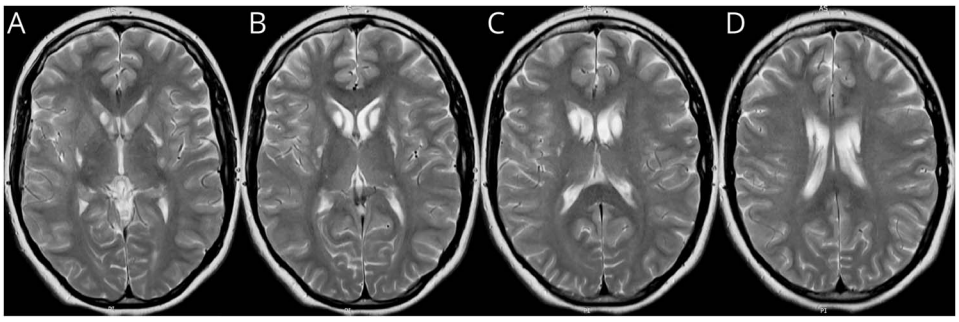
All 15 individuals underwent at least 1 MRI study and 7 of them had multiple (up to 4) MRIs (Table 2). There was a very broad range regarding the age (from day of birth until the 5th

Figure 2 MRI of Individual 7



Axial T2w MRI of individual 7 at early infantile (A–D) and at toddler age (E–H), illustrating marked cerebral atrophy and a "mottled" T2 hyperintense pattern of the caudate and putamen (encircled).

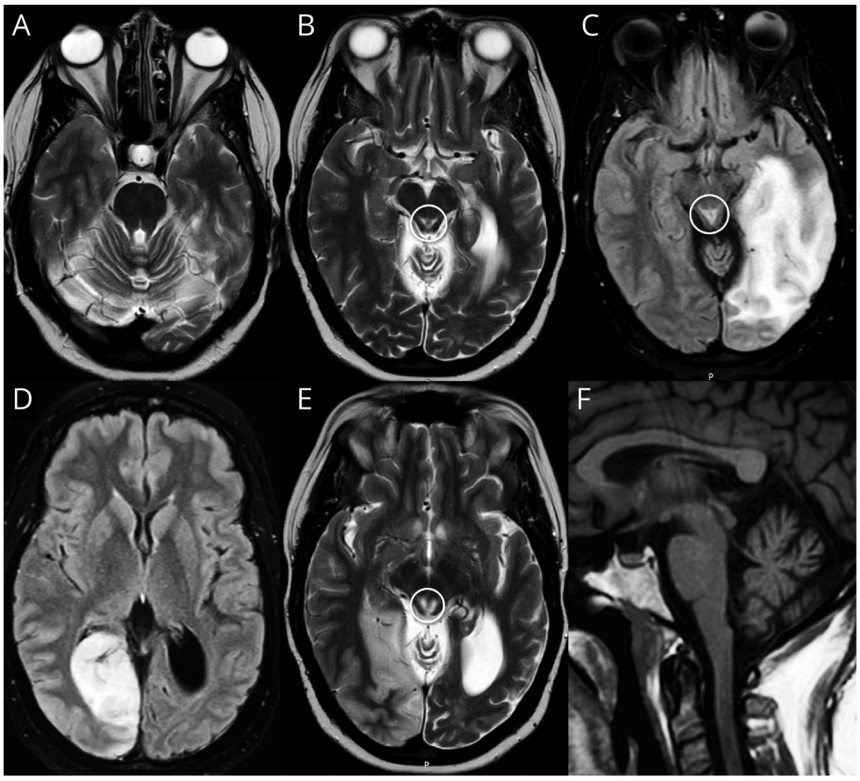
Figure 3 MRI of Individual 14



Axial T2w MRI of individual 14 at pre-adolescent age demonstrating signal changes in the head and body of the caudate nucleus (A–D) and atrophy of the putamen with a "putaminal eye" on the left side (A).

