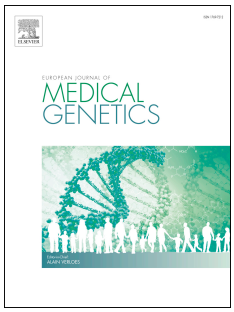

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PII: S1769-7212(23)00002-2

DOI: https://doi.org/10.1016/j.ejmg.2023.104696

Reference: EJMG 104696

To appear in: *European Journal of Medical Genetics*

Received Date: 21 August 2022

Revised Date: 30 November 2022

Accepted Date: 7 January 2023

Please cite this article as: R.L. Poole, M. Badonyi, A. Cozens, N. Foulds, J.A. Marsh, S. Rahman, A. Ross, J. Schooley, V. Straub, A.J. Quigley, D. FitzPatrick, A. Lampe, Expanding the neurodevelopmental phenotype associated with *HK1* de novo heterozygous missense variants, *European Journal of Medical Genetics* (2023), doi: https://doi.org/10.1016/j.ejmg.2023.104696.

CRediT Author Statement

Rebecca L. Poole: Conceptualization, Investigation, Methodology, Formal analysis, Writing- original draft, Writing- review and editing, Visualization, Project Administration. Mihaly Badonyi: Formal analysis, Investigation, Writing- review and editing, Visualization. Alison Cozens: Investigation, Writing- review and editing. Nicola Foulds: Investigation, Writing- review and editing, Joseph A. Marsh: Formal analysis, Investigation, Writing – review and editing, Visualization. Shamima Rahman: Writing- review and editing. Alison Ross: Investigation, Writing- review and editing. Joanna Schooley: Investigation, Writing- review and editing. Volker Straub: Investigation, Writing- review and editing. Alan J. Quigley: Formal analysis, Investigation, Writing- review and editing, Visualization. David FitzPatrick: Conceptualization, Investigation, Methodology, Formal analysis, Writing – review and editing. Anne Lampe: Conceptualization, Investigation, Writing- review and editing, Supervision.

Expanding the neurodevelopmental phenotype associated with *HK1* de novo heterozygous missense variants

Authors

Rebecca L. Poole[1], Mihaly Badonyi[2], Alison Cozens[3], Nicola Foulds[4], Joseph A. Marsh[2], Shamima Rahman[5], Alison Ross[6], Joanna Schooley[4], Volker Straub[7], Alan J. Quigley[8], David FitzPatrick[2], Anne Lampe[1]

Authors' affiliations

[1] South East of Scotland Clinical Genetics Service, Western General Hospital, Crewe Road South, Edinburgh, EH4 2XU, UK

[2] Medical Research Council Human Genetics Unit, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, EH4 2XU, UK

[3] Royal Hospital for Children and Young People, 50 Little France Crescent, Edinburgh Bio Quarter, Edinburgh, EH16 4TJ, UK

[4] Wessex Clinical Genetics Services, University of Southampton NHS Foundation Trust, Southampton, UK

[5] Genetics and Genomic Medicine Department, UCL Great Ormond Street Institute of Child Health, London, WC1N 1EH, UK

[6] North of Scotland Regional Genetics Service, Clinical Genetics Centre, Ashgrove House, Aberdeen Royal Infirmary, Foresterhill, Aberdeen, AB25 2ZA

[7]John Walton Muscular Dystrophy Research Centre, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, NE1 3BZ, UK

[8] Paediatric Imaging Department, Royal Hospital for Children and Young People, 50 Little France Crescent, Edinburgh Bio Quarter, Edinburgh, EH16 4TJ, UK

Corresponding author

Dr Rebecca Poole, ST4 Clinical Genetics, South East of Scotland Clinical Genetics Service, Crewe Road South, Western General Hospital, Edinburgh, EH4 2XU

Telephone 0131 537 1116 Email: rebecca.poole@nhs.scot

Abstract

Neurodevelopmental disorder with visual defects and brain anomalies (NEDVIBA) is a recently described genetic condition caused by de novo missense *HK1* variants. Phenotypic data is currently limited; only seven patients have been published to date. This descriptive case series of a further four patients with de novo missense *HK1* variants, alongside integration of phenotypic data with the reported cases, aims to improve our understanding of the associated phenotype. We provide further evidence that de novo *HK1* variants located within the regulatory-terminal domain and alpha helix are associated with neurological problems and visual problems. We highlight for the first time an association with a raised cerebrospinal fluid lactate and specific abnormalities to the basal ganglia on brain magnetic resonance imaging, as well as associated respiratory issues and swallowing/feeding difficulties. We propose that this distinctive neurodevelopmental phenotype could arise through disruption of the regulatory glucose-6-phosphate binding site and subsequent gain of function of HK1 within the brain.

Keywords

HK1 protein, human; disorder, neurological; basal ganglia

Main Manuscript

Introduction

Glycolysis is the process by which glucose is converted to pyruvic acid, generating net adenosine triphosphate (ATP) (Grüning and Ralser, 2021). The enzyme hexokinase (HK) catalyzes the first step of glycolysis and phosphorylates glucose to form glucose-6-phosphate (G6P) (Jamwal et al., 2019).

