

## BRIEF COMMUNICATION

**Familial deep cavitating state with a glutathione metabolism defect**John Rendu<sup>1</sup> , Laetitia Van Noolen<sup>2</sup>, Catherine Garrel<sup>2</sup>, Julie Brocard<sup>3</sup>, Isabelle Marty<sup>3</sup>, Christelle Corne<sup>2</sup>, Julien Fauré<sup>1,\*</sup> & Gérard Besson<sup>4,\*</sup><sup>1</sup>Univ. Grenoble Alpes, Inserm, U1216, CHU Grenoble Alpes, Grenoble Institut Neurosciences, 38000, Grenoble, France<sup>2</sup>CHU Grenoble Alpes, 38000, Grenoble, France<sup>3</sup>Univ. Grenoble Alpes, Inserm, U1216, Grenoble Institut des Neurosciences, 38000, Grenoble, France<sup>4</sup>Department of Neurology, CHU, Grenoble Alpes, 38000, Grenoble, France**Correspondence**

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**Abstract**

Adult genetic disorders causing brain lesions have been mostly described as white matter vanishing diseases. We present here the investigations realized in patients referred for psychiatric disorder with magnetic resonance imaging showing atypical basal ganglia lesions. Genetic explorations of this family revealed a new hereditary disease linked to glutathione metabolism.

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**Introduction**

Brain cavitations are observed in conditions such as stroke, multiple sclerosis, infectious, or genetic diseases.<sup>1–4</sup> For most of the inherited diseases, early presentations are linked to mitochondrial defect, and in adults, these lesions have been essentially described in vanishing white matter diseases.<sup>4–8</sup> Following investigations in a family referred to our center for psychiatric and/or neurologic disorders, we describe here a specific combination of cystic lesions in basal ganglia with a metabolic profile resulting in a positive sulfite urine test. Genetic and metabolic explorations suggest a new hereditary syndrome due to a loss of function in the *NIT1* gene impairing glutathione metabolism.

**Clinical Reports**

The index case (II.4 Table 1 and Fig. 1A), a 34 years old man, was admitted in the emergency ward for a sudden left hemiparesis. He had a history of a nonsevere mental retardation, and was admitted 2 years before in psychiatry unit for an acute psychotic episode followed by generalized seizures. Upon clinical examination, a pure motor left hemiparesis was diagnosed and a stroke was suspected. A computed tomography scan (CT scan) and a magnetic resonance imaging (MRI) T1 and T2 (0.5 T) showed bilateral small cystic lesions affecting basal ganglia. Arteriography was normal as well as electroencephalography (EEG), electromyography (EMG), Doppler ultrasound, and cardiac echocardiography. Among biological analysis, a dipstick

**Table 1.** Clinical, biochemical, and genetic findings in family members.

	Current age/age of death		Clinical phenotype	Brain MRI defects	Urine sulfite dipstick test	Genetic status for <i>NIT1</i>
I.1	Age of death: 77	N.R.		N.A.	N.A.	N.A.
I.2	84	N.R.		N.A.	–	c.457G> A +/- c.670dupA -/-
II.1	69	N.R.		N.A.	N.A.	N.A.
II.2	67	N.R.		N.A.	N.A.	N.A.
II.3	66	N.R.		N.A.	–	c.457G> A -/- c.670dupA -/-
II.4	Age of death: 57	Mental retardation, Psychotic episodes, Seizures		Basal ganglia cystic lesions	+	N.A.
II.5	63	Sudden coma with cardiovascular collapse, Neuropsychological deterioration		Basal ganglia cystic lesions	+	c.457G> A +/- c.670dupA +/-
II.6	61	N.R.		N.A.	–	c.457G > A -/- c.670dupA -/-
II.7	60	Schizophrenia		Basal ganglia cystic lesions	+	c.457G > A +/- c.670dupA +/-
II.8	58	N.R.		N.A.	–	c.457G > A -/- c.670dupA -/-
II.9	Age of death: 17	Suicide		N.A.	N.A.	N.A.

N.R., not reported yet; N.A., not available or tested; – tested negative, + tested positive.

urine test for sulfites (MQuant, VWR®) was positive. The clinical status of the patient progressively worsened over a 20 years period with a severe neuropsychological deterioration and a spastic tetraplegia. He died in 2011, no autopsy was performed. In 2014, one of his brother (II.5, Table 1 and Fig. 1A) was admitted in the intensive care for a sudden coma with cardiovascular collapse. He was a 58 years old man with a history of diabetes mellitus and had lived an active professional life. The CT and the MRI (3 T) showed the same abnormalities as the patient II.4 (Fig. 2) and CT. The urine sulfite test was also positive. The patient progressively recovered but presented with a neuropsychological deterioration (BREF: 14/18, MMSE: 22/30).