decade at the first MRI. Aiming to get meaningful—although small—subgroups, we merged the results of the first MRI study of every individual, using age groups. (1) MRI in the *neonatal period* (0–4 weeks; n = 4; individuals 1, 3, 5, and 8), likely reflecting an early and severe disease course. Besides the finding of agenesis of the corpus callosum in individual 5, these (early) images were normal or had only mild non-specific findings. The observation period is too short to assess potential brain atrophy in 2 children, while evolving brain atrophy was apparent in 2 other individuals. (2) MRI in the *infantile/early toddler period* (1–18 months; n = 4; individuals 2, 4, 6, and 7). The findings were dominated by marked brain atrophy. Of note, individual 6 had no malformation in contrast to his sibling (individual 5, with agenesis of the corpus callosum; see above). His MRI showed severe brain atrophy, swelling of the globus pallidus, and signal alterations in the crura cerebri (Figure 1). In individual 7, the putamen and caudate nucleus had a remarkable "mottled" appearance (Figure 2). All children in this group had global brain atrophy. (3) MRI at *school age* (n = 3; aged 4–5 years at their first MRI; individuals 10, 12, and 14). The images of individual 10 showed only mild cerebellar atrophy at school age. Individual 12 (at school age) was found to have cerebellar atrophy and bilateral involvement of the putamen with a putaminal eye

Figure 4 MRI of Individual 15



MRI of individual 15 in the middle of the fourth decade (A–C, and 1 year later D–F). (A) T2w axial view demonstrating marked cerebellar atrophy affecting the vermis and hemispheres. (B and C) Images taken during the first stoke-like episode showing periaqueductal signal changes (encircled in B, C) and a large FLAIR hyperintense area in the left hemisphere. (D and E) MRI at 45 years of age demonstrating a new stroke-like lesion in the right hemisphere; the left lateral ventricle is dilated due to tissue loss following the first stroke. (E) Additional signal changes in the inferior hypothalamic region. (F) T1w sagittal midline view demonstrating cerebellar vermis atrophy.

Table 3 Results of CSF Studies

Individual	1	1	1	1	2	4	5	6	7	8	8	8	9	10	12	14	15
Age at lumbar puncture	Neonate	Neonate	Early infant	Early infant	Infant	Toddler	Infant	Infant	Preadolescent	Neonatal	Early infant	Infant	Adult	School	School	(Pre) adolescent	Adult
CSF glucose (mmol/L)	3.0	1.9*	1.5	1.5	2.0*	2.3	2.0	2.5*	2,4	2.1*	1.6	1.4	2.0	1.9	2.1	2.2	3.3
Age-specific RV for CSF glucose	1.9–5.6	1.9–5.6	1.7–5.6	1.7–5.6	1.9–4.9	2.4–4.2	2.8–4.9	2.4–4.9	2.6–4.3	1.9–5.6	1.9–4.9	1.9–4.9	2.7–4.4	2.5–4.0	2.5–4.0	2.6–4.3	2.8–4.4
CSF lactate (mmol/L)	6.1	5.4	4.6	4.3	6.6	6.0	4.4	8.6	6.4	Na	5.3	4.9	6.9	5.8	5.5	6.8	Na
Age-specific RV for CSF lactate	0.9–2.5	0.9–2.5	0.9–2.2	0.9–2.2	1.0–2.2	1.0–2.0	1,1–2.2	1.1–2.2	1.0–2.2		1.0–2.2	1.0–2.2	1.2–2.2	1.1–2.1	1.1–2.1	1.2–2.2	
Blood glucose (mmol/L)	Na	4.9	4.9	5.9	5.0	3.4	Na	Na	5.2	Na	4.0	2.4	4.6	Na	4.2	4.2	5.3
CSF/blood glucose ratio	Na	0.31	0.39*	0.51	0.40*	0.70	Na	Na	0.37	Na	0.40*	0.58	0.43	Na	0.49	0.52	0.60
Age-specific RV for CSF/ blood glucose ratio	0.42–1.10	0.42–1.10	0.36–1.20	0.36–1.20	0.39–1.10	0.44–0.84			0.39–1.10	0.42–1.10	0.39–1.10	0.39–1.10	0.46–0.90		0.45–0.84	0.47–0.83	0.46–0.88

