European Journal of Medical Genetics 59 (2016) 204–209

Contents lists available at ScienceDirect

European Journal of Medical Genetics

journal homepage: http://www.elsevier.com/locate/ejmg

A novel homozygous splice site mutation in NALCN identified in siblings with cachexia, strabismus, severe intellectual disability, epilepsy and abnormal respiratory rhythm

Moran Gal [a, *], Daniella Magen [b, c], Younan Zahran [d], Sarit Ravid [i], Ayelet Eran [e], Morad Khayat [f], Chen Gafni [f], Erez Y. Levanon [a, **], Hanna Mandel [g, h, ***]

[a] The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan Israel
[b] Pediatric Nephrology Institute, Rambam Health Care Campus, Haifa, Israel
[c] Laboratory of Molecular Medicine, Rappaport School of Medicine, Technion, Haifa, Israel
[d] Department of Pediatric Medicine, Clalit Health Services, Ibillin, Israel
[e] Department of Radiology, Health Care Campus, Haifa, Israel
[f] The Genetic Institute, Emek Medical Center, Afula, Israel
[g] Metabolic Unit, Rambam Health Care Center, Haifa, Israel
[h] Rappaport School of Medicine, Technion, Haifa, Israel
[i] Pediatric Neurology Unit and Epilepsy Service, Meyer Children's Hospital, Rambam Health Care Campus, Haifa, Israel

ARTICLE INFO

Article history:
Received 6 November 2015
Received in revised form
16 February 2016
Accepted 19 February 2016
Available online 23 February 2016

Keywords:
Cachexia
NALCN gene
Intellectual disability
Abnormal respiratory rhythm
Seizures

ABSTRACT

We studied three siblings, born to consanguineous parents who presented with severe intellectual disability, cachexia, strabismus, seizures and episodes of abnormal respiratory rhythm. Whole exome sequencing led to identification of a novel homozygous splice site mutation, IVS29-1G > A in the *NALCN* gene, that resulted in aberrant transcript in the patients. *NALCN* encodes a voltage-independent cation channel, involved in regulation of neuronal excitability. Three homozygous mutations in the *NALCN* gene were previously identified in only eight patients with severe hypotonia, speech impairment, cognitive delay, constipation and Infantile–Neuroaxonal-dystrophy- like symptoms. Our patients broaden the clinical spectrum associated with recessive mutations in *NALCN*, featuring also disrupted respiratory rhythm mimicking homozygous *Nalcn* knockout mice.

1. Introduction

Over the last decade we have followed and studied three affected siblings, born to consanguineous parents, in whom the most striking clinical findings were, severe hypotonia, lack of developmental milestones and regression, seizures, episodes of abnormal respiratory rhythm, progressive strabismus and cachexia. Since the symptoms were neither specific, nor indicative of known autosomal recessive disorders, we took the approach of whole-exome sequencing (WES) in a single affected individual to identify the underlying molecular pathology. A novel splice site mutation leading to frame shift and premature stop codon was found in the sodium leak channel, nonselective *NALCN* gene (MIM 611549).

NALCN encodes a non-selective cation channel that provides leak conductance into cells, to drive their membrane excitability. Its current was found to be activated by various types of neurotransmitters such as Acetylcholine (Swayne et al., 2009), neurotensin and substance P (Lu et al., 2009), and it is expressed predominantly in the brain (Lee et al., 1999). Its ortholog in Drosophila (Dmα1U) is highly expressed in the synaptic regions, suggesting a role in neurotransmission. Deletion of the gene in various animal models leads to abnormal respiratory rhythm (B. Lu et al., 2007), behavior phenotypes such as impaired circadian rhythm (Lear et al., 2013) or locomotion (Lu and Feng, 2011), all caused by abnormal neuronal activity. De-novo heterozygote mutations in NALCN have been

* Corresponding author.
** Corresponding author.
*** Corresponding author.Metabolic Unit, Rambam Health Care Center, Haifa, Israel.
E-mail addresses: morangal30@gmail.com (M. Gal), s_ravid@rambam.health.gov.il (S. Ravid).

http://dx.doi.org/10.1016/j.ejmg.2016.02.007
1769-7212/

found to cause dominant CLIFAHDD syndrome in 18 patients, (Chong et al., 2015; Aoyagi et al., 2015; Fukai et al., 2016), whereas recessive mutations have been described in only three families so far, leading to a different neurological phenotype (Al-Sayed et al., 2013; Köroğlu et al., 2013). The patients described herein expand the phenotypic spectrum associated with recessive *NALCN* gene mutations.

2. Clinical report

A consanguineous Israeli Moslem family, consisting of three affected infants and one healthy girl was studied (Fig. 1). The first sibling (Fig. 1,V-2) was a full-term male born after normal pregnancy with birth weight of 3090 g and Apgar score of 5/8. He had meconium-stained amniotic fluid associated with transient respiratory distress, and an asymptomatic VSD. At one month of age he presented with a high pitched cry, roving eye movements, and irritability on gentle touching. At the age of 6 months his weight 3425 g (−5.4SD), his head circumference was 37 cm, (<<5 centile), and he had severe hypotonia with increased tendon reflexes. Since the age of 9 months he displayed internal squint but still had a social smile. Physical examination at the age of 10 months revealed a cachectic infant (5.5 kg, −6.5SD), with persistence of truncal hypotonia, reduced spontaneous movements of the legs and purposeless hand movements. Tendon reflexes were no longer elicited. Severe constipation was reported. Routine blood studies including complete blood count, renal, liver, muscle and thyroid function tests were all within normal limits. Metabolic screening including plasma lactate, amino acids, very long chain fatty acids (VLCFA), isoelectric focusing of transferrin, acylcarnitine profile in dry blood spots, urinary amino and organic acids profiles were unremarkable. A skeletal muscle biopsy revealed normal histology and normal activity of the five mitochondrial respiratory chain complexes. Brain MRI at the age of 10 months was normal. The patient died suddenly at home at the age of 20 months.