There are four different hexokinase isozymes found in humans: HK1; HK2; HK3; and HK4. These are each coded for by separate genes (Jamwal et al., 2019). HK1 is encoded by the *HK1* gene. Although ubiquitously expressed, *HK1* is referred to as the 'brain-type hexokinase' due to its strong expression in neurons (Okur et al., 2019). The majority of HK1 is bound to the outer mitochondrial membrane (Rosano et al., 1999). The HK1 protein is a symmetrical dimer, with each monomer consisting of an N-terminal (regulatory) domain and C-terminal (catalytic) domain, connected by an alpha helix (Aleshin et al., 1998, Rosano et al., 1999). G6P acts as a product inhibitor, binding to both N- and C- terminal domains, with resultant competitive inhibition of ATP binding. In turn, G6P inhibition can be relieved by binding of inorganic phosphate (P_i), which competitively inhibits G6P (Sui and Wilson, 2002).

HK1 variants have been associated with several different clinical conditions. Hemolytic anemia due to hexokinase deficiency (OMIM 235700), and hereditary motor and sensory neuropathy, Russe type (OMIM 605285, also known as Charcot-Marie Tooth type 4G) are both caused by biallelic heterozygous or homozygous *HK1* variants (Rijksen et al., 1983, Rogers et al., 2000, van Wijk et al., 2003, Claramunt et al., 2007, Hantke et al., 2009, Sevilla et al., 2013, Dongerdiye et al., 2021, Sasaki et al., 2022). A specific heterozygous *HK1* variant, c.2539G>A p.(Glu847Lys), has been associated with autosomal dominant retinitis pigmentosa 79 (OMIM 617460) (Sullivan et al., 2014, Wang et al., 2014). More recently, four heterozygous *HK1* missense variants were associated with neurodevelopmental disorder with visual defects and brain anomalies (NEDVIBA, OMIM number

618547) (Okur et al., 2019). Two of these variants were recurrent in two unrelated families: c.1370C>T p.(Thr457Met) and c.1334C>T p.(Ser445Leu).

The current study aims to further delineate the phenotype associated with de novo heterozygous *HK1* missense variants, through investigation of clinical data from a further four previously unreported individuals.

Methods

Four patients with constitutional de novo *HK1* variants were recruited via the Deciphering Developmental Disorders (DDD) study (04/MRE05/50) (2015) and an *HK1* complementary analysis protocol (CAP). The inclusion criteria were the presence of a de novo heterozygous *HK1* missense variant in genomic DNA and the absence of other pathogenic or likely pathogenic variants. The variants were identified through the standard DDD trio whole exome sequencing pipeline and were reported with reference to the canonical transcript NM_000183.3. All families had given informed consent to participate in the study. Magnetic resonance imaging (MRI) were available for three patients and images were reviewed by a consultant pediatric radiologist.

Standardized phenotypic and anthropometric data were collected by clinical review by an experienced clinical geneticist. Anthropometric Z scores were calculated with reference to UK 1990 and United Kingdom-World Health Organization (UK-WHO) Child Growth Standards (Freeman et al., 1995, Cole et al., 1998). The variants were classified using the American College of Medical Genetics and Genomics (ACMG) framework (Richards et al., 2015).

The structure of hexokinase 1 was obtained from the Protein Data Bank (PDB) (Berman et al., 2000) under the PDB code 1qha. Structural analysis and visualization of the molecule were performed in UCSF ChimeraX version 1.1 (Petterson et al., 2021). In silico predictions of pathogenicity were derived using SIFT, PolyPhen, CADD, REVEL, Grantham, Mutation Taster and Missense 3D (Supplementary Table 1).

Results

Decipher ID 300246

ID 300246 was a 16 year old female. She was born at 40 weeks with low Apgar scores and was admitted to the Neonatal Unit (NNU) for 48 hours. She developed a social smile at 7 weeks, sat independently on time and walked at 1 year and 1 month of age. She was unsteady on her feet but was able to walk independently. Development of speech was delayed, and she had dysarthria. At 16 years of age, she had a severe intellectual disability. On examination at 16 years, she had coarse facial features with down slanting palpebral fissures, a broad nasal base, upturned nasal tip with anteverted nares, smooth philtrum, and prominent upper gums. Growth parameters were normal (Table 1). During early adolescence, she had mild visual impairment, which deteriorated over subsequent years to severe visual impairment with abnormal saccadic eye movements. Electroretinogram findings were suggestive of retinitis pigmentosa. In her early twenties, she developed bradykinesia, followed by cogwheel rigidity, brisk reflexes, dysarthria, gait ataxia and limb dystonia. Episodes of drowsiness and poor respiratory drive were observed following periods of illness. These episodes of drowsiness progressed to loss of consciousness, and she had a reduced respiratory drive, requiring ventilation in intensive care. Her condition was progressive, and she could not be weaned off ventilatory support. She died age 25 years. Results of investigations showed elevated CSF lactate, as detailed in Table 1 and abnormal MRI brain imaging with volume loss and cystic degeneration of putamina and caudates bilaterally (Figure 1A-C). Postmortem brain findings confirmed longstanding lesions with neuronal loss, gliosis and microvacuolation. Other investigative results are as shown in Table 1. The presumptive diagnosis was of a likely mitochondrial disorder. Microarray and full sequencing of mitochondrial DNA (mtDNA) was normal. Trio whole exome sequencing identified a de novo, likely pathogenic, heterozygous *HK1* missense variant, c.1334C>T p.(Ser445Leu) (Supplementary Table 1).

