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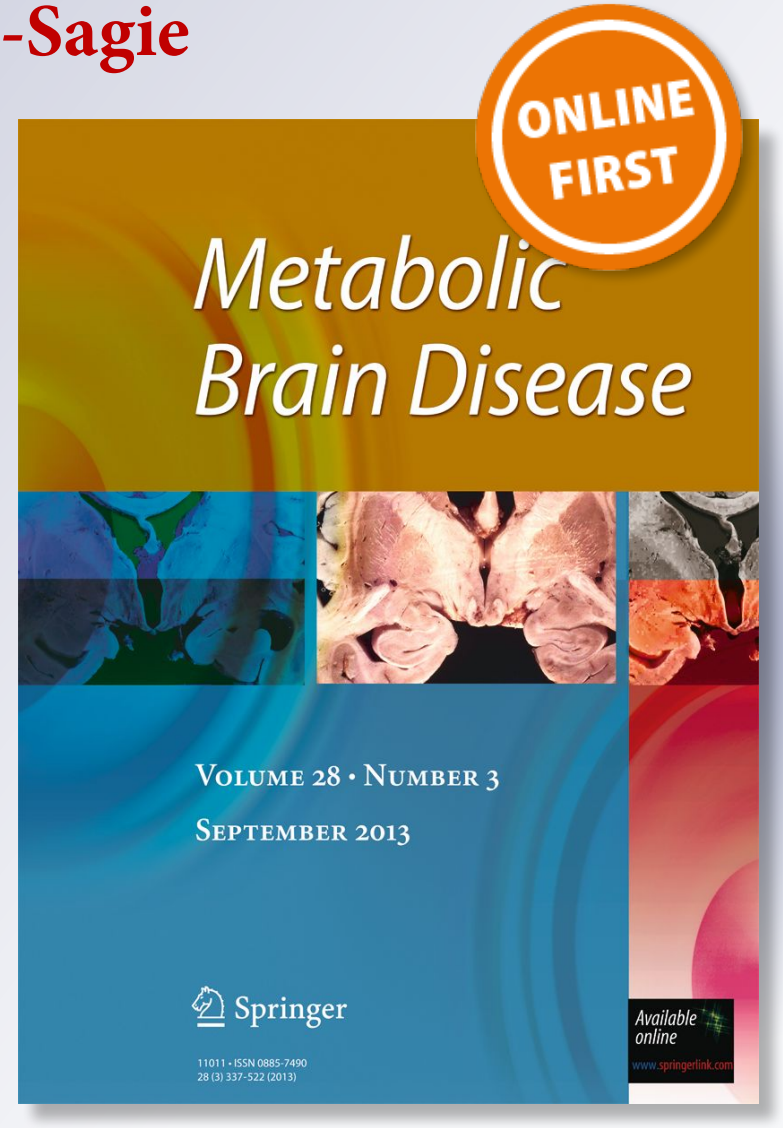

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ORIGINAL ARTICLE

Metabolic stroke in a patient with bi-allelic *OPA1* mutations

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Abstract

*OPA*1 related disorders include: classic autosomal dominant optic atrophy syndrome (ADOA), ADOA plus syndrome and a bi-allelic *OPA1* complex neurological disorder. We describe metabolic stroke in a patient with bi-allelic *OPA1* mutations. A twelve-year old girl presented with a complex neurological disorder that includes: early onset optic atrophy at one year of age, progressive gait ataxia, dysarthria, tremor and learning impairment. A metabolic stroke occurred at the age of 12 years. The patient was found to harbor a de novo heterozygous frame shift mutation c.1963_1964dupAT; p.Lys656fs (NM_015560.2) and a missense mutation c.1146A > G; Ile382Met (NM_015560.2) inherited from her mother. The mother, aunt, and grandmother are heterozygous for the Ile382Met mutation and are asymptomatic. The co-occurrence of bi-allelic mutations can explain the severity and the early onset of her disease. This case adds to a growing number of patients recently discovered with bi-allelic *OPA1* mutations presenting with a complex and early onset neurological disorder resembling Behr syndrome. To the best of our knowledge metabolic stroke has not been described before as an *OPA1* related manifestation. It is important to be aware of this clinical feature for a prompt diagnosis and consideration of available treatment.