Abbreviations: m = months; Na = not available; RV = reference value; w = weeks; y = years.
Values in green boxes are normal. In red boxes, values are abnormal, i.e., below the 5th percentile (CSF glucose and CSF/blood glucose ratio) or above the 95th percentile (CSF lactate) of age-related reference values. Asterisks mark normal values of CSF glucose and CSF/blood glucose ratio (above the 5th percentile), which are still below the 10th percentile of age-specific reference ranges. All age-specific reference ranges are from Leen et al.[16] Age ranges: neonate 0–4 wk, early infant 1–2 mo, infant 2–6 mo, late infant 6–12 mo, toddler 1–3 y, preschool 3–4 y, school 4–10 y, (pre)adolescent 10–18 y, young adult 18–30 y, and adult 30–50 y.

pattern and signal changes within the caudate heads, while globus pallidus and thalamus were not affected. Cerebellar atrophy was seen. Individual 14 had a first MRI at school age with evidence of mild signal changes in the caudate heads and a localized change in 1 putamen. At early (pre)adolescent age (Figure 3), the signal changes were very prominent in the caudate heads with extension into the body of the caudate nucleus. In addition, the putamina were atrophic, with a putaminal eye on 1 side. Remarkably, this individual has not developed cerebellar atrophy so far. (4) MRI in *adulthood* (older than 18 years; n = 4; individuals 9, 11, 13, and 15). The findings among adult individuals were variable but overall relatively mild; cerebellar atrophy was noted in all 4. It is important that MRI studies in individual 15 (at the middle of the 5th decade) after a subacutely developed clinical attack with headache and a hemianopsy at the right side showed, apart from cerebellar atrophy, an area of left parieto-occipital cortical ischemia not compatible with a vascular territory (Figure 4). One year later, after another subacute attack with the hemianopsia presenting at the opposite side, the MRI showed a cortical ischemia also of the right parieto-occipital area. The neuroimaging findings were found to be progressive when follow-up MRIs were available (individuals 1, 6, 7, 8, 14, and 15; Figures 1, 2 and 4).

CSF Analysis

Results of CSF analyses were available for 12 of 15 cases (*not* in individuals 3, 11, and 13). Two individuals underwent multiple lumbar punctures; altogether, data were available for 17 CSF samples (Table 3). This does not include blood lactate concentrations, CSF cell count, and CSF protein levels because these values were generally within reference ranges. While blood glucose concentrations were normal, CSF glucose was below the 10th percentile of age-specific reference values[16] in 15/17 (88%) samples and below the 5th percentile of age-specific reference values in 11/17 (65%) samples. The CSF/blood glucose ratio was available for 13 samples and found to be below the 10th percentile of age-specific reference values in 7 (54%) of these samples; in 4/13 (31%) occasions, the ratio was below the fifth percentile. CSF lactate was measured in 15 CSF samples and found to be far above age-specific references values in all of them (100%).

Discussion

Although our study is too small to draw firm conclusions on genotype-phenotype correlations, we noticed that all individuals with the most severe clinical and radiologic phenotype (neonatal and infantile age group) harbored the same monoallelic *HK1* variant c.1370C>T (replacing threonine 457 by a methionine.) These individuals presented in the first months of life with a DEE and showed virtually no achievement of any developmental milestones. They have a greatly shortened life expectancy. On the contrary, of the 4 individuals with the c.1334C>T and the (single) individual with the c.1240G>A variant who already reached adult age, 4 of the 5 showed an attenuated disease course, starting with an apparently static encephalopathy with global developmental difficulties, which later unexpectedly turned into a progressive disorder in their early 20s. Of note, both the c.1370C>T and the c.1334C>T variant are situated in the interdomain helix, while the c.1240G>A is located in the HK1 N-terminal subdomain.

Remarkably, retinitis pigmentosa was found in 3 individuals with the biphasic course. In this context, we considered it noteworthy that RP79, a disorder limited to the retina without any neurologic (or systemic) involvement, is caused by 1 specific monoallelic *HK1* variant, namely c.2539G>A (p.Glu847Lys),[17] which is located outside the catalytic pocket in the HK1 C-terminal catalytic subdomain. It is unknown why this specific variant affects only the retinal tissue.