Decipher ID 278818

ID 278818 was a 15.5 year old male. There were no antenatal complications, and he was born at 39 weeks. He was readmitted a few days after birth with feeding difficulties, an uncoordinated swallow and hypotonia. Social smile was achieved at 6 weeks but other developmental milestones were not reached. He had a severe intellectual disability. As an infant, he required feeding via a nasogastric tube and subsequently received a gastrostomy and Nissen fundoplication at four months of age. Whilst an infant, he also had episodes of extensor spasm, which progressed to tonic seizures with focal onset as a child. Seizures were initially managed with anti-epileptics (polytherapy) but were refractory. He was trialed on a ketogenic diet, which, whilst reducing his seizure frequency, was not tolerated; he experienced pale, sweaty episodes with high blood sugars. Other neurological problems included central hypotonia and peripheral hypertonia. He had cortical visual impairment and nystagmus, with disconjugate eye movements. Scoliosis was managed with bracing. He had frequent lower respiratory tract infections and copious respiratory secretions requiring regular suctioning. He was also noted to have episodes of hypothermia, bradycardia and a low respiratory rate, which could occur both during periods of illness and when he was otherwise well. On examination, at 15.5 years, there were no dysmorphic facial features. Pectus carinatum and macroglossia were previously noted as a child. Growth was globally restricted with microcephaly (-5.6 standard deviations (SD)), short stature (-3.29 SD) and low body weight (-3.35 SD) (Table 1). He had an elevated CSF lactate, (Table 1) and abnormal MRI brain imaging, with frontal lobe volume loss and likely cystic change within the putamina and caudate heads (Figure 1D-F). Results of other investigations are shown in Table 1. Microarray, common mtDNA mutation screening and mtDNA sequencing were normal. Trio whole exome sequencing identified a de novo, likely pathogenic, heterozygous *HK1* missense variant, c.1370C>T p.(Thr457Met) (Supplementary Table 1) Prior to this genetic diagnosis, he had a working diagnosis of Leigh Syndrome, in view of the elevated CSF lactate and MRI brain changes. He died in his sleep aged 16 years and the cause of death is not known.

Decipher ID 300984

ID 300984 was a 28 month old male, born at 31 weeks. Antenatal scans were normal, except for polyhydramnios identified on the day of delivery. He was admitted to the Neonatal Unit and required intubation and ventilation. He was hypotonic and had a V-shaped soft palate cleft and a natal tooth. Following extubation, he continued to experience recurrent episodes of respiratory distress requiring reintubation and ventilation and/or continuous positive airway pressure (CPAP). He spent 12 weeks in the Neonatal Unit. He was later dependent on CPAP and had significant respiratory secretions requiring mobile suction. He had severe gastro-esophageal reflux disease (GORD) and a gastrostomy and Nissen fundoplication were performed at 8 months. Infantile spasms and abnormal movements were noted from age 6 months. A ketogenic diet was trialed for refractory epilepsy, however there were difficulties maintaining adequate ketosis due to intercurrent respiratory illness. After one year, the ketogenic diet was stopped due to lack of benefit. There was no report of any adverse effects from the ketogenic diet. He had roving, nystagmoid eye movements and a variable divergent squint with no meaningful eye movements. There was no abnormal retinal pigmentation or lens opacities. He had severe global developmental delay; he was able to vocalize in response to familiar voices and laugh and had little spontaneous movement. Assessment of hearing showed flat tympanometry bilaterally. Further hearing assessment was not performed. He had a small PDA (patent ductus arteriosus) which fully resolved, with normal ECG and echo at 10 months of age. A 24-hour tape showed a wide variability in heart rate, with five brief episodes of sinus bradycardia. On examination at 28 months, he had single palmar creases, with deep creases of the hands and feet, mild camptodactyly but no joint contractures. He had short stature and was microcephalic with overfolding of ears and no facial dysmorphisms. He had an elevated CSF lactate (Table 1) and abnormal MRI brain imaging (Figure 1G-J). Results of other investigations are as shown in Table 1. Microarray was normal and common mtDNA mutations were excluded. Trio whole exome sequencing identified a de novo, likely pathogenic, heterozygous *HK1* missense variant, c.1370C>T p.(Thr457Met) (Supplementary Table 1). Prior to this finding, he previously had a diagnosis of a likely mitochondrial syndrome. His disorder was progressive, with increasingly frequent breakthrough seizures and episodes of

apnea. He developed a respiratory tract infection and died age 31 months. He did not undergo a post mortem examination.

Decipher ID 274401

ID 274401 was a 13 year old male who was born at 39 weeks. The pregnancy was complicated by bleeding during the second trimester and reduced fetal movements. He was hypotonic from birth and initially fed via a nasogastric tube for one day but subsequently bottle fed. By 12 months of age, feeding difficulties had worsened and he underwent gastrostomy insertion at 14 months. He had dysphagia over the first few years of life, which had fully resolved by 13 years of age. He had global developmental delay and autistic behavior. He smiled at 16 weeks, sat independently at 1.5 years and walked independently at 1.8 years. He spoke his first words at 2.3 years. On examination at 13 years, he walked independently and showed muscle weakness including a Gowers sign. He had generalized fluctuating muscle weakness, hypotonia and a history of falling when fatigued. He could develop ptosis by the end of the day and complained of occasional blurring of vision. He had asymmetrical scapular winging and a lumbar hyperlordosis. Facial features included a prominent forehead, epicanthic folds, and a small nose with anteverted nares. His hands and feet were normal and there were no skin lesions. Growth parameters were normal (Table 1). Lactate was reported to be slightly increased as an infant, however specific values were not available. MRI was normal in the neonatal period and results of other investigations are given in Table 1. Microarray was normal. Trio whole exome sequencing identified a de novo, heterozygous *HK1* missense variant of uncertain significance, c.1969G>A p.(Asp657Asn) (Supplementary Table 1). He had a current working diagnosis of autism spectrum disorder and fluctuating muscle problems, with a differential diagnosis of a congenital myasthenic syndrome.