Keywords Mitochondrial disease · Neurodegenerative disorder · Optic atrophy · Behr syndrome · Metabolic stroke · Next generation sequencing

Background

Optic atrophy is the result of retinal ganglion cell death and is commonly associated with abnormal oxidative phosphorylation. Inherited optic neuropathies can be due either to mitochondrial or nuclear gene defects. Sometimes it can be the only pathological feature of a mitochondrial disorder (i.e Leber Hereditary Optic Neuropathy).

Several mechanisms have been proposed for the pathogenesis of mitochondrial optic neuropathies. These include energetic failure, oxidative stress, glutamate toxicity, abnormal mitochondrial dynamics and axonal transport, and susceptibility to apoptosis (Carelli et al. 2009).

Early onset optic atrophy plus other neurologic manifestations, have been described in various mitochondrial disorders such as LHON 'plus' (Leber Hereditary Optic Neuropathy),

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OPA1 -ADOA 'plus' (Autosomal Dominant Optic Atrophy) and Costeff syndrome (OPA3).

Autosomal dominant optic atrophy was first described by Kjer in 1957 (Kjer 1957). Mutations in the OPA1 gene encoding a dynamin like mitochondrial GTPase, were first described as causing non syndromic autosomal dominant optic atrophy (ADOA) by Delettre et al. in 2000 (Delettre et al. 2000). Later, OPA1 'plus' was recognized as a neurologic disorder with extra- ocular manifestations such as myopathy, ataxia, deafness, peripheral neuropathy, or spastic paraparesis (Amati-Bonneau et al. 2009).

In the last few years, several cases of bi-allelic *OPA1* mutations have been described as causing an infantile onset optic atrophy with more severe neurological manifestations. We describe the occurrence of a metabolic stroke as a new manifestation of bi-allelic OPA1 mutation.

Methods

Whole exome sequencing was performed on the patients' DNA. The sample was enriched with Sureselect Human All Exome v.2 kit 50 Mb (Agilent, Santa Clara, CA, USA). Sequencing was carried out on HiSeq2000 (Illumina, San diego, CA, USA) as 100-bp paired–end runs. Reads were aligned with the human reference genome (assembly GRCh37/hg19). Pipeline was performed using the Genoox platform based on BWA (version 0.7.16) for read alignment and GATK HaplotypeCaller (version 3.7) and FreeBayes (version 1.1.0) for variant calling.

Dataset files including the annotated information were analyzed with the filtering steps as previously described [Sagie et al. 2018]. Conformation and familial segregation were performed using direct Sanger sequencing (3500 Genetic Analyzer Applied Biosystems).

Results

Case History: (the highlights are presented in Fig. 1).

A twelve-year old girl presented at the age of 18 months to the metabolic neurogenetic clinic due to decreased vision and global developmental delay. The mother had noticed abnormal eye movements since early infancy. An ophthalmic examination revealed bilateral optic atrophy with vertical and eyelid nystagmus. Visual evoked responses (VER) were pathologic due to low responses while Electroretinogram (ERG) was described as normal.

She had mild delay in acquisition of motor and language milestones. She started to walk independently at the age of 19 months. Gait ataxia was first diagnosed at the age of 24 months. Her gait deteriorated slowly during the years but she has sustained the ability to walk independently for short distances.