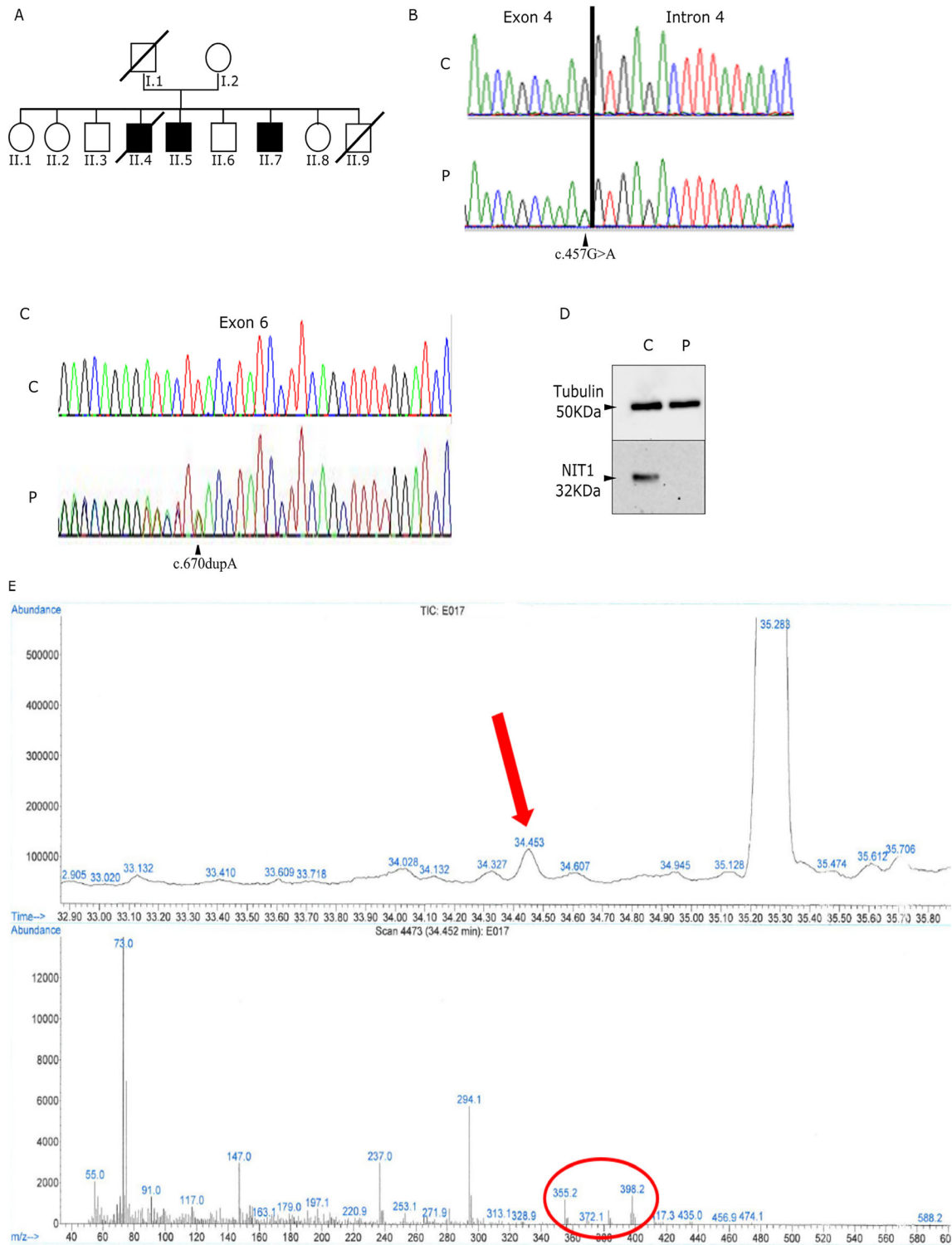
Due to a recurrent phenotype, a familial investigation was undertaken. A third brother of the family was in a psychiatric institution for a schizophrenia diagnosed 40 years ago (II.7). His clinical examination was normal, but the CT scan and the MRI (3 T) found the same brain lesions together with a positive urine sulfite test. The mother (I.2) and three others brothers and sisters (II.3,

II.6, II.7), had no symptoms nor positive sulfite test in urine samples.

An inherited recessive disorder was suspected and an exome study was performed on individuals II.7 and II.5 DNA. After excluding “OMIM” diseases with a special attention on genes involved in cavitation encephalopathy and sulfite metabolism, two rare variants were found in the *NIT1* gene (Nitrite-like protein 1, NM\_005600): an heterozygous c.457G > A;p.Gly153Arg variation and an heterozygous c.670dupA;p.Thr224Asnfs\*41 variation (Fig. 1B–C). The familial study showed that asymptomatic brothers and sisters, whose DNA was available, had neither of the variants, and that the c.670dupA was inherited from the asymptomatic mother. The father’s DNA was not available, but molecular cloning of a PCR fragment encompassing both variations from patient II.5 showed that they were initially present on different alleles of the gene (not shown).