Study	Okur et al. 2019	Okur et al. 2020	Okur et al. 2021	Okur et al. 2022	DDD	DDD	DDD	Okur et al. 2023	Okur et al. 2024	Okur et al. 2025	DDD
ID	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 300246**	**Patient 278818**	**Patient 300984**	**Patient 5**	**Patient 6**	**Patient 7**	**Patient 274401**
Variant type	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense
cDNA (ENST00000359426.7)	c.1241G>A	c.1252A>G	c.1334C>T	c.1334C>T	c.1334C>T	c.1370C>T	c.1370C>T	c.1370C>T	c.1370C>T	c.1370C>T	c.1969G>A
Protein consequence	p.(Gly414Glu)	p.(Lys418Glu)	p.(Ser445Leu)	p.(Ser445Leu)	p.(Ser445Leu)	p.(Thr457Met)	p.(Thr457Met)	p.(Thr457Met)	p.(Thr457Met)	p.(Thr457Met)	p.(Asp657Asn)
ACMG class	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Uncertain significance
Inheritance	De Novo	De Novo	De Novo	De Novo	De Novo	De Novo	De Novo	De Novo	De Novo	De Novo	De Novo
Patient alive/ deceased	Alive	Alive	Alive	Alive	Deceased	Deceased	Deceased	Alive	Deceased age 1y	Deceased age 1y	Alive
Sex	Female	Female	Male	Male	Female	Male	Male	Male	Male	Female	Male
Working diagnosis prior to genetic result	N/A	N/A	N/A	N/A	Mitochondrial disorder	Leigh syndrome	Likely mitochondrial syndrome	N/A	N/A	N/A	ASD, congenital myasthenic syndrome
Prenatal Growth											
Birth weight (kg)	N/A	N/A	N/A	N/A	2.976	2.6	1.55	N/A	N/A	N/A	3.487
Birth weight/ SD	N/A	N/A	N/A	N/A	-0.95	-2.03	-0.35	N/A	N/A	N/A	0.3
Gestation (wks)	N/A	N/A	N/A	32	40	39	31	N/A	N/A	N/A	39
Birth OFC (cm)	N/A	N/A	N/A	N/A	N/A	33	29.5	N/A	N/A	N/A	>25th centile
Birth OFC/ SD	N/A	N/A	N/A	N/A	N/A	-1.73	0.17	N/A	N/A	N/A	N/A
Pregnancy, Birth and Newborn Period											
Antenatal complications	No	Cystic brain lesions found at 7m gestation, later resolved	No	Prematurity	Induced 40 +3 wks; C-section fetal distress	No	Polyhydramnios on day of delivery; prematurity	No	No	No	Bleeding second trimester; reduced fetal movements
Jaundice	N/A	N/A	N/A	N/A	Yes	No	Yes	N/A	N/A	N/A	No

Hypoglycaemia	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Poor feeding	N/A	N/A	N/A	N/A	No	Yes	Yes	N/A	N/A	N/A	Yes
Hypotonia	N/A	N/A	N/A	N/A	Yes	Yes	No	N/A	N/A	N/A	Yes
Other neonatal complications	N/A	Torticollis, clonus and spasticity; poor weight gain	N/A	N/A	48 hour NNU admission	Readmitted with feeding difficulties; uncoordinated swallow	3 m NNU admission requiring respiratory and feeding support; V-shaped soft palate cleft; natal tooth	N/A	N/A	Failure to thrive	
Postnatal Growth											
Age at last assessment	34y	9y	14y	44y	16y	15y 6m	28m	8y	1y	1y	13y
Height/ SD	N/A	N/A	N/A	N/A	0.35	-3.29	-2.86	N/A	N/A	N/A	0.23
Weight/ SD	N/A	N/A	N/A	N/A	1.76	-3.35	1.35	N/A	N/A	N/A	1.63
OFC/ SD	N/A	N/A	N/A	N/A	0.39	-5.6	-2.26	N/A	N/A	N/A	N/A
Development											
GDD /Intellectual disability	Yes	Yes	Yes	Yes	Yes - severe	Yes- severe	Yes - severe	Yes	Yes	Yes	Yes
Social smile	N/A	N/A	N/A	N/A	7 wks	6 wks	N/A	N/A	N/A	N/A	16 wks
Sitting independently	Delayed	2y	8m	8m	Achieved within normal range	Not achieved	Not achieved	12m	Not achieved	Not achieved	1.5y
Walking independently	27m	Not achieved	18m	16m	1y 1m	Not achieved	Not achieved	12m	N/A	N/A	1y 10m
First words	Delayed	14m	2y	18m	Very delayed	Not achieved	Not achieved	Delayed	N/A	N/A	2y 3m
Level of development achieved	N/A	Only a few words, significant limited expressive language	Speaks in sentences. Word finding difficulties	N/A	Walks independently for short distances	Social smile	Listens and responds to familiar voices; laughs; baby vocalisations; little spontaneous movement	Dysarthric speech	N/A	N/A	Walks independently
Other Clinical Features											