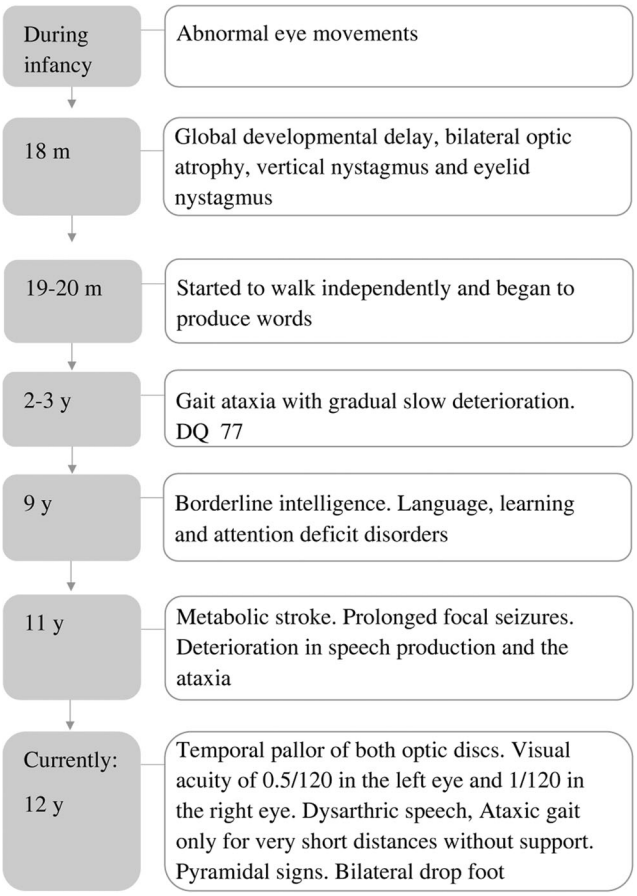

Fig. 1- Case history highlights. m-months; y-years

She began to produce words at the age of 20 months. She had further mild delay in syntactic abilities. Her general DQ was 77 at the age of 3 years, but her IQ test performed at the age of 6 years showed a total IQ score of 100. However, at the age of 8 years and 10 months her intelligence was scored as borderline. She was diagnosed with learning disorder, language and speech disorder, attention deficit and low self-esteem.

Brain MRI at the age of one year was within normal limits. MRI performed at the age of four years showed atrophy of the optic nerves, and mild central and deep white matter multifocal T2 hyperintensities. Similar findings were demonstrated on an MRI performed at the age of 9 years.

At the age of 11 years she had a prolonged seizure during a febrile illness with a cluster of focal seizures and myoclonic jerks, that were aborted with antiepileptic medications. She was hospitalized due to recurrent vomiting and an encephalopathic state that continued for a few days after the cessation of seizures. EEG performed during the hospitalization showed slow parietooccipital activity with intermixed spikes. Blood tests during her hospitalization revealed a normal blood count and liver function tests and CK. A mildly elevated arterial

blood lactate- 3.29 (normal range- 0-1.8), normalized after a week to 0.71. CSF Lactate was not measured.

MRI during the hospitalization revealed thin optic nerves and chiasma that were also evident in her previous MRIs. (Fig. 2a-b) A right parietal, acute infarct with restricted diffusion and edema was noted (Fig. 2c-g) as well as mild signal abnormalities in the right thalamus (Fig. 2f), MRA was normal. A follow up MRI a year later, demonstrated parenchymal loss in in both parietal lobes, more significantly on the right (Fig. 2h).

She went through a rehabilitation program with slow improvement in her speech and motor functions. Currently at the age of 12 years: she is alert and communicative. She speaks in long sentences but with moderate dysarthria. She is able to walk for very short distances without support and she uses a walker for longer ones. She attends a regular class with an aid and uses a computer adapted for the blind. She has learning difficulties. Neurologic examination shows cerebellar signs: ataxic gait, dysarthria and dysmetria. She scored 18 out of 40 on the SARA (Scale for Assessment and Rating of Ataxia). Pyramidal signs with exaggerated DTRs were noted but she also has bilateral drop foot attributed to a peripheral neuropathy (EMG was not performed at family request).

Her last ophthalmic examination at the age of 11 years and 3 months demonstrated temporal pallor of both optic discs. Visual acuity of 0.5/120 in the left eye and 1/120 in the right eye.

Laboratory and genetic studies:

A thorough investigation performed at the age of 20 months was normal and included: metabolic screen, EEG, cardiology and audiology evaluations.

A muscle biopsy performed at 21 months of age demonstrated normal macroscopic appearance and electron microscopy. No ragged red fibers were noticed, there was a slight decrease in complexes 1, 2 and 4 activities.

Molecular evaluation was negative for LHON point mutations and mtDNA sequencing was normal, Southern blot showed no mitochondrial deletions and chromosomal microarray analysis CMA was normal.