The 2nd sibling (Fig. 1, V-3) was a full term female born after a normal pregnancy, with a birth weight of 3000 gr, head circumference of 34 cm (50 centile) and Apgar score of 8/9. At the age of 3 months a neurodevelopmental delay was suspected due to lack of social eye contact and truncal hypotonia. At 6 months she had good eye contact and a social smile, but truncal hypotonia persisted. Her head circumference growth rate seemed to slow down (was 40.6 cm, 10 centile) and her weight was 5.8 kg (−1.6SD) despite of good calories intake. Since the age of 7 months she suffered from severe constipation. By the age of 11 months she seemed to be very thin, lying in frog position but could lift her legs against gravity. She had internal strabismus, horizontal and vertical nystagmus, and purposeless hand movements. Startle reflex was noted in response to even fine touch, with increased tendon reflexes. At the age of 27 months she had pronounced emaciation, weighed 6.5 kg (−5.5SD), lost social eye contact and did not gain any developmental milestone. Since the age of 4.5 years she had generalized tonic–clonic seizures which responded to Vigabatrin therapy.

At the age of 6 years, while seizures seemed to abate, her parents reported on recurrent episodes of abnormal respiratory rhythm consisting of apneas (lasting 10–12 s), alternating with deep fast breathing. Such episodes were also witnessed by several physicians. Due to severe malnutrition (−7SD), a gastrostomy tube was introduced, but the child remained cachectic (Fig. 2). Of note, no specific dysmorphic features existed except for pronounced internal strabismus and emaciation. Routine blood count and biochemical studies as well as metabolic screening and CSF protein analysis were unremarkable. The patient's pronounced malnutrition and constipation led us to suspect mitochondrial neurogastrointestinal encephalopathy (MNGIE) disease and study thymidine and deoxyuridine serum levels, which were normal. The combination of cachexia, developmental retardation and regression also raised the possibility of Cockayne syndrome, which was ruled out by a normal transcription coupled DNA repair test. Brainstem evoked response audiometry (BERA) and visual evoked potential (VEP) were normal. Brain MRI performed at the age 3 of years demonstrated two T2 high intensity foci on the dorsal brainstem, anterior to the fourth ventricle (Fig. 3). This finding raised the possibility of a mitochondrial disease. However, muscle biopsy revealed normal histology and normal activity of all five mitochondrial respiratory chain complexes. Currently, at the age of 8 years, she features cachexia and strabismus with no progress in her physical or developmental achievements.

The 3rd affected sibling (Fig. 1, V-4) was a full term female born after normal pregnancy with a birth weight 3200 gr., head circumference of 34.3 cm and an Apgar score of 9/9. She was not brought to medical attention until 7 months of age when her mother felt that she also might suffer from the same familial

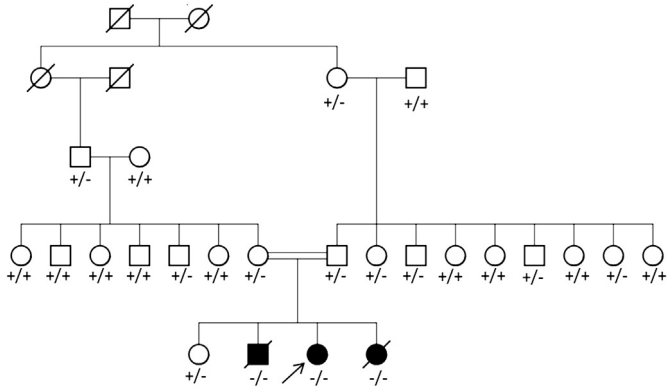

Fig. 1. The kindred studied. The Proband, whose whole exome was sequenced, is indicated with an arrow. Genotypes are presented as "+" for the wild-type allele and "−" for the NALCN- c.G3390A mutated allele.

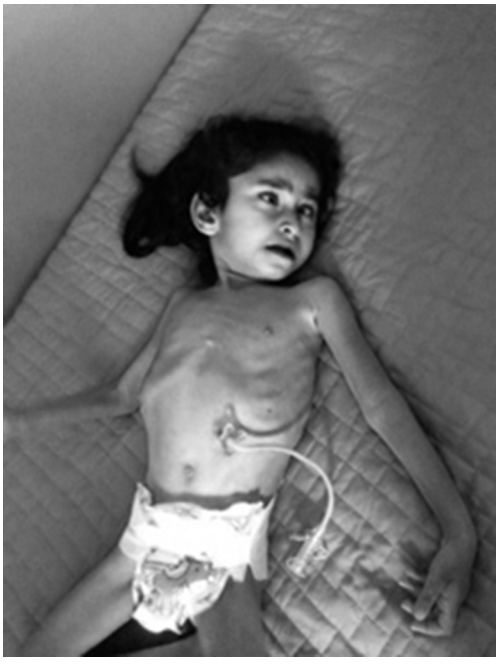

Fig. 2. Patient V-3, at age 8 years. This picture depicts the strabismus and severe malnutrition.