Swallowing/ feeding difficulties	N/A	N/A	N/A	N/A	No	Required NGT as infant; gastrostomy at 4m; required suction for secretions	Required NGT as infant; gastrostomy at 8m	Bulbar weakness; mild drooling; swallowing dysfunction;	Yes	Yes, gastrostomy	Dysphagia during early years; gastrostomy at 14m; no swallowing or choking issues at 13y
Gastro-esophageal reflux disease	N/A	N/A	N/A	N/A	No	Nissen fundoplication at 4m	Nissen fundoplication at 8m	N/A	Yes	Yes	No
Epilepsy	N/A	N/A	N/A	No	No	Episodes of extensor spasm as an infant; tonic seizures with focal onset as a child; refractive to polytherapy	Infantile spasms and abnormal movements from 6 m; refractory epilepsy, some response to clonazepam	N/A	Seizures; infantile spasms	Seizures; infantile spasms	No
Ketogenic diet	N/A	N/A	N/A	N/A	No	Trialed - decreased seizure frequency but had pale, sweaty episodes with high blood sugars	Trialed- no benefit	N/A	N/A	N/A	N/A
Other neurological problems	Ataxia; anxiety	Truncal hypotonia; limb hypertonia (lower>upper); brisk DTRs; some contractures in hamstrings	Ataxia; staring spells; tingling in legs (EMG normal); hypotonia; will hug and kiss strangers	No	Bradykinesia and cogwheel rigidity from early 20s; brisk reflexes; dysarthria; slurred speech; gait ataxia; limb dystonia.	Central hypotonia, peripheral hypertonia	Hypotonia	Progressive neurological decline; abnormal tone; mild wide spaced gait; recent onset right sided weakness	Limb hypertonia	Hypertonia	Hypotonia; falls when fatigued; generalized fluctuating weakness; Gowers sign
Scoliosis	Kyphoscoliosis	Yes	N/A	N/A	No	Bracing, declined surgery	N/A	N/A	N/A	N/A	Lumbar hyperlordosis; asymmetrical scapula winging

Visual impairment	Retinitis pigmentosa; peripheral vision loss and gaze abnormality	Cortical visual impairment; strabismus; astigmatism	Retinitis pigmentosa; cone-rod dystrophy; optic atrophy; photophobia	Retinitis pigmentosa (age of diagnosis 7 years); optic atrophy	Visual acuity 6/9 - progressed to severe visual impairment; ERG suggestive of retinitis pigmentosa; some restriction to upward gaze abnormal saccadic eye movements	Cortical visual impairment; nystagmus; disconjugate eye movements	Roving nystagmoid eye movements; variable divergent squint; no abnormal retinal pigmentation; no lens opacities	Bilateral optic atrophy	Optic atrophy; nystagmus	Optic atrophy	Ptosis at end of day; occasional blurring of vision
Respiratory symptoms	N/A	N/A	N/A	N/A	From early 20s, progressive episodes of drowsiness, LOC and poor respiratory drive	Frequent LRTIs, respiratory secretions requiring mobile suction	Dependent on CPAP, intermittently required full ventilation; respiratory secretions requiring mobile suction	N/A	Laryngo-tracheomalacia; died due to respiratory infection	Laryngo-tracheomalacia; respiratory distress; died due to respiratory infection	Recurrent pneumonia; FVC in sitting 62%, in lying 53%. Fewer respiratory problems over the past few years.
Hearing impairment	N/A	N/A	N/A	N/A	No. Recurrent ear infections.	No - passed newborn hearing test	Flat tympanometry on left and right, further hearing assessment not performed	N/A	N/A	Yes	No
Progressive disorder	N/A	N/A	N/A	N/A	Yes	Yes	Yes	Yes	N/A	N/A	No
Facial dysmorphic features	N/A	Right earlobe crease; frontal bossing; epicanthal folds; anteverted nose; bulbous nasal tip; preauricular pit; thin upper lip	No	No	Coarse features; down slanted palpebral fissures; broad nasal base; upturned nose with anteverted nares; smooth philtrum; prominent upper gums	No	No	Flat occiput; slight synophrys; widely spaced teeth	No	No	Prominent forehead; epicanthic folds; small nose with anteverted nares

