Natural History of Branched-Chain- Keto-acid Dehydrogenase Kinase (BCKDK) deficiency

Study leaders:

MD PhD. Angels Garcia Cazorla

Neurology and Clinical Biochemistry Departments, Hospital Sant Joan de Déu, Passeig Sant Joan de Déu 2, 08950 Esplugues de Llobregat Barcelona, Spain

MD PhD Trine Tangeraas Paediatric and Adolescent Medicine, Oslo University Hospital, Sognsvannsveien 20, 0424 Oslo, Norway

MD Juliana Ribeiro Constante

Neurology and Clinical Biochemistry Departments, Hospital Sant Joan de Déu, Passeig Sant Joan de Déu 2, 08950 Esplugues de Llobregat Barcelona, Spain

jribeiro@fsjd.org

Collaborating study centres:

Prof Lars Mørkrid. lamo2@ous-hf.no

Department of Medical Biochemistry, Oslo Univ Hosp, Oslo, Norway

PhD Paul Hoff Backe

Paul.Hoff.Backe@rr-research.no

Department of Medical Biochemistry, Oslo Univ Hosp, Oslo, Norway

MD Irene Machado

Hospital Universitario Clínico San Cecilio Av. del Conocimiento, s/n, 18016 Granada

ireso77@yahoo.es

PhD Alfonso Oyarzábal

aldeoyarzabal@fsjd.org

Neurometabolic Unit and Laboratory of Synaptic Metabolism, Sant Joan de Déu Hospital Passeig de Sant Joan de Déu, 2, 08950, Esplugues de Llobregat, Barcelona

Emma Footitt Metabolic Medicine Department, Great Ormond Street Hospital for Children UK
Emma.Footitt@gosh.nhs.uk,
James Davidson Metabolic Medicine Department, Great Ormond Street Hospital for Children UK
James.Davison@gosh.nhs.uk
Marjorie Dixon. Metabolic Medicine Department, Great Ormond Street Hospital for Children UK
Marjorie.Dixon@gosh.nhs.uk.
Julia Neugebauer Charité Universitätsmedizin Berlin, Germany
julia.neugebauer@charite.de
Nathalie Weinhold Charité Universitätsmedizin Berlin; Germany
natalie.weinhold@charite.de
Clarissa Bueno Sabara Hospital Brazil
clarissa.bueno@hc.fm.usp.br
Gaia Novarino. Institute of Science and Technology Austria
gaia.novarino@ist.ac.at
Paul El-Fishawy,Child and Adolescent psychiatry, New Haven Connecticut,USA
paul.el-fishawy@yale.edu
Nouriya Abbas Al-Sannaa, Pediatrics Services Division Johns Hopkins Aramco Healthcare
nouriya.sannaa@jhah.com
Caroline.Martinez, The Mount Sinai Hospital, Madison Ave New York City USA
caroline.martinez@mssm.edu
Burcu Ozturk-Hismi, Pediatrics and Inherited Metabolic Disorders,Marmara University School of Medicine, Department of Pediatrics, İstanbul, Turkey.
bhismi@gmail.com
Robert Currier, Genetic Disease Screening Program, California Department of Public Health, Richmond, California, USA.
Robert.Currier@ucsf.edu
Evren Gumus, Department of Medical Genetics, Faculty of Medicine, University of Harran, 63000 Sanliurfa, Turkey.
evreng@ymail.com

Further study centres can be included during the study period 2020-2022.

Supporting body:

The project emerges from the European Reference network for rare inherited metabolic diseases (MetabERN). MetabERN is a European non-profit network established by the EU to facilitate access to the best available care and address the needs across the border of all patients affected by any rare inherited metabolic disease and their families.

Summary

Only a very few cases with the ultra- rare disease Branched-chain keto-acid dehydrogenase kinase (BCKDK) deficiency have so far been characterised in literature. Individuals with this disease have low levels of branched chain amino acids (BCAA) in plasma and in the brain due to accelerated catabolism of these essential amino-acids.

BCAA`s are crucial for proper brain development and function. The symptoms of the disease such as delayed psychomotor development, behavioral and communication profiles overlap in part with other disease entities such as autism spectrum disorders and therefore the disease is most likely under-diagnosed and the phenotypic expression is probably broader than described hitherto. For a few patients the first clue to diagnosis have been through the discovery of low levels of BCAA`s in plasma and spinal fluid. However, as low levels of BCAA may pass unrecognized in the plasma amino-acid profile, the majority of known cases have been ascertained by next generation sequencing studies.