Exome sequencing revealed two variants in *OPA1* c.1963_1964dupAT; p.Lys656fs (NM_015560.2) and c.1146A > G; Ile382Met (NM_015560.2). The variants were validated by Sanger sequencing performed at our clinical laboratory. Familial segregation showed that the Ile382Met variant was inherited from the asymptomatic mother. This variant was previously reported in the literature. Further family investigation revealed that the asymptomatic aunt, and grandmother also carried the mutation. The c.1963_1964dupAT; p.Lys656fs variant arose

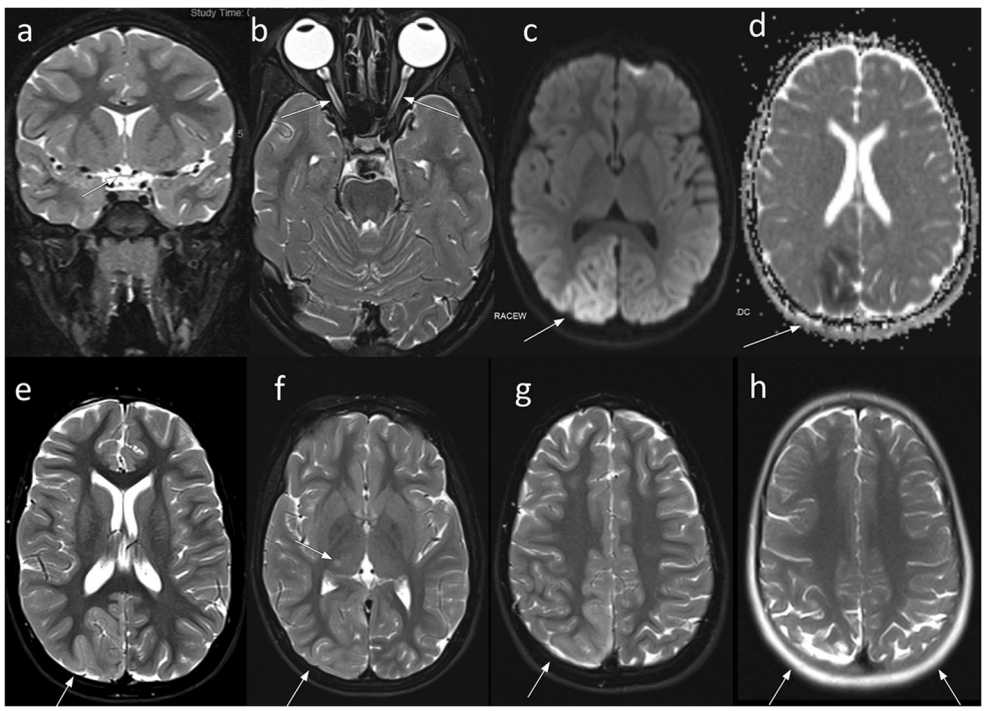

Fig. 2– Brain MRI of the patient. a-g were performed at the age of 11 years. **a** Coronal T2 with fat saturation, demonstrating thin optic chiasma. **b** Axial T2 with fat saturation, demonstrating thin optic nerves. **c** Axial DWI demonstrating restrictive diffusion in the right parietal lobe. **d** Axial ADC demonstrating restrictive diffusion in the right parietal lobe. **e** Axial T2 demonstrating hyperintense signal and cortical swelling in the right parietal lobe. **f** Axial T2 demonstrating hyperintense signal and cortical swelling in the right parietal lobe and mild hyperintensity in the right thalamus. **g** Axial T2 demonstrating right parietal cortical edema and parenchymal swelling. **h** Axial T2 at the age of 12 years showing moderate right parietal atrophy and mild left parietal atrophy