Episodes of hypothermia/bradycardia/low respiratory rate	N/A	N/A	N/A	N/A	Progressive episodes of drowsiness & reduced respiratory drive requiring ventilation, unable to be weaned off ventilator	Episodes of hypothermia/ bradycardia/ low respiratory rate	5 brief episodes of sinus bradycardia on 24 hr tape	N/A	N/A	N/A	No
Other features	5th finger clinodactyly; pes planus	Bilateral hip dislocation	FMF (Het MEFV mutation)	Hypertension	Episodes of hypertension; normal puberty; sociable and happy; hypermobile; small joints; short 5th toes.	Pectus carinatum; macroglossia	Butterfly 11 thoracic vertebrae; single palmar creases; deep palmar and plantar creases; overfolding of ears; mild camptodactyly; episodes of screaming at night	Full cheeks (steroid usage); truncal obesity; high blood vs zero CSF glutamine	Small umbilical hernia		Autistic behavior
Investigations											
CSF lactate (mmol/L) Normal range 1.2-2.1mmol/L	N/A	N/A	N/A	N/A	8.38	6.13	5.5	High	N/A	N/A	N/A
Plasma lactate (mmol/L) Normal range 0.5-2.2 mmol/L	N/A	N/A	N/A	N/A	1.4	2.04	2.4	High	N/A	N/A	N/A
Ratio	N/A	N/A	N/A	N/A	6	3	2.3	N/A	N/A	N/A	N/A
Corresponding blood glucose (mmol/L) Normal range 4-7.8mmol/L	N/A	N/A	N/A	N/A	4.4	5.2	4.7	N/A	N/A	N/A	N/A

MRI brain	Abnormal signal in caudate pathway and putamen	Cerebral and cerebellar atrophy; thin corpus callosum; periventricular leukomalacia; possible grey matter heterotopia	Cystic lesion on brain	N/A	Bilateral symmetrical increased signal and atrophy in bilateral basal ganglia involving putamina, caudate and midbrain nuclei	Marked atrophy of frontal lobes; increased signal within bilateral basal ganglia predominantly lentiform nuclei. Symmetrical high signal intensities in posterior suprapontine stem	Early infant period- dilated lateral ventricles with scalloped margins consistent with PVL; absent septum pellucidum; thin corpus callosum. Repeat- progressive atrophy and ventricular dilatation	Lesions in brainstem (concerning for demyelinating disease vs autoimmune process vs Guillain Barre syndrome)	Abnormal autopsy findings; volume loss along the white matter adjacent to the atria and frontal horns of the ventricle; atrophy of pons and brainstem; bilateral subdural fluid collection	Ventricular dilatation involving occipital & temporal horns of lateral ventricles, prominence of subarachnoid, possibility of underlying atrophy & migrational abnormality	Normal in neonatal period
EEG	N/A	Normal	Normal	N/A	Normal	Tonic seizures with focal onset right hemisphere	Severely abnormal- hypsarrhythmia	Mild diffuse slowing	Consistent with early myoclonic epileptic encephalopathy	Abnormal (verbal disclosure)	Occasional sharp waves on EEG over left posterior temporal region
Echo	N/A	N/A	N/A	N/A	Normal	Normal	Small PDA, subsequently closed, normal echo at 10 m	N/A	N/A	N/A	Normal
Other imaging	N/A	N/A	N/A	N/A	N/A	Normal renal US and liver US	Normal renal US; cystic periventricular leukomalacia changes on neonatal cranial US	N/A	N/A	N/A	Muscle MRI normal
Muscle biopsy	N/A	N/A	N/A	N/A	Non-specific myopathic appearances; some features consistent with possible underlying mitochondrial disorder; mitochondria respiratory chain enzymes - evidence of reduced complex 2 and 3 activity	No mitochondrial respiratory chain deficiency	No mitochondrial respiratory chain deficiency	N/A	N/A	N/A	Non-specific changes
Pyruvate dehydrogenase enzyme testing	N/A	N/A	N/A	N/A	Upper end of normal	Normal	Normal	N/A	N/A	N/A	N/A

Pyruvate carboxylase enzyme testing	N/A	N/A	N/A	N/A	N/A	Normal	Normal	N/A	N/A	N/A	N/A
Microarray	N/A	N/A	N/A	N/A	Normal	Normal	Normal	N/A	N/A	N/A	Normal
Mitochondrial DNA	N/A	N/A	N/A	N/A	Full sequencing - normal	Common mitochondria DNA mutations excluded; mitochondrial DNA sequencing normal	Common mitochondrial DNA mutations excluded	N/A	N/A	N/A	N/A
Other					Post mortem brain- longstanding lesions with neuronal loss, gliosis and microvacuolation Widespread Alzheimer's type 2 astrocytes; basic neurometabolic screen normal; heterozygous for POLG c.22436>C (p.Trp748Ser) mutation - RNA follow up has not identified a 2nd pathogenic mutation.	Lysosomal enzymes normal					Many neuromuscular disease genes negative, PWS negative

Table 1 – Summary of genotypic and phenotypic data for 11 patients with de novo missense *HK1* variants (previously reported patients and the current study).

Abbreviations: N/A, not available; y, years; m, months; wks, weeks; hrs, hours; SD, standard deviation; OFC, occipital frontal circumference; NGT, nasogastric tube; DTR, deep tendon reflexes; EMG, electromyography; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; PVL, periventricular leukomalacia; EEG, electroencephalography; US, ultrasound; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; US, ultrasound; NNU, neonatal unit

