BRAT1–related disorders: phenotypic spectrum and phenotype-genotype correlation from 97 cases

Running title: Phenotypic spectrum of *BRAT1*-related disorders

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ABSTRACT

Purpose: Biallelic variations in *BRAT1* were initially described by Puffenberger et al. in 2012 in two patients with epileptic encephalopathy and rigidity. In 2015, Hanes et al. reported recessive variations in this gene in patients with developmental delay and cerebellar atrophy, with or without epilepsy. Since then, 37 patients from 26 families have been reported in the literature.

Methods: Thanks to an international collaboration, we collected clinical and molecular data from 57 new patients allowing us, together with data from the literature, to describe a large series of 97 patients and to study phenotype-genotype correlations.

Results: Data analysis of our cohort showed two distinct clinical phenotypes. In the first group, 59 patients from 45 families exhibited a neonatal epileptic encephalopathy presentation. Prenatal signs were present in 22 of them (22/44; 50%) with abnormal movements reported in 32% of cases (14/44) and IUGR in 20% (9/44). Most of these newborns had no psychomotor acquisition (57/58; 98%) and all of them had epilepsy or myoclonic movements (59/59; 100%) with a very early age of onset (median age 1 day). The neurological examination showed microcephaly (42/46; 91%) and limb rigidity (53/57; 93%). Brain MRI showed cerebral atrophy in 57% of cases (31/54). In most cases, death occured prematurely with a median age of 113 days (6 days - 23 years).

In the second group, 38 patients from 29 families presented a milder phenotype, with acquisition of walking in 76% of them (29/38) and acquisition of language in 68% (26/38). On clinical examination, cerebellar ataxia was found in 27 patients (27/38; 71%), axial hypotonia in 23 patients (23/29; 79%), and microcephaly in 11 patients (11/33; 33%). Epilepsy was inconstant (7/38; 18%) with onset in childhood (median age 3 years). Brain MRI showed cerebellar atrophy in 100% of patients (37/37). No patient in this group died.

Study of phenotype-genotype correlation showed that patients with epileptic encephalopathy harbor two predicted amorphic variations in the majority of cases (27/45; 60%) and a recurrent inframe deletion or duplication in just over ten percent of cases (5/45; 11%). In contrast, most patients with cerebellar ataxia phenotype present at least one missense (24/29; 83%).

Conclusion: In summary, *BRAT1* biallelic variations are associated with two different clinical presentations. The most severe picture, observed in patients with two amorphic variations, is

associated with epileptic encephalopathy and early death. On the opposite, patients with at least one missense variant have a moderate phenotype including variable intellectual disability, ataxia and cerebellar atrophy.

Key words: BRAT1, RMSFL, NEDCAS, epileptic encephalopathy, cerebellar ataxia

INTRODUCTION

Recessive variations in *BRAT1* (BRCA1-associated protein required for ATM activation-1) were first described in human pathology by *Puffenberger et al.* in 2012, after sequencing the exome of two patients from an endogamous population of Amish origin in Pennsylvania. These patients had a combination of severe drug-resistant epilepsy, limb rigidity, brain injury and early death (1). In 2015, Hanes et al. reported the case of a patient with heterozygous composite *BRAT1* variations and a less severe clinical phenotype combining global developmental delay with cerebellar atrophy (2). *BRAT1* biallelic variations have therefore been associated with two distinct clinical pictures: the rigidity and multifocal seizure syndrome (RMFSL) and a neurodevelopmental disorder associating cerebellar atrophy with or without seizures syndrome (NEDCAS). A correlation between the severity of the disorder and the type of *BRAT1* mutations have been suggested, biallelic truncating variations appearing to be associated with a more severe phenotype (3,4).

To our knowledge, 40 patients from 29 families have already been described with recessive variations in *BRAT1* (1,2,4–25). We report here a cohort of 57 additional patients from 45 families, describe the clinical features of the *BRAT1*-related disorders and discuss genotype-phenotype correlations.

PATIENTS AND METHODS

Patients

Individuals with *BRAT1* biallelic pathogenic variants were collected from Australia, Canada, France, Germany, Italy, Iran, Israel, United Kingdom and USA. Clinicians and biologists from the different centers were contacted through GeneMatcher and during European congresses. Clinical and genetic data were transmitted by the referring clinicians in a detailed table (Supp data S1).