Of note, the 3 different phenotypes (RP79, congenital hyperinsulinism, and NEDVIBA) related to monoallelic *HK1* variants are caused by recurrent variants, while HMSNR is caused by 1 recurrent homozygous variant. Furthermore, also biallelic *HK1* variants are known in association with anemia. *HK1* is a human gene in which variants lead to exceptionally many distinctive and very diverse clinical phenotypes.

Retrospective analyses of all available images allowed us to recognize some common MRI patterns in this series of individuals, especially after merging the results for different age groups. The number of (serial) MRIs was too small to make subgroups based on the genotype. We found that cerebral MRI did not show gross brain malformations, except in 1 individual with agenesis of the corpus callosum. Individuals who (clinically) presented in the first months of life had (near)-normal images, although they were clinically severely affected and often had a fatal disease course. We assume, given the progressive (clinical) course of the disease in these individuals and the findings in all other age groups, that these early MRI studies simply "lag behind" the clinical course. Severe brain atrophy was a very dominant finding in the MRI studies of the second age group, i.e., infants. In age groups 3 and 4 (juvenile and adult), we noted involvement of specific brain structures, namely basal ganglia and cerebellum, but we also found remarkable variability between individuals.

The *putamen* was affected in 5 individuals, showing a pattern highly reminiscent of the so-called putaminal eye in 4 of them (case 4 at 16 months; case 12 at 4 years; case 14 at 7 years; and case 9 at 25 years). The term "putaminal eye" was coined in the image analysis of individuals with MEGDEL syndrome (3-MEthylGlutaconic aciduria, Deafness, Encephalopathy, Leigh-like syndrome, MIM#614739)[18] and refers to a spared tissue section in an otherwise abnormal T2 hyperintense putamen. This sign is stage associated and disappears with disease progression in MEGDEL syndrome.[19] A putaminal eye is no longer considered pathognomonic for MEGDEL syndrome because it was also observed in single cases with Aicardi-Goutières syndrome, mitochondrial complex 1 deficiency, and *SLC19A3*-associated disease.[20] Putaminal eyes

have also been recognized in 2 other individuals with de novo *HK1* variants (figure 1 in ref 14). An explanation for the temporary sparing of the central putaminal area in MEGDEL syndrome and the other disorders is lacking. We hypothesize that a distinct form of mitochondrial dysfunction is the common underlying mechanism.

Only in 1 individual (individual 6), the *globus pallidus* and *mesencephalic structures* were markedly affected; this occurred in a late stage of the disorder, in which severe supratentorial atrophy had already occurred. We postulate that this imaging pattern was not seen more often because other individuals may already have succumbed or may not have undergone MRI during the final disease stages. Involvement of the *caudate nucleus* is variable in our cohort. It was never seen in MRI studies in the neonatal and infantile age groups nor in adult individuals, and it never occurred as an isolated finding. In the context of brain atrophy, the *thalamus* may show some nonspecific volume loss and T2 hyperintensity, but otherwise, this structure is spared in all images. Atrophy of the *cerebellum* is a common nonspecific finding in many inborn metabolic and genetic disorders.[21] It was not seen in neonatal-onset cases and their follow-up (as far as imaging was available), but otherwise found in almost all MRI studies. Apart from atrophy, no other cerebellar (including deep cerebellar nuclei) abnormalities were seen.

Stroke-like episodes were encountered in the oldest individual (individual 15), the only individual with the c.1240G>A variant. Previous reports in 2 individuals with an identical variant showed similar cerebellar atrophy but without stroke-like episodes.[13,15] Whether stroke-like episodes are part of the imaging spectrum associated with de novo *HK1* variants in general or with the c.1240G>A variant particularly remains to be determined.

In this study, we describe the characteristics of a series of 15 individuals with monoallelic *HK1* variants. Of interest, we detected only 3 different *HK1* variants in our cohort, all of which had been reported before[13-15]and therefore likely represent mutational hot spots. Of note, to date, a total of 6 monoallelic *HK1* variants (c.1240G>A, c.1241G>A, c.1252A>G, c.1334C>T, c.1370C>T, and c.1969G>A) have been reported to cause an NDD phenotype, all are thought to lead to gain of function.