Figure 1 - MRI brain imaging

ID 300246. **A** - Axial T2 age 25y: cystic degeneration seen as bright T2 signal and volume loss of the putamina (asterix) and caudate heads (white arrows) bilaterally. **B**- Axial T2 age 25y: abnormal foci of increased T2 signal within the midbrain (white arrow), putaminal abnormal signal can be seen on the left (asterix). **C** - Coronal FLAIR age 25y: volume loss and cystic degeneration of putamina (white arrow) and caudates (black arrow) bilaterally with associated gliosis. **ID 278818. D** - Axial T2 at 6y7m: frontal lobe volume loss, ventricular enlargement and partially deficient septum pellucidum (asterix), larger frontal extra-axial spaces and bilateral abnormally increased T2 signal within putamina (white arrow) and caudate heads (black arrow) (likely cystic change). **E** - Axial T2 age 6y7m: abnormal high T2 signal within the dorsal midbrain (white arrow) and mainly frontal volume loss. **F** - Sagittal T1 at 6y7m: marked thinning of the corpus callosum (white arrows), supratentorial volume loss (worse frontally) slender brain stem with small pons (white asterix) and enlarged ventricles. **ID 300984. G** - Axial T2 at age 6m: mainly frontal lobe volume loss, prominent extra-axial spaces with bilateral subdural hygromas (white arrows), ventricular enlargement (assumed secondary to volume loss) and absence of septum pellucidum (asterix). **H** - Coronal T2 at age 6m: ventricular enlargement with irregular ventricular contour, enlarged extra-axial spaces with bilateral subdural hygromas (white arrows), generalized volume loss. **I** - Axial T2 age 2y6m: generalized volume loss most prominent frontally and ventricular enlargement suggesting progressive volume loss. **J** - Sagittal T1 at 2y6m: marked thinning of corpus callosum (white arrows), supratentorial parenchymal volume loss and ventricular enlargement.

Clinical characteristic	Frequency of clinical characteristic			
	Current study	Previous reports	Total	
Global developmental delay/ intellectual disability	4/4	7/7	100%	11/11
Raised CSF lactate	3/3	1/1	100%	4/4
Respiratory issues	4/4	2/2	100%	6/6
Visual impairment	3/4	7/7	91%	10/11
MRI brain anomalies	3/4	6/6	90%	9/10
Abnormal tone	4/4	5/6	90%	9/10
Speech and language difficulties	3/4	4/4	88%	7/8
Swallowing/feeding difficulties	3/4	4/4	88%	7/8
Scoliosis	2/3	2/2	80%	4/5
Ataxia	1/1	3/4	80%	4/5
Progressive course of disease	3/4	1/1	80%	4/5
Poor feeding as neonate	3/4	N/A	75%	3/4
Neonatal hypotonia	3/4	N/A	75%	3/4
Gastroesophageal reflux disease	2/4	2/2	67%	4/6
Abnormal EEG	3/4	3/5	67%	6/9
Seizures	2/4	2/3	57%	4/7
Dysmorphic facial features	2/4	2/6	40%	4/10
Hearing impairment	1/4	1/1	40%	2/5

Table 2 - Frequency of clinical characteristics associated with de novo *HK1* missense variants reported in current study and previous reports

Abbreviations: CSF, cerebrospinal fluid; EEG, electroencephalogram; N/A, not available.

Protein Structural Analysis

Analysis of the hexokinase-1 crystal structure (PDB ID: 1qha) revealed proximity of the following four variants to the regulatory glucose-6-phosphate (G6P) binding site, with likely disruption of the G6P binding site: c.1241G>A p.(Gly414Glu); c.1252A>G p.(Lys418Glu); c.1334C>T p.(Ser445Leu) and c.1370C>T p.(Thr457Met) (Figure 2).

Figure 2 - Structure of hexokinase-1 dimer and the sites of missense *HK1* variants associated with neurodevelopmental phenotype clustered around the regulatory glucose-6-phosphate (G6P) binding site.

Discussion

This descriptive case series of four previously unreported patients with heterozygous, de novo, missense *HK1* variants provides further insight into the emerging neurodevelopmental phenotype associated with *HK1* missense variants. We provide further evidence that de novo *HK1* variants are associated with neurological problems and visual problems. We highlight for the first time an association with a raised CSF lactate and specific abnormalities of the basal ganglia on brain MRI, as well as associated respiratory issues and swallowing/feeding difficulties.

We have integrated phenotypic data from our case series with seven previously reported patients with de novo missense *HK1* variants, comprising a total of 11 patients (Table 1). Three patients had the recurrent c.1334C>T p.(Ser445Leu) *HK1* variant, and five patients had the recurrent c.1370C>T p.(Thr457Met) *HK1* variant. All variants were de novo and classified as likely pathogenic, except for one, (c.1969G>A p.(Asp657Asn), which was a de novo variant of uncertain significance. Integration of these data allowed us to identify the frequency of different clinical characteristics in the current study, previous reports and in the total number of reported patients (Table 2).

All the de novo *HK1* variants reported here, except for c.1969G>A p.(Asp657Asn) (ID 274401) are located within the hexokinase large subdomain in the N-terminal regulatory domain or within the interdomain alpha helix. However, the variant of uncertain significance, c.1969G>A p.(Asp657Asn), is the only de novo *HK1* variant located within the hexokinase large subdomain 2 in the C-terminal catalytic domain. Whilst limited conclusions can be made from one individual patient, there were some interesting observed differences in phenotype between this patient (ID 274401) and the other 10 reported patients in the case series. Whilst feeding difficulties and dysphagia were present in infancy, patient ID 274401 had no swallowing issues at 13 years of age. Furthermore, there was no history of cortical visual impairment or seizures, and his MRI brain was normal. It was not known whether this patient had a raised CSF lactate. In terms of future variant classification, we feel it is important to point out that we suspect that this patient's variant has previously been included in a

publication by Turner et al. (supplementary table 2, row 5190, column G) so should not be double counted (Turner et al.,2019).