In addition, we reviewed the patients described in the literature (1,2,4–8,10–20,22,23,26,27) to compare the phenotype to the one reported in our patients (Supp data S2).

Patients 38, 39 and 40 were previously reported briefly by Valence et al. (3) and patients 19 and 20 by Cornet et al. (28). However, we included them in our new cohort because we obtained a detailed clinical description from the referring clinicians.

Methods

All variations were identified by gene panel sequencing or exome sequencing (ES) and confirmed by Sanger sequencing, in the respective regional centers. In all cases, the parents were tested to confirm that the variations found in the proband were in trans. Written informed consent was obtained for genetic testing and use of photographs (if applicable).

RESULTS

Clinical data

Fifty-seven patients belonging to 45 unrelated families were identified with biallelic *BRAT1* pathogenic variants. Twenty-five were male (44%) and 32 were female (56%). The median age at last follow-up was 3 years 6 months (min:11 days; max: 28 years).

The families were of Algerian, Caribbean, Egyptian, English, French, German, Italian, Iranian, Kazakhstani, Latino, Libanese, Thai, Tunisian, Turkish, North African and Sub-Saharan African descent. Among the 45 families, 19 were consanguineous (42%).

Patients were divided into two subgroups according to their phenotype: on one side, newborns with epileptic encephalopathy, without psychomotor acquisitions, who died prematurely, and on the other side, patients presenting with developmental delay and cerebellar atrophy with or without epilepsy.

"Epileptic encephalopathy" group (Table 1)

Thirty-one patients from 25 families were included in this group, composed of 14 boys and 17 girls. Consanguinity was noted in 9 families (9/25; 36%). Median age at last clinical examination was 80 days (11 days - 14 years).

Prenatal signs were found in 50% of cases (15/30) with IUGR in 9 patients (9/30; 30%), abnormal movements in 8 patients (8/30; 27%), hydramnios in two patients (2/30; 7%) and oligohydramnios in one (1/30; 3%). Seven patients were born prematurely (7/30; 23%), including one at 27 weeks of gestation + 6 days. Congenital microcephaly was reported in 6 patients (6/26; 23%), axial hypotonia in 8 (8/21; 38%) and peripheral hypertonia in 16 (16/21; 76%).

On a neurodevelopmental perspective, none of these children had any psychomotor acquisition (30/30; 100%) and early death occurred in 26 patients (26/31; 84%) with an average age of 15 months (median: 3 $^{1/2}$ months; 11 days – 23 years old). Four patients in this cohort are still alive at the time of the study, two of whom are less than one month old.

All patients had epilepsy (31/31; 100%). Seizures were mostly drug-resistant (25/28; 89%). Mean age of onset was 47 days (median: 3 days) ranging from in utero to 15 months. The epilepsy of patient 7, who was born prematurely, was considered to start at day 1 corrected age.

Brain MRI showed signs of cerebral atrophy in 55% of them (16/29), but also cerebellar atrophy (8/29; 28%), abnormalities of the corpus callosum (7/29; 24%) and delayed myelination (6/29; 21%) (Figure 1A). One patient had a pattern of brain injury typical of neonatal hypoxic-ischemic injury (patient 29). Brain MRI was normal in 7 patients (7/29; 24%).

On clinical examination, a majority of patients had microcephaly (20/23; 87%) and peripheral hypertonia or spasticity (27/30; 90%). Axial hypotonia was present in 50% of them (15/30). Half of the patients had dysmorphic features (14/26; 54%), but none of them seemed specific. Photographs of the patients are shown in Figure 2A.

Other associated findings were cardiac anomalies in two patients and unilateral clubfoot in three patients.

"Cerebellar ataxia" group (Table 1)

Twenty-six patients from 20 families were included in this group, decomposed of 11 boys and 15 girls. Consanguinity was noted in half of the cases (10/20; 50%). Median age at last clinical examination was 7 years and 7 months (22 months - 28 years).

There were no prenatal signs, except in one patient who had oligohydramnios (1/21; 5%). Half of the newborns had neonatal hypotonia (12/24; 50%) and only 1 had neonatal microcephaly (1/24; 4%).