Some known mechanisms may, at least partially, explain these hot spots. CpG dinucleotides have, on average, a 10-fold higher mutability than non-CpG dinucleotides.[22] In addition, the trinucleotide sequence is another factor significantly determining mutability rate: the mutation rate among the 64 possible trinucleotide sequences may differ up to 75-fold between the most and least mutable trinucleotide.[23] The c.1334C>T variant affects an ACG and the c.1370C>T variant a TCG trinucleotide. Analyzing these variants within a bigger DNA sequence context would be interesting; however, we are not aware of any prediction tools that could predict mutational hot spots in genes. We will not examine the mechanisms of evolution and selection in terms of gene mutability. From these perspectives, the c.1334C>T and c.1370C>T *HK1* variants both arise in vulnerable trinucleotides. Of interest 2 of our cases (individuals 5 and 6 in Table 1) carrying the c.1370C>T variant were siblings, and the same was true in a previously reported sibling pair.[13] We can only speculate about contributing factors.

Spermatogenic cell-specific type 1 hexokinase (HK1S) is abundant in mouse sperm and located mainly in the principal piece of the sperm flagellum, where other spermatogenic cell-specific glycolytic enzymes have been found.[24] Three variant transcripts of *HK1* that are expressed specifically in spermatogenic cells have different 5′ untranslated regions and encode the protein HK1S in which the porin-binding domain of HK1 is replaced by a novel N-terminal spermatogenic cell-specific region. Because HK1S seems to be important for the fitness/motility of sperm cells, a *HK1* gain-of-function variant might support its own selection for the germline de novo mode of inheritance. Of note, both in a previous publication[13] and here, sibling pairs with identical de novo variants are reported, suggesting germline mosaicism, which could further strengthen the presented hypothesis.

Of interest, and for the first time, we report that CSF glucose is low in almost all CSF samples of individuals with de novo *HK1* variants, often even below the 5th percentile of age-specific reference values, under normoglycemic conditions. In line with this, the CSF/blood glucose ratio is low on many occasions.

A low CSF glucose concentration and low CSF/blood glucose ratio are considered highly specific biomarkers for glucose transporter type 1 deficiency syndrome (GLUT1DS, MIM#606777), a neurologic disorder that is caused by defective transport of glucose into the CNS (i.e., GLUT1 haploinsufficiency) due to heterozygous *SLC2A1* variants.[16,25] The disorder caused by de novo *HK1* variants seems to share this remarkable CSF profile with GLUT1DS. It is important that, however, CSF lactate was found (far) above age-specific references values in all individuals in this study and in 3 previously reported individuals,[14] while CSF lactate is within normal ranges or even decreased in individuals with GLUT1DS16. Thus, considering both CSF glucose and CSF/blood glucose ratio and lactate concentrations, the biomarker profiles of individuals with *HK1* and *SLC2A1* variants differ essentially.

Two questions remain: why individuals with de novo *HK1* variants have (1) increased CSF lactate concentrations and (2) low CSF glucose concentrations and CSF/blood glucose ratio (similar to GLUT1DS)? These CSF abnormalities may directly link to the underlying disease mechanism suggested by Poole et al.[14] In their study, the authors suggested, based on structural protein analysis, that certain missense variants (c.1241G>A, c.1252A>G, c.1334C>T, c.1370C>T) within

HK1 may disrupt the regulatory glucose-6-phosphate (G6P) binding site, which is responsible for inhibition of HK1 by its own product (G6P). Protein changes within this site may therefore lead to decreased inhibition by G6P and thus gain of function of HK1. According to this hypothesis, excessive glucose phosphorylation and metabolic flux through the glycolytic pathway would play a central role in the underlying disease mechanism. We can imagine that under these conditions, cerebral glucose consumption may simply be greater than cerebral glucose supply (via GLUT1), thus leading to a decreased CSF glucose and CSF/blood glucose ratio. Furthermore, overactive glycolysis would result in the accumulation of glycolytic intermediates including its end product, pyruvate, and therefore also lactate in the brain. An increased flux through the glycolytic pathway would—at least theoretically—also lead to increased concentrations of some of its metabolites such as dihydroxyacetone phosphate and glyceraldehyde 3-phosphate or their byproduct methylglyoxal, which are thought to drive mitochondrial dysfunction and linked to the development of neurodegenerative disorders.[26,27]