HK1 is associated with several other autosomal dominant and recessive disorders, which, interestingly, do not display all the associated features described above. Autosomal dominant retinitis pigmentosa 79 is associated with the heterozygous missense variant c.2539G>A p.(Glu847Lys), located within the C-terminal domain, and is not associated with any extraocular symptoms; individuals have normal intellect and normal exercise tolerance (Sullivan et al., 2014, Wang et al., 2014). Furthermore, in both autosomal dominant retinitis pigmentosa and hereditary motor and sensory neuropathy, Russe Type, there is no alteration in hexokinase activity (Hantke et al., 2009, Sullivan et al., 2014, Wang et al., 2014). Hemolytic anemia due to hexokinase deficiency is associated with biallellic or homozygous HK1 variants, and has not been associated with the variants identified in this study. Furthermore, hemolytic anemia was not known to be present in any patients in the current study, nor in those previously reported by Okur et al. (Okur et al., 2019).

Three out of four patients in the current study had a raised CSF lactate (average value 6.7mmol/L), with an average CSF lactate: plasma lactate ratio of 3.8. All three of these patients had variants within the N-terminal domain and interdomain alpha helix. One patient previously reported by Okur et al. (Patient 5), was also identified as having a raised CSF lactate (Okur et al., 2019). This patient had the same variant (c.1370 C>T, p.(Thr457Met)) as two of our patients with a raised CSF lactate. The same three patients in the current study had anomalies on MRI brain, with specific changes occurring in the basal ganglia. It is noteworthy that one patient previously reported by Okur et al. (Patient 1) also had abnormal basal ganglia signaling (Okur et al., 2019).

There are several different theories to explain why these variants could cause this specific phenotype. Okur et al. measured normal hexokinase activity in red blood cells of two patients, suggesting that the disease mechanism is not due to a loss of hexokinase enzymatic activity. Their group proposed that heterozygous missense *HK1* variants could cause this different phenotype via

a gain-of-function effect and subsequent accumulation of HK1 protein within the brain. Furthermore, intracellular accumulation of misfolded proteins is a well-recognized process involved in the pathophysiology of several different neurological disorders (Okur et al., 2019).

Our structural analysis suggests that the missense variants within the N-terminal regulatory domain and interdomain alpha helix may disrupt the regulatory G6P binding site. Since this binding site is responsible for product inhibition of HK1, disruption of G6P binding could result in an inability to auto-regulate, leading to gain of function and constitutive glucose phosphorylation. Since HK1 is abundant in neurons, overproduction of glycolytic intermediates could result in the accumulation of pyruvate in the brain. Furthermore, there are some phenotypic similarities between our reported patients and patients with pyruvate dehydrogenase deficiency, for example, global developmental delay, progressive course of disease, abnormal tone, and structural brain changes (Patel et al. 2012). A potential shift from aerobic to anaerobic respiration could explain preferential fermentation of pyruvate to lactate, rather than entry into the Krebs cycle - electron transport chain pathway. Alternatively, HK1 gain of function could also result in increased CSF lactate due to cell death or detrimental mitochondrial function occurring predominantly within the brain. It would be interesting to know whether other previously reported individuals had a raised CSF lactate, and further review of imaging looking for basal ganglia anomalies could also identify further individuals with a similar phenotype. Such findings would reduce the possibility of ascertainment bias in the current study.

Two patients in the current study were trialed on a ketogenic diet. Interestingly, in one patient, high blood sugars and sweatiness were reported. Since the ketogenic diet is normally associated with an increased risk of hypoglycemia, the observation of hyperglycemia could be explained by possible inhibition of hexokinase activity. Other explanations could include general impaired glucose tolerance due to mitochondrial dysfunction, or compensation from the other non-brain-specific hexokinases. It would be interesting to find out more about the effect of the ketogenic diet in other patients with de novo missense *HK1* variants.

In summary, this descriptive case series expands the neurodevelopmental phenotype associated with de novo, heterozygous, missense *HK1* variants and increases the total number of reported patients to 11. This work highlights further clinical features for the first time, including a raised CSF lactate and specific changes to the basal ganglia on MRI brain imaging compatible with Leigh syndrome spectrum. De novo missense variants in *HK1* should be included in the differential diagnosis of Leigh syndrome spectrum. Whilst further work is needed to determine the course of this condition and genotype-phenotype correlation, our work suggests that variants within the N-terminal and interdomain alpha helix are associated with a progressive neurodevelopmental condition. Protein structural analysis supports the theory that heterozygous missense variants located within the N-terminal domain and interdomain alpha helix result in a distinctive neurodevelopmental phenotype through disruption of the regulatory G6P binding site and subsequent constitutive activation of HK1 within the brain.

Acknowledgements and Funding Sources

We would like to thank the patients and their families for their participation in this research study, and local clinicians for their assistance and support. The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003]. This study makes use of DECIPHER (http://decipher.sanger.ac.uk), which is funded by Wellcome. See Nature PMID: 25533962 or www.ddduk.org/access.html for full acknowledgement.

Disclosure of Conflict of Interest

The authors have no conflicts of interest to declare.

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Supplemental Data

Supplementary Table 1