All patients showed developmental delay and intellectual disability ranging from mild to severe. Ninety-six percent of patients acquired sitting (25/26) and 81% acquired walking (21/26). Among them, 38% were only able to walk a few steps (8/21) and 52% required support (11/21). Ataxia was present in 81% of cases (16/21). Language skills were present in 17 patients (17/26; 65%). Twelve of them were able to say only a few words or short sentences. Two siblings acquired almost normal language late in life (Patients 32 and 33). Eight patients had dysarthria (8/10; 80%).

Epilepsy was present in 2 patients (2/26; 8%), with an age of onset at 3 and 5 years. Pharmacoresistance is noted for one patient.

Brain MRI showed cerebellar atrophy in all patients (26/26; 100%) and abnormalities of the corpus callosum in 5 (5/26; 19%) (Figure 1B).

Facial dysmorphism was reported in 7 of 20 individuals (35%) (Figure 1B). Mean height was –0,66 SD (standard deviation) with 6 patients under -2 SD (6/21; 29%). Microcephaly (OFC <

-2SD) was observed in 6 patients (6/22; 27%); hypotonia was noted in 17 of 20 patients (85%) and hypertonia of the limbs in 6 patients (6/26; 23%).

Of the 22 patients for whom data are available, fundus oculi revealed optic atrophy in 3 cases (14%) and signs of retinopathy in 6 (27%). Nystagmus was reported in 15 patients (15/19; 79%) and strabismus in 6 (6/13; 46%).

None of the patients in this group had died at the time of publication.

Molecular findings

BRAT1 variants were compound heterozygous in 22 families (22/45; 49%) and homozygous in the others (23/45; 51%).

We identified 38 variants in our cohort, including 16 missense, 9 frameshift, 6 non-sense, 4 in-frame deletions, 1 in-frame duplication and 2 intronic variations (Figure 3). Twenty-eight of these variants were novel.

Twelve variations were recurrent in our cohort: p.(Val214Glyfs*189) was found in 10 families, p.(Leu99Thrfs*92) in five families, p.(Arg120His) and p.(Gln132_Ala141dup) in four families, p.(Pro309_Gln310del) and p.(Glu522Lys) in three families, p.(Gln132_Ala141del), p.(Ala164Val), p.(Arg268His), p.(Cys401*), p.(Gln438Argfs*51) and p.(Phe709Thrfs*17) in two families. Six of these recurrent variants have already been reported in the literature.

New missense variations identified in this study were classified as probably or possibly damaging by the PolyPhen-2 and SIFT prediction tools, except for the p.(Arg120Cys) and p.(Ala136Thr) variations, for which predictions are mixed (Supp data S3). The missense variation p.(Arg268His) have already been reported once (16) and have a predicted effect on splicing, according to SPiP and the literature (Supp data 3). It was therefore considered as a splice variant in our study. Five other missense variations may also have an effect on splicing but with a lower risk of occurrence and were considered as missense (Supp data 3). None of these variants is present in a homozygous state in GnomAD.

DISCUSSION

BRAT1, located at 7p22.3, encodes a nuclear protein that interacts with the tumor suppressor protein BRCA1 (breast cancer 1) and ATM (*ataxia telangiectasia mutated*). It has a role in DNA repair, particularly through ATM, a protein necessary for the establishment of an early response to double-stranded DNA degrading agents, such as ionizing radiation. ATM deficient cells are thus extremely sensitive to these radiations. It has been shown that ionizing radiation induces an immediate phosphorylation of the Ser1981 residue of ATM, allowing the activation of this protein (29). BRAT1, which binds to ATM at double-strand breaks, is required for its activation (29). Further functional analyses showed that BRAT1 also interacts with the catalytic subunit of the DNA-dependent protein kinase DNA-PK (DNA-PKcs), the major player in DNA double-strand break repair (30). BRAT1 also plays a role in cell cycle and growth, through the protein SMC1, but also through its contribution to the stability and regulation of mTOR (30,31). Finally, BRAT1 is involved in apoptosis through the production of reactive oxygen species (32).

In the literature, we retrieved the data from 40 patients including 28 patients from 20 families with an epileptic encephalopathy phenotype and 12 patients from 9 families with a cerebellar ataxia phenotype. By combining these data and the results of our series, we were able to study a very large cohort of 97 patients from 74 families with biallelic variations in *BRAT1*, which is quite significant for such a rare condition. The study of large cohorts in rare diseases is always very valuable to better understand the natural history of the disease, to consider possible therapeutic perspectives and to adapt the genetic counselling of families.