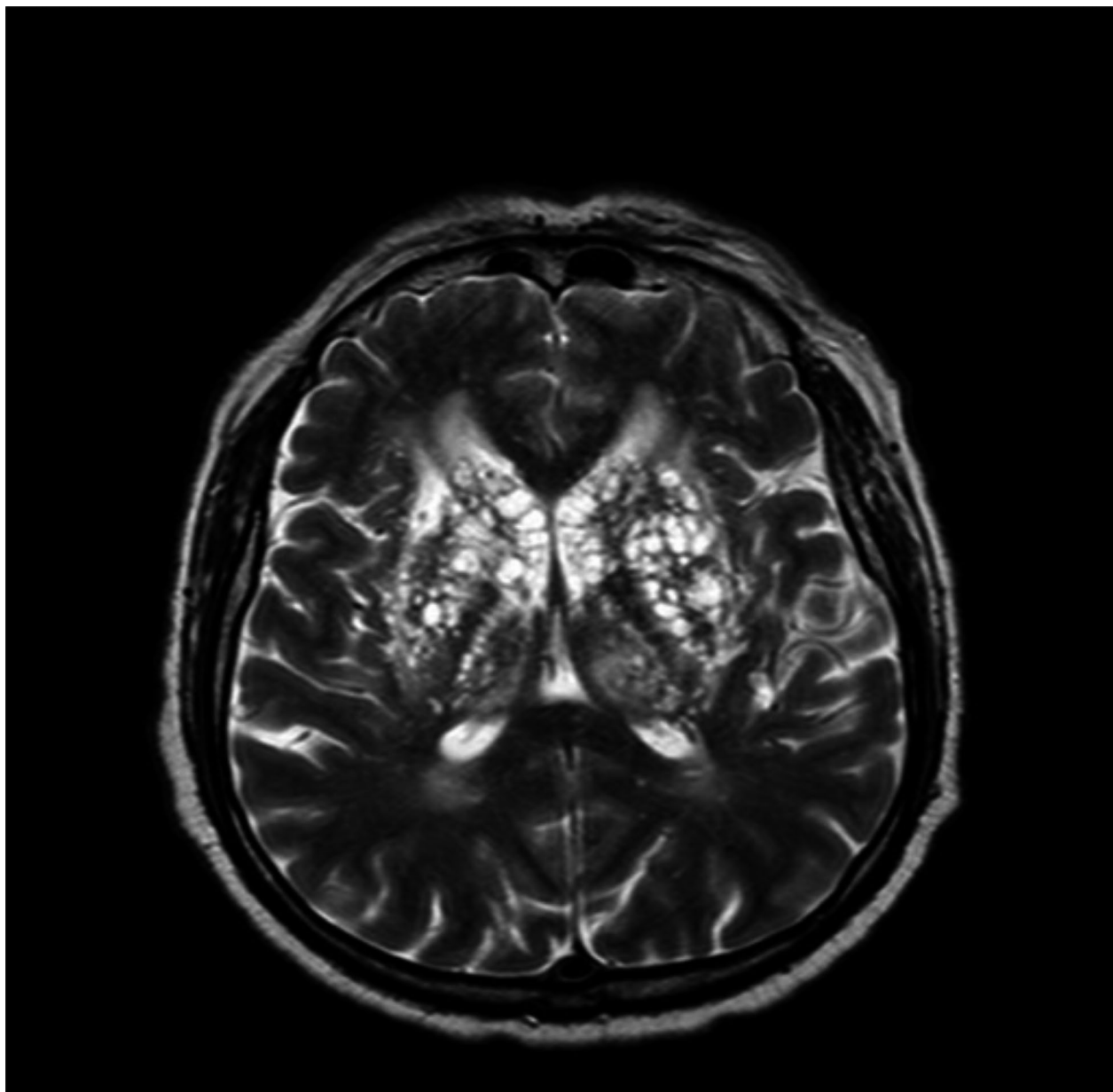
The c.670dupA variant is predicted to induce a loss of function of the *NIT1* allele due to a frameshift in the coding sequence. The c.457G > A variant induces the replacement of a highly conserved Glycine to an Arginine.