The primary aim of this study is to characterize the phenotypic, biochemical and genetic spectrum of BCKDK deficiency in terms of age and symptoms at presentation, diagnostic investigations, treatment provided and outcome of the disease. Currently, the available treatment for this disease is protein enrichment and BCAA supplementation in the diet, suggested to be moderate beneficial for the few patients that have been published. However, most patients have started treatment only after several years with symptoms and therapy may be more potent if started earlier. A second aim of this study is therefore to re-examine the newborn screening (NBS) results if available, to investigate if this disease can be diagnosed early in life and potentially ameliorate the disease course.

Background and pathophysiology:

Mutations in the BCKDK (branched-chain keto-acid dehydrogenase kinase) gene (MIM #614901) were for the first time described in humans as a cause of comorbid intellectual disability, autism, and epilepsy in 2012 by Novarino et al (1). In this first paper 3 consanguineous families with 2 affected patients each (a total of 6 patients) were reported as part of an autism/epilepsy study. All of them had abnormally low plasma levels of branched-chain amino acids (BCAAs). These families were of Egyptian and Turkish ancestry. Later on, in 2014, García-Cazorla et al. reported 2 additional patients from unrelated families with an overlapping phenotype to that reported by Novarino et al and in this paper the patient`s the neurobehavioral and biochemical outcome was somewhat improved after treatment with high protein diet and regular supplementation of BCAA`s (2) .

The reported phenotype in these two publications included global developmental delay/intellectual developmental disability, epilepsy, behavioral and communication problems. Several patients were reported with microcephaly but it was not established from these publications whether microcephaly when present, was congenital (as an indicator of brain development disturbance starting in utero) or acquired. As is often the case in new disease descriptions, the presented cases had rather severe phenotypes. It is possible that milder phenotypes exist in the disease spectrum and who are even more amenable to treatment but have not been detected so far.

The BCAA (Leucine, Isoleucine and Valine) are essential amino-acids (AA) crucial for protein synthesis, nutrient signals and key in several other metabolic functions including maintaining proper AA balance in the brain (3). BCAAs cannot be stored in any form other than protein; excess of BCAAs must therefore be removed. The BCAA catabolic pathway includes a deamination step followed by the irreversible oxidative decarboxylation of the deaminated products—branched-chain α-keto acids (BCKAs)—to their corresponding acyl-CoA esters. This is catalyzed by mitochondrial BCAA amino transferase and the branched-chain α-keto acid dehydrogenase complex (BCKD)(4). Human BCKD is a macromolecular machine composed of four catalytic subunits encoded by four different genes: E1α-BCKDHA (MIM #608348), E1β- BCKDHB (MIM #24861), E2-DBT (MIM #248610), and E3-DLD (MIM #238331). These four subunits are assembled to form a mitochondrial located complex that shows strong structural homology

to the pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase complexes.

The branched-chain ketoacid dehydrogenase (BCKDH) complex catalyzes the irreversible, rate-limiting step in the catabolism of branched-chain amino acids (BCAAs), and BCKDK encodes a kinase that phosphorylates and thus inactivates the E1α subunit of this complex (5) (6). Mutations in any of the three subunits of the BCKDH complex lead to toxic accumulation of BCAAs and their metabolites and are the cause of Maple Syrup Urine Disease (MSUD), characterized by severe neurological complications when untreated (7-9). MSUD is treated by dietary restriction of BCAAs. Mutations in the BCKDK on the other hand, lead to significant depletion of BCAA and results in a neurodevelopmental disease. The proposed and logical treatment has so far been to replenish low levels of BCAAs in plasma (and CSF) by initiating a high protein diet and providing frequent supplementation of BCAAs several times a day including a night drip when possible. Despite BCAA enriched treatment may normalize plasma BCAA levels in patients (1), the therapy when initiated late has only shown modest clinical improvements in the small number of patients described. It is possible that supplementation of BCAAs with normalization of plasma BCAAs levels does not lead to complete brain BCAAs restoration. Moreover, plasma amino acids compete with each other for transport across the BBB. As have been previously shown in BCKDK knockout mice (1, 10), a decrease in plasma BCAAs could lead to a perturbation in human brain concentrations of not only BCAAs but also affect transportation of other LNAAs (large neutral amino acids). These amino acids (AA) are precursors for important neurotransmitters. Additionally, some of these AA behave also as signaling molecules in the brain and probably contribute to the neurological phenotype. Fibroblasts isolated from patients with BCKDK have showed secondary altered mitochondrial ultrastructure, bioenergetics depletion and mitochondrial dysfunction (11, 12). To this date no additional patients since 2014 have been reported in the medical literature and there are many questions that need to be explored regarding this disease.