When we compared our data with those of the literature, they proved to be comparable (Table 1 and Supp data S4). In total, 59 patients from 45 families harbored an epileptic encephalopathy phenotype (59/97; 61%) and 38 patients from 29 families had a milder phenotype of cerebellar ataxia (38/97; 39%).

Epileptic encephalopathy (EE) group

Patients of the EE group have a very severe phenotype, with antenatal signs in 50% of cases (22/44) such as abnormal movements (14/44; 32%), IUGR (9/44; 20%), hydramnios (2/44; 5%) or oligohydramnios (2/44; 5%). They all had epilepsy, with a very early onset (in utero-15 months; median: 1 day). Microcephaly was present in 91% of cases (42/46), spasticity/peripheral hypertonia in 93% (53/57) and axial hypotonia in 52% (29/56). Brain MRI

revealed cerebral atrophy in 57% of cases (31/54) and cerebellar atrophy in 26% (14/54). The evolution was marked by the absence of psychomotor acquisitions (57/58; 98%) for all patients but one who acquired head control at 1 year old and briefly sat. Death occurred before the age of 18 months in the large majority of cases (47/56; 84%). Only 5 patients in this group are still alive today; two of them are less than 1 month old.

Cerebellar ataxia (CA) group

In contrast, the patients in this group showed few prenatal signs (only 1 patient with oligohydramnios). All of them had intellectual disability except one described as borderline. Most of them were able to sit (97% of cases; 37/38) and walk (76%; 29/38). Walking was possible with support in 52% of cases (15/29) and was ataxic in 80% of cases (20/25). Twenty-six patients (26/38; 68%) had language skills. Brain MRI showed cerebellar atrophy for all of them (37/37; 100%). None of these patients died.

Genotype-phenotype correlation

Among the 38 patients with a phenotype of cerebellar ataxia, 31 present at least one missense variation (81%). Seven other patients presented with the frameshift variation p.(Val214Glyfs*189) or p.(Leu99Thrfs*92) in trans of a splice variation (7/38; 18%). No patients in this group presented with two amorphic variations (Table 2).

Among the 59 patients with an epileptic encephalopathy phenotype, 36 harbor two amorphic variations (61%), 6 the recurrent variations p.(Gln132_Ala141del) or p.(Gln132_Ala141dup) (10%) and 6 others a splice variation in trans of an amorphic one (10%). Seven patients present one amorphic and one hypomorphic variation (12%) and only 3 had two hypomorphic variations (5%) (Table 2).

These observations allowed us to draw genotype-phenotype correlations for *BRAT1-related disorders* (Table 3) Indeed, patients carrying 2 frameshift or nonsense variations or the homozygous recurrent variations p.(Gln132_Ala141del)/p.(Gln132_Ala141dup) all have a severe phenotype of epileptic encephalopathy (43/43;100%). On the opposite, hypomorphic genotypes with two missense variants or other inframe deletion were more likely associated with a cerebellar ataxia presentation (31/41; 76%). Among the 10 patients with a hypomorphic genotype and a severe condition, it is interesting to note that one patient who died prematurely presented with neonatal lactic acidosis and cerebral lesions suggestive of hypoxia on brain MRI (Patient 12). The severity of his clinical presentation could thus be related to a perinatal

complication or a dual genetic diagnosis that has not been proven so far. The phenotype of the patients carrying splice variations appear variable: 54% presented with epileptic encephalopathy (7/13) and 46% with cerebellar ataxia (6/13). Splice variations are generally considered as amorphic, but a milder phenotype could be explained by a residual effect of an alternative transcript.

Functional studies would be valuable to confirm the amorphic or hypomorphic effect of *BRAT1* variations in particular for splice variations. It would also be interesting to study the functional consequence of the recurrent variations p.(Gln132_Ala141del) and p.(Gln132_Ala141dup). We know indeed that the NDFIP1 domain is necessary for BRAT1 translocation in the nucleus (33). The duplication and recurrent deletion of 10 amino acids in this domain could lead to a conformational defect that makes the nuclear action of BRAT1 impossible, explaining why these variations may act as amorphic ones.

An intrafamilial variability was suggested for *BRAT1* related disorders (14). However, our large series does not confirm these preliminary data, siblings always belonging to the same phenotypic group.

In conclusion, we report here the largest cohort of patients with biallelic *BRAT1* pathogenic variations and describe