**Figure 1.** (A) Pedigree of the family. (B and C) Sequencing for the c.457G > A and c.670dupA variants upper part shows a control sequence (C) and the lower part shows the sequence from patient II.5 (P). (D) *NIT1* protein detection. Proteins were extracted from skin cutaneous control (C) or patient II.5 (P) fibroblasts and separated on SDS-PAGE. Anti-Nit1 antibody (Abcam®) or anti-Tubulin antibody (loading control) was used for the western blot. (E) GC/MS detection of a dGSH derivative in the urine of patient II.4 as described.<sup>9,10</sup> The compound only present in the patient urine is marked by an arrow on the chromatogram (upper part) with retention time of 34.45 min. It was identified as dGSH by mass spectrometry (lower part) by the presence of two specific peaks (circle). The mass spectrum of the compound is similar to the one present in the urine of *Nit1*-Knock Out mice and corresponds to a dGSH derivative (*m/z* 398 for the apparent molecular ion, *m/z* 383 for M-15, and *m/z* 355) as previously described<sup>9</sup>. The same peaks were observed in urine of his brothers (II.5 and II.7) but were undetectable in the other siblings tested and the mother.



It is also located on the last nucleotide of exon 4 and predicted to affect the splicing of *NIT1* mRNA, which was next demonstrated by sequencing of the *NIT1* transcript extracted from a skin biopsy of patient II.5 (data not

shown). Overall, the combination of the two variations should lead to a loss of expression of the *NIT1* gene. Accordingly, the level of Nit1 protein was undetectable by western blot in fibroblasts from patient II.5 (Fig. 1D).



**Figure 2.** Brain MRI of the patient II.5. Axial T2-weighted image showing deep cavitating state (3 T).

The *NIT1* gene encodes a highly conserved enzyme whose amidase function is required to catalyze the recycling of deaminated glutathione (dGSH), a side product of several metabolic reactions, into 2-ketoglutarate and cysteinylglycine leading to glutathione.<sup>9</sup> Interestingly, the knock-out of the gene in mice is responsible for the accumulation of dGSH that is excreted in urine.<sup>9</sup> A qualitative organic acid analysis profile<sup>10</sup> on urine samples of members of the family identified in individuals mutated for *NIT1* an unusual compound with a mass spectrum identical to dGSH (Fig. 1E). Due to the link between oxidative

stress and the *NIT1* pathway, the activity of superoxide dismutase (SOD) as markers of cellular overproduction of free radicals was tested on fibroblasts of the patient. The cytoplasmic SOD (Cu/Zn SOD) activity was not changed, whilst the activity of the mitochondrial SOD (MnSOD) showed a twofold increase as compared to control samples (relative amount of SOD activity 2.0, SEM  $\pm$  0.1513  $P = 0.0027$  student  $t$  test).

Altogether, our study shows in symptomatic patients of the family loss of function mutations in the *NIT1* gene, which probably induce an accumulation of dGSH

in their organism as well as oxidative stress at the cellular level.

## Discussion

We describe here the phenotype and genetic analysis of three brothers with a history of neuropsychological deterioration. All share specific brain damage identified by MRI and a positive urine sulfite test. The cavities detected by MRI in symptomatic members of the family are different from cavitary lesions frequently reported in adults,<sup>4,11</sup> especially because they locate in the basal ganglia. They may correspond to the so-called “cerebral porosis” in historical classification of lacunar lesions of the brain.<sup>1</sup> To date, the only known pathologies showing brain damage together with a positive urinary sulfite test are sulfite oxidase deficiency and molybdenum cofactor deficiency.<sup>12,13</sup> These pathologies were excluded and we identify here a novel nosological entity.

A whole-exome analysis showed that patients harbored compound heterozygous mutations in the *NIT1* gene. The loss of function of the gene led to the accumulation of dGSH, which was detected in the urine of patients, and probably responsible for the positive urine sulfite test. As recently published,<sup>9,14</sup> dGSH may represent a toxic metabolite, whose accumulation would interfere with the glutathione metabolism and in the long term possibly induce redox damages. Accordingly, an increase in the mitochondrial SOD activity, which is described as an adaptive response to reactive oxygen species-induced damage, was detected in fibroblasts from the patient. Other pathogenic effect linked to *NIT1* deficiency, such as modification of Wnt/beta-catenin pathways,<sup>15</sup> could be involved in patients phenotype.

Overall, we described here a novel autosomal recessive metabolic disorder due to mutations in the *NIT1* gene which associates neurological signs, basal ganglia lesions as well as positive urine sulfite test, the latter being easily used to screen for the disease in cohorts of psychiatric patients. Although a characteristic feature of the pathology is the accumulation of the glutathione metabolism intermediate dGSH due to *NIT1* loss of function, the pathophysiological mechanisms that could involve a progressive toxicity of dGSH itself and/or increased oxidative stress need to be further investigated to understand neuronal dysfunctions.

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## Author Contributions

JR: conception and design study, data acquisition, and drafting. LVN: conception and design study, data acquisition, and drafting. CC: conception and design study, data acquisition, and drafting. CG: data acquisition and drafting. JB: data acquisition. IM: data acquisition. JF: conception and design study, data acquisition, and drafting. GB: conception and design study, data acquisition, and drafting.

## Conflict of Interest

Nothing to report.

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