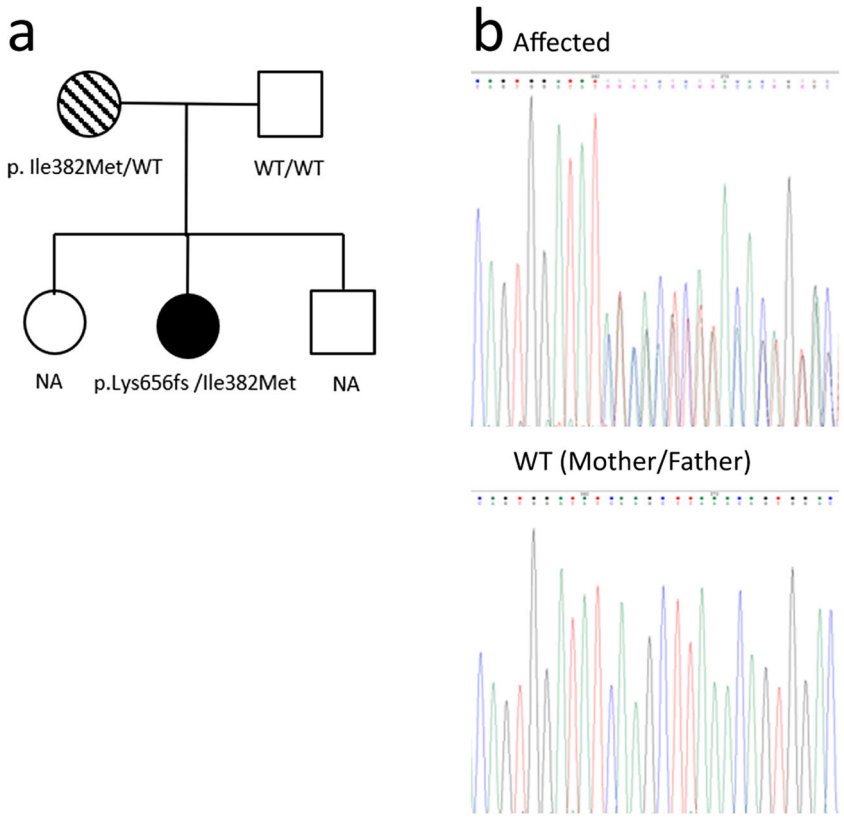

Fig. 3 – Molecular diagnosis of the patient. **a** Pedigree of the family. **b** Sanger confirmation analysis of the mutation c.1963_1964dupAT; p.Lys656fs (NM_015560.2) in the OPA1 gene, Carrier heterozygosity in the affected individual and wild type (WT) in both parents were confirmed. Not available (NA)

de novo and is a truncating mutation. This variant is absent from the databases (gnomAD, EXAC). (Fig. 3)

Discussion

The OPA1 gene encodes a dynamin related protein of the large GTPase superfamily that locates to the inner mitochondrial membrane. It is involved in mitochondrial dynamics and mtDNA maintenance. Impaired *OPA1* cells show abnormal mitochondrial morphology, distribution and function.

Pathogenic heterozygous mutations in *OPA1* have originally been associated with autosomal dominant optic atrophy (ADOA), which is the most common form of non-syndromic, early onset slowly progressive inherited mitochondrial blindness.

"ADOA plus syndrome" refers to a subgroup of patients with optic atrophy who develop an additional more complex neurodegenerative disorder, usually starting from the 3rd decade. (Nasca et al. 2017).

Bi-allelic *OPA1* mutations have been linked to an early onset complex neurodegenerative disorder (Nascaet al. 2017; Bonneau et al. 2014; Bonifert et al. 2014; Carelli et al. 2015; Schaaf et al. 2011). The clinical phenotype usually resembles Behr syndrome- a disease characterized by early-onset optic atrophy accompanied by neurologic features, including ataxia, pyramidal signs, spasticity and mental retardation (Bonneau et al. 2014).

Our patient carries bi-allelic mutations: a previously described missense mutation p.Ile382Met; NM_015560.2 inherited from the healthy mother and a de novo novel frame shift mutation c.1963_1964dupAT; p.Lys656fs (NM_015560.2).

Recently it has become clear that the Ile382Met variant is not pathogenic per se, but acts as a phenotypic modifier.

The modifying effect of the Ile382Met mutation is emphasized by the finding that the variant does not lead to disease even in the homozygous state (Bonifert et al. 2014). The de novo frame shift mutation causes premature truncation of the open reading frame, leading to mRNA decay and to complete loss of function of the mutated allele. It was suggested that compound heterozygous early onset cases of Behr syndrome might be associated with a pathogenic variant and a milder hypomorphic one (Bonneau et al. 2014).

Although the p.Ile382Met mutation is asymptomatic by itself, when combined with a pathogenic variant it induces a severe pathological condition compatible with a recessive mode of inheritance.