To explain the decreased CSF glucose concentrations and low CSF/blood glucose ratio, we considered that HK1 is the rate-limiting enzyme of the glycolytic pathway and that increases of its activity may significantly affect brain glucose metabolism. Alternatively, we postulate that increased concentrations of the abovementioned glycolytic products may inhibit GLUT1 expression in the CNS (including the blood-brain barrier) to decrease glucose availability for the glycolytic pathway. The existence of such a protective feedback mechanism may be of vital importance, especially for organs such as the brain, which are not protected by insulin-regulated glucose influx, and has been demonstrated under experimental conditions in different cell cultures.[28,29] Taken together, both in HK1-related disease and in GLUT1DS, hypoglycorrhachia occurs. The HK1 clinical phenotype is a progressive mitochondrial disease presumably due to forced glycolysis with impaired autoregulation presenting more like a Leigh syndrome with predominantly neurodevelopmental issues and seizures. By contrast, in GLUT1DS, glycolysis is impaired by too little substrate and presents with seizures, but the movement disorder is the other dominating clinical feature. In addition, lactate may contribute to this. While lactate is low in GLUT1DS reflecting the complete shortage of all kind of energy sources, it is high and presumably contributing to the observed damage in HK1 deficiency.

From a therapeutic viewpoint, it would be extremely important to know whether part of the neurologic disorder is indeed the consequence of insufficient GLUT1 transport capacity because ketogenic diet therapy is generally very effective in individuals with GLUT1DS (in whom defective glucose transport into the CNS is the leading disease mechanism). In our cohort, 5 of the individuals had been on ketogenic diet therapy. One was having more seizures when not in ketosis, and ketogenic diet therapy was therefore continued until his early death. In the other 4 patients, ketogenic diet therapy was started upon the suspicion of GLUT1 deficiency. The diet had no obvious effect on the seizures and/or the EEG, and it was stopped after several weeks when GLUT1 deficiency had been ruled out. In addition, Poole et al.[14] reported 2 individuals who had been on ketogenic diet therapy without improvement, but more data are needed to draw firm conclusions on this topic.

Taking into account the spectrum of the progressive neurologic disorder and MRI characteristics including Leigh-like phenotypes, and stroke-like episodes, and the high CSF lactate concentrations, the HK1-associated NDD as reported in this study may be classified among the group of mitochondrial disorders. Categorizing this phenotype as such may facilitate early recognition and aid variant interpretation. Similarly, the severe early-onset, drug-resistant epilepsy of all cases with the c.1370C>T variant allows for classification as a DEE and vice versa adds HK1-associated NDD to the differential diagnoses of the clinical syndrome of DEE.

Finally, we would like to stress that this disorder seems to be only the second disorder (after GLUT1DS) with a remarkably low CSF glucose concentration and low CSF/blood glucose ratio.

Our study has several limitations: the cohort includes only a small number of individuals. MRI was performed at various ages, i.e., likely in different disease stages, using different imaging protocols, and many individuals had no follow-up studies. Likewise, lumbar punctures were performed at different ages and disease stages under different circumstances. Nevertheless, we feel that the results, as discussed earlier, may add valuable novel insights and may guide future research. A rational next step could be to find out whether these individuals indeed have a CSF profile that would fit with 1 or both proposed disease mechanisms (overactive glycolysis and deficient glucose transport into the CNS, respectively, for additional biomarkers, see also.[30] We feel that only a deeper understanding of the underlying biochemical defect in this disorder would make it more amenable for (rational) therapeutic approaches, manipulating cerebral glucose handling in general or HK1 enzyme activity particularly.

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Continued

Appendix *(continued)*

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