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Case Report Open Access

"Myoclonic Absences" and other Novel Findings in Warburg Micro Syndrome: Clinical Report of an Expanding *RAB18* Phenotype

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Introduction

Warburg Micro syndrome (WARBM) is a rare autosomal recessive disease. In 1993 Warburg first described in an inbred Pakistani family three children suffering from microcephaly, microcornea, congenital cataract, retinal dystrophy, optic nerve atrophy, cognitive disability, hypothalamic hypogenitalism and corpus callosum agenesis [1]. Onward, in addition to severe growth and psychomotor delay, more connotative features are reported in facies (low anterior hairline, narrow forehead, short nose with prominent root, blepharophimosis, large ears, short prominent philtrum, and micrognathia), in skeletal deformities (hallux valgus, overlapping toes, clinodactyly, camptodactyly, arachnodactyly) and in neurologic disorders (spastic paresis and progressive motor neuropathy) [2-5]. Polymicrogyria emerges as the most frequent cortical malformation, however other gyration abnormalities or various brain alterations such as ventriculomegaly and cerebellar vermis hypoplasia are possible [5]. Electroencephalogram (EEG) abnormalities as well as tonic-clonic, partial and myoclonic seizures are described [3-7].

WARBM is caused by mutations of *RAB3GAP1, RAB3GAP2* [8] or *RAB18* genes [4] in about 50% of cases, *RAB3GAP1* being the most common gene involved. Rarely the disease is linked to *TBC1D20* gene, which is known to probably act through *RAB18* in autophagosome maturation [9]. Phenotype does not correlate for a specific gene but rather for severity of mutation: *RAB3GAP1*, *RAB3GAP2* and *RAB18* loss of function mutations cause an undifferentiated WARBM whereas milder ones produce Martsolf syndrome. *De facto*, these two conditions assemble a sort of nosologic continuum [5].

We describe the clinical and instrumental findings of two children born to consanguineous parents and carriers of a novel *RAB18* mutation. In association with typical WARBM dysmorphic and neurological signs, the firstborn had a peculiar epileptic phenotype including "myoclonic absences (MA)" and previously undescribed findings at brain magnetic resonance imaging (MRI).

Case Report

The first-born

A one-year-old child was brought to our attention for a severe motor and intellectual delay. He was initially the sole son of two healthy first cousins from Morocco. The family history was negative for neurological and genetic disorders. Pregnancy elapsed with intrauterine growth restriction which required a caesarean section at 34 gestational weeks. At birth, the newborn had a weight of 1370 grams and an Apgar score of 7/9. He had an apnoeic event at 2 days of life and a neonatal hyperbilirubinemia peak of 12 mg/dL on the fifth day.

At the time of our first assessment (12 months of age) he had neither postural control nor language; he showed poor orientation, worse to visual stimulations compared to sounds and touch. He had microcephaly, narrow face, sparse eyebrows, frontal hypertrichosis, palpebral pseudoptosis, ogival palate, bulbous nose, short philtrum, asymmetric large ears and tapering fingers. The child was also suffering from microphthalmia, microcornea, small pupils, bilateral cataract, cryptorchidism and congenital talipes equinovarus. Abdomen and cardiac ultrasound were negative.

A brain MRI had been performed at three months of age (Figure 1). In addition to a widespread white matter hyperintensity on T2-weighted images-likely related to a physiological hypomyelinization-, it showed bilateral frontal polymicrogyria consisting of smoothed sulci and multiple cortical microgyria. The gyration abnormality extended posteriorly to the opercular regions and to the most cranial sections of both parietal lobes. The sylvian fissures were enlarged and the corpus callosum was hypoplastic. At subsequent MRI examinations, performed at 30 months and 8 years of age, pericerebral subarachnoidal spaces were increased and the frontal horns of the lateral ventricles were wider; supratentorial white matter was poorly represented and hyperintense on T2-weighted images, consistent with an incomplete or delayed myelinization. Corpus callosum was hypotrophic. Last MRI performed at 8 years' detected in the infra-tentorial district an enlargement of cisterna magna with a superior hypotrophy of vermis, a mild dilatation of the fourth ventricle and a hypotrophy of middle cerebellar peduncles. Peri-ventricular white matter marked hyperintensity on T2-weighted images persisted, suggesting a focal marked hypo-dysmyelination.

At three years of age he underwent lens aspiration and after a few years the surgical correction of a secondary corectopia was required. The optic correction of aphakia with glasses couldn't improve his vision due to the coexistence of exotropia, nystagmus, bilateral corneal haze, and a progressive bilateral optic atrophy.

First wakefulness EEG (12 months of age) revealed low-amplitude fast rhythms prevailing frontally and a central-parietal focus of high voltage 4-6 Hz waves. During sleep, bisynchronous discharges of irregular high amplitude spike-waves or polyspike-waves were detectable on the frontal-central-temporal regions. Visual evoked potentials (VEPs) were progressively abolished whereas electroretinogram (ERG) was normal; brainstem auditory evoked responses (BAEPs) showed delayed responses.

Since he was 2 years old he suffered from recurrent focal

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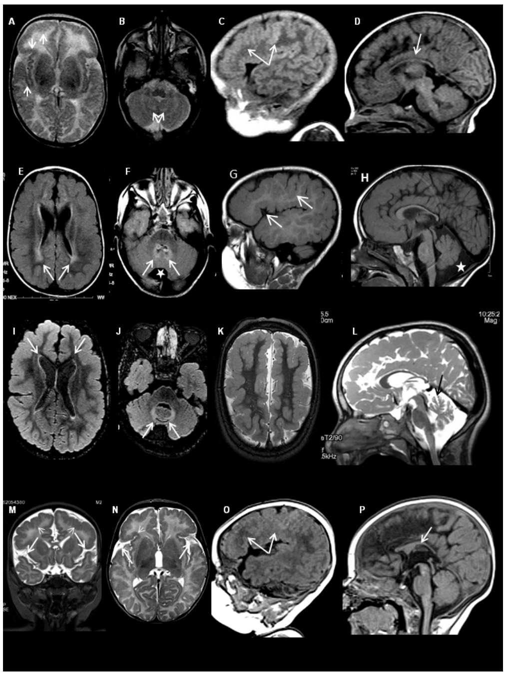

First born: 1st MRI at 3 months: A and B, axial T2-weighted images; C and D, parasagittal and sagittal T1-weighted images. 2nd MRI at 30 months: E and F, axial T2-FLAIR images; G and H, para-sagittal and sagittal T1-weighted images. 3rd MRI performed with a 3,0 Tesla machine at 8 years of age: I and J, axial T2-FLAIR images; K, axial FSEIR (STIR) image; L, sagittal T2-weighted image.

1st MRI (A, B, C, D). The white matter appears hyperintense on T2 images (T2-FLAIR images not acquired) due to particular age of the myelinization in supratentorial (A) and infratentorial district (arrows in B). The frontal, parietal and fronto-opercular cortex (arrows in A and C) is thickened and associated with numerous short sulci, consistent with polimicrogiria. The corpus callosum is globally present but hypotrophic (arrow in D). MRI at 30 months (E, F, G, H). The white matter shows an improved myelinization, except for scattered areas in the supratentorial peri-ventricular and deep white matter (arrows in E). The white matter surrounding the IV ventricle persists markedly hyperintense on T2 (arrow in F). It is confirmed the presence of polymicrogiric cortex, that extends in fronto-parietal and opercular regions (G). The corpus callosum evolved in myelinization and better shows a global hypotrophic aspect (H). The cisterna magna is enlarged (star in F and H), with no sure evidence of hypotrophy of the cerebellum. MRI at 8 years (I, J, K, L). Improved myelinization is seen on T2 in the supratentorial white matter, but associated with an enlargement of the frontal horns of the lateral ventricles (arrows in I). A persistent lack of myelinization is appreciable in the cerebellar deep white matter (arrows in J), associated at this age to an widening of the IV ventricle. The extension of the polymicrogiric cortex (K) is well evaluable and the global hypotrophy of the corpus callosum (L) is better defined thanks to high resolution images acquired by means of a 3,0 Tesla machine. In the contex of a global slight enlargement of the subarachnoidal peri-cerebral spaces (K and L), it is appreciable a hypotrophy of the cerebellar, in particular of the vermis (black arrow in L).

Younger brother: MRI performed at three months of age: M, coronal T2-weghted image; N, axial T2-weighted image; O and P parasagittal and sagittal T1-weighted image. In the context of a wide hyperintensity on T2 of the white matter, due to hypomyelinization for the particular age of the child, it is detectable an enlarge subarachnoidal spaces at fronto-temporal level (arrows in M, N), a polymicrogiric cortex in the frontal lobes (arrowheads in M) extended to the opercular cortex (arrowhead in N) of a wide sylvian fissure (arrows in N) and to the parietal lobes (arrows in P). The corpus callosum is present but globally hypotrophic (Q), with limited visualization of the rostrum and the splenium related to the at the moment global hypomyielinization. No infra-tentorial abnormality is detectable.

Figure 1: Brain magnetic resonance images in two brothers with MICRO syndrome. First born A-L; younger brother M-Q.

epileptic status mainly related to fever. Simultaneously EEG disclosed asynchronous parietal-occipital low voltage spikes or spike-waves moderately increased by sleep. Carbamazepine treatment was introduced with a temporary effect.

The clinical picture evolved to a spastic tetraparesis with axial hypotonia and limbs dystonic movement disorder. He had dysphagia and severe intellectual disability. The visual acuity was *motu manu* in the right eye and light perception in left eye. He could produce sporadic vocals, social smile and motor stereotypies of arms. A demyelinating limbs neuropathy arose, with a predominantly motor involvement.

At 6 years' tonic seizures supervened in wellness. Carbamazepine was thus replaced with Levetiracetam leading to a good response. Starting from the age of 8 years, the child had repeated daily MA lasting 10-12 seconds. Clinically the seizures were characterized by loss of consciousness, right head deviation and generalized rhythmic myoclonias superimposed on progressive tonic limbs abduction. They were related to an EEG paroxysmal discharge of generalized and regular high amplitude 3 Hz spike-waves (Figure 2). Interictal EEG showed an intensification of the frontal-central-parietal focus of 3-4 Hz high amplitude activity, at times intermingled with synchronous or asynchronous spike-waves discharges. Clonazepam and Valproate were added to treatment and afterwards no further epileptic events occurred nor epileptiform activities were observed. Karyotype, array-CGH and Sanger sequencing of tubulin and GPR56 genes detected no alterations. Over the years, the patient had two brothers: a healthy child and an affected one reported below.

The younger brother

The patient was born full term through an urgent caesarean section required by IUGR and fetal distress. Neonatal weight was 1900 grams and the extra uterine life adaptation was adequate. Postnatal sucking was ineffective. At our first evaluation, the child was one-year-old and he had no developmental, postural or praxic skill appropriate for age. He had microphthalmia, bilateral cataract, microcornea, a central corneal opacity, small pupils and iris dysgenesis. Clinical features also included microcephaly, frontal hypertrichosis, sparse and up-slanted eyebrows, hypotelorism, short palpebral fissures, bulbous nose, arched palate, hypogenitalism and a sacral Mongolic patch. He had axial hypotonia, limbs spasticity and a dyskinetic movement disturbance of face and

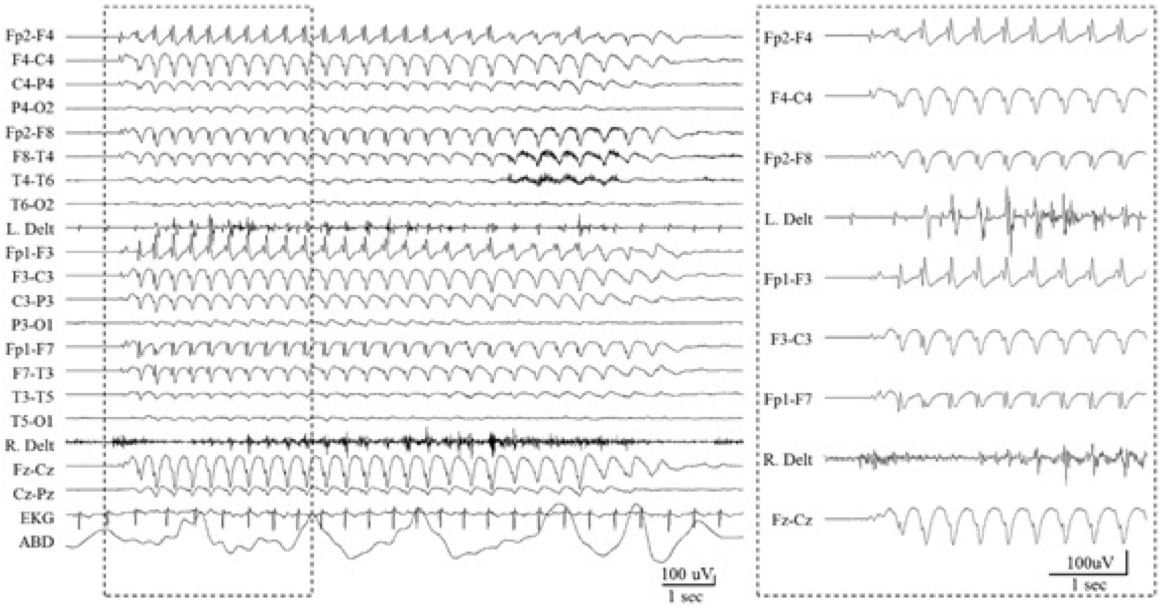

Figure 2: EEG documentation of “myoclonic absences” in the firstborn. High amplitude generalized 3 Hz sharp-waves are related to limbs myoclonias and post-myoclonic silences superimposed on a progressive tonic contraction (see electromyography).

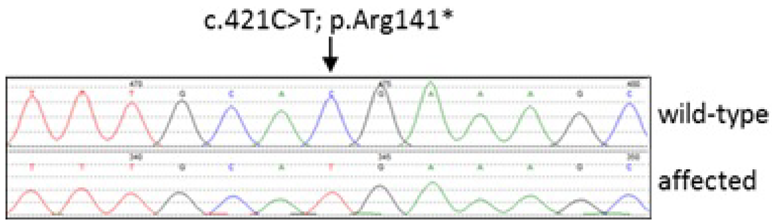

Figure 3: An affected child carries a homozygous nonsense variant in *RAB18*. Electropherogram from affected child (lower trace) shows a homozygous c.421C>T; p.Arg141* variant confirmed by direct sequencing. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence NM_021252. Peptide residue numbering reflects protein primary structure with p.Met1 corresponding to the first methionine in the reference sequence NP_067075.

arms. Gaze direction was possible in response to tactile and auditory stimuli. No language was expressed. ERG was normal and VEP had no response; BAEPs were bilaterally delayed. Nerve conduction velocity (NCV) detected a limb demyelinating neuropathy, with a mainly motor impairment.

Despite early cataract surgery at one month of life, he had a severe visual impairment and fundus examination – as far as possible for small pupils – disclosed hypoplasia and pallor of optic disc. A pronounced thinning of the optic nerves was found by neuroradiology, confirming the presence of an optic hypoplasia.

A brain MRI performed at three months’ (Figure 1) detected a slight fronto-temporal enlargement of subarachnoidal spaces with a mild dilatation of lateral ventricles. A polymicrogiric gyration of both frontal lobes was associated, extending to the opercular cortex of a wide sylvian fissure and to the parietal lobes, in particular on the left side. Corpus callosum appeared hypoplasic, with poor visibility of rostrum and splenium due to an age-related global hypomyelinization. No infra-tentorial abnormalities were identifiable.

At 3 years of life, EEG disclosed in wakefulness frontal fast rhythms and central 2-3 Hz activity of high and medium amplitude; during sleep, high voltage and asynchronous sharp-waves were recognizable on frontal-temporal regions. He had no seizures.

Genetics

In both affected brothers a homozygous nonsense *RAB18* variation c.421C>T (p.Arg141) was detected by Sanger sequencing (Figure 3). Parents and the healthy brother were heterozygous. This variation was never reported as known mutation (HGMD professional) or detected in healthy databases (db SNP. 1000 Genomes Project, NHLBI GO Exome Sequencing Project, Exome Aggregation Consortium, MLPA). Nevertheless, this mutation introduces a premature stop codon after 141 amino acids, it is predicted to be deleterious by all consulted bioinformatic webtools (MutationTaster, PolyPhen-2, LRT, SIFT, Mutation Assessor, CADD) and its evidence of pathogenicity is very strong according to the criteria of the American College of Medical Genetics and Genomics [10].

Discussion

Micro Warburg syndrome is a rare autosomal recessive disease caused by loss-of-function mutations of *TBC1D20*, *RAB3GAP1*, *RAB3GAP2* or *RAB18*. Recent evidences suggest the absence of a clear gene-specificity: *TBC1D20* and *RAB3GAP1/2* transcripts may affect through different pathways the same target, i.e. the intracellular localization and functioning of *RAB18* [8]. To date, only five *RAB18* mutations have been described [3,7], therefore this report could further expand WARBM phenotype and particularly in relation to this gene.

First, in both brothers NCV detected a limb demyelinating neuropathy, with a mainly motor impairment as only sporadically reported in other WARBM patients with RAB gene mutations [3,6,7].

Secondly, in the younger child an optic hypoplasia was observed, unlike most of WARBM subjects usually suffering from a progressive optic atrophy. Moreover, both of our patients had a central opacity of cornea, never evidently reported before and possibly specific for *RAB18*-related microphthalmia.

Thirdly, in the firstborn brain MRI showed over the time the appearance of a marked peri-ventricular white matter hyperintensity on T2-weighted images, suggestive of a focal hypo-dysmyelination. This finding seems consistent with a white matter disorder rather than a myelination delay as previously reported in the literature. In the younger brother only one brain-MRI was performed at three months of life and no data are available on neuroradiological evolution.

Lastly, the first-born child had an epileptic phenotype characterized at onset by focal seizures, then by generalized tonic seizures and finally by MA. No seizure had occurred in the second-born up to the last follow up at 3 years of age, when EEG showed in sleep high voltage and asynchronous frontal-temporal paroxysms.

Seizures with "myoclonic" components were previously described in other patients with pathogenic *RAB18* mutations [3,6,7]. In particular, 6 out of 13 patients had epilepsy, 3 with generalized tonic clonic seizures and the other 3 with myoclonic seizures. In two siblings reported by [2], the age at onset of myoclonic seizures was respectively 5 and 3 years. They were very frequent (20-25 episodes per day) and refractory to multiple anticonvulsants. In the female described by Handley [5] myoclonic seizures were described since two years of age. In all of patients ictal and interictal EEG disclosed diffuse spike and wave discharges.

MA are generalized seizures first described by Tassinari et al, with an average age of onset of 7 years. MA can represent the prevailing ictal expression of certain idiopathic epilepsies and can also occur, more often along other seizures types, in association with structural brain lesions or genetic syndromes [10-12].

Conclusion

It is documented for the first time MA in WARBM and we wonder whether the seizures with "myoclonic" features previously reported could actually be MA. In that case, MA may be considered as a significant component of WARBM epileptic phenotype, particularly in relation with *RAB18* and considering its involvement in neurotransmitters release.

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