OPA1 belongs to the dynamin family, it shares three conserved regions: a GTPase domain, a middle domain and a carboxy-terminal coiled-coil domain also called GTPase effector domain (GED). The missense mutation p.Ile382Met is located in the dynamin GTPase domain, like more than two-thirds of the mutations reported in the OPA1 gene (Chao et al. 2016), while the frame shift mutation p.Lys656fs is located in the dynamins' conserved middle domain.

Homozygous *OPA1* mutations have also been described in the most severe phenotype of fatal infantile mitochondrial encephalomyopathy (Spiegel et al. 2016).

The neuroradiologic features of *OPA1* - related disease consist of cerebral and cerebellar atrophy, white matter signal abnormalities and thin optic nerves. Leigh-like features have also been described (Roubertie et al. 2015; Rubegni et al. 2017). However, metabolic stroke similar to that seen in Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like Episodes (MELAS) (Kuriyama and Igata 1985) has never been previously reported.

Metabolic stroke differs from ischemic and hemorrhagic ones. It begins with metabolic dysfunction that leads to a rapid onset of lasting focal brain lesions in the absence of large vessel rupture or occlusion. These lesions may not follow a defined arterial territory distribution, have a predilection to the posterior areas of the brain, and may spread progressively (Testai and Gorelick 2010).

Although metabolic stroke is typically described in MELAS, it has been reported in other mitochondrial cytopathies inherited by both the mitochondrial and nuclear genome (i.e Leigh disease, Kearns Sayre syndrome and polymerase gamma (POLG) – associated encephalopathy). It has also been reported in other inborn errors of metabolism such as organic acidurias (Brinjikji et al. 2011; Testai and Gorelick 2010).

The pathophysiology of metabolic stroke is incompletely understood. Current literature suggests a combination of neuronal mitochondrial energy failure and a cerebrovascular angiopathy with dysregulated perfusion. Impaired nitric oxide flux that results in vasospasm is postulated as a major cause of stroke in MELAS.

In POLG encephalopathy it was also suggested that the primary pathogenic event is neuronal energy failure due to respiratory chain dysfunction induced by mtDNA depletion (Hikmat et al. 2017). mitochondrial DNA depletion has been described in 2 sisters that carried a homozygous mutation in OPA1 gene (Spiegel et al. 2016). They had a severe encephalomyopathy and died at the end of their first year of life. Muscle biopsies showed significant mtDNA depletion, with a 78% decrease compared to controls. Increased mitophagy and excessive mitochondrial fragmentation were detected in primary human cultures of fibroblasts from patients with biallelic OPA1 mutations. The authors found a significant increase in cells that were depleted of mtDNA (Liao et al. 2017). DNA depletion might have had a role in the pathogenesis of the stroke in our case but unfortunately this could not be checked.

Recent work has demonstrated a beneficial effect of L-arginine in adult patients with MELAS for acute treatment and prevention. Its therapeutic benefit is postulated to result from arginine acting as a nitric oxide donor to reverse vasospasm. It is suggested that abnormal nitric oxide flux represent a common pathogenic factor across a diverse group of mitochondrial respiratory chain disorders and arginine has been given as a therapeutic drug in metabolic strokes for adults and children due to various mitochondrial diseases with encouraging results (Ganetzky and Falk 2018).

To our knowledge metabolic stroke has not been previously described as an *OPA1* related manifestation. We suggest to be alert to the possibility of a metabolic stroke in bi-allelic *OPA1* patients and to consider arginine treatment in the acute phase.

Currently there is no curative treatment for OPA1 related disease. It is common practice to treat with a mitochondrial "cocktail" which includes coenzyme Q10 as Ubiquinol or Idebenone. At present gene therapy in optic nerve diseases is being extensively studied. There are already clinical trials in LHON patients and in an OPA1 animal model (Chun and Rizzo 2017). Therefore, it is important to keep updated with the current literature.

Conclusions

This is the first case presenting a metabolic stroke in a patient with an *OPA1* related disease due to bi-allelic mutations - a novel de novo truncating mutation and a known *OPA1* modifier mutation.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest interests.

Informed consent Informed consent was obtained from the parents for publication in this study.

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