Unpublished data suggest low levels of BCAA can be detected by revisiting newborn screening (NBS) profile in patients with BCKDK. Anonymous searches in large retrospective NBS cohorts (Norwegian n = 197630; Swedish n = 298731, Illinois n = 177316) have demonstrated its potential (Lars Mørkrid, personal communication). The advent of the Region 4 Stork post-analytical interpretive tool (R4S) founded by Piero Rinaldo at the Mayo Clinic has enhanced interpretations of NBS conditions by profile analysis (13, 14). By its successor, the Collaborative Laboratory Integrated Reports (CLIR 2.0) a "BCKDK tool" has been developed, available at the website https://clir.mayo.edu/ .
Detection and early treatment initiation in newborns with BCKDK deficiency may improve prognosis.

Aim of the study

Primary aim:

- To describe the BCKDK phenotype, biochemical and genetic spectrum, treatment, development and clinical outcome in individuals affected by BCKDK deficiency

Secondary aims:

- To evaluate the clinical and developmental response to BCAA supplementation in patients
- To describe the complete biochemical phenotype of BCKDK deficiency
- Elucidate the opportunity to detect BCKDK deficiency by newborn screening .

- To understand the pathophysiology of the neurodevelopmental phenotype.

Recruitment and information

Health Care Providers of the MetabERN network and other health professionals outside Europe following patients with this disease will be invited to ask their respective patient(s) (or their legal representatives on behalf of the patient) with BCKDK deficiency to participate in the study. Metabolic physicians will inform the participants about the study in both an oral and written form. The majority of patients ascertained with BCKDK to date are intellectually disabled. Therefore it will be essential to invite caregivers/guardians/next kin to consent on their behalf. Participants to this study will be recruited during 2020-2022.

Methods:

Baseline data will be collected from participants through participating centers and includes age at diagnosis and at last follow-up, gender, clinical presentation, growth, developmental and behavior, biochemical and genetic test results, neurophysiology, neuroimaging, and neurophysiological data, pharmacological treatment in case of epilepsy and where performed, neurodevelopmental test results. We'll register the clinical response to the treatment when performed. We will retrieve aminoacids and acylcarnitine profile from newborn screening results if available Algebraic combinations of specific age and birthweight adjusted markers (valine, isoleucine, leucine, alanine, phenylalanine, tyrosine) together with post-analytical algorithms developed at (CLIR, Mayo Clinic, Rochester, MN). In case of CSF availability from participants we'll perform a detailed multi-omic study (metabolomics and proteomics).

Merknad [TT1]: Do we need a control group for NBS data and must this be stated somewhere

Merknad [TT2]: Could it be cases where NBS bloodspots are available but where analysis of all AA or AC are not included in the particular NBS repertoar, should we include an option to send a couple of NBS punches to be analysed in Barcelona as part of the biological samples or not necessary?

Course of the study

Participation to this study does not imply any extra time or intervention for the patient/caregiver as all data that will collected for this study are available from previous visits at the patient`s local hospital.

Data protection:

In accordance with the General Data Protection Regulation the controller San Joan de Deu Children`s Hospital in Barcelona and Oslo University Hospital in Norway and the project managers MD PhD Angeles Garcia –Cazorla, telephone +34628891314 email agarcia@sjdhospitalbarcelona.org or MD PhD Trine Tangeraas, telephone +47-97980764 email ttangera@ous-hf.no, are independently responsible to ensure that the processing of personal data concerning health has a legal basis. This project has legal basis in accordance with the EUs General Data Protection Regulation, article 6 no. 1a, article 9 no. 2a

Pseudonymous patient data and biological samples if available (plasma, cerebrospinalfluid, CSF) will be collected by the coordinating study centre led by Angeles Garcia Cazorla and entered to a protected database and biobank, respectively, at San Joan de Deu Children`s Hospital in Barcelona

Merknad [TT3]: How do we solve that data are in fact sent by email which is forbidden at least in Norway despite pseudonymised data (due to the ultra rare disease and genetic results patients may be identified): To apply the IRB in Norway I need to answer secure transfer of data, suggestions on how to come around this to avoid disapproval from IRB?....

Benefits and risk of participation:

Participation to this study does not imply any direct benefit for the participant beyond the indirect knowledge gained for the disease that may enhance better therapy in the future. There are no direct side effects or physical discomforts from participating since no extra blood samples or investigations will be performed.

Study design:

This is an open, non-interventional, multicentre observational study (retrospective/cross sectional).

Inclusion criteria:

Children and adults with confirmed diagnosis (biochemical/clinical and/or genetic) of BCKDK deficiency

Exclusion criteria

Individuals with severe unrelated comorbidity that may influence the phenotypic expression of BCKDK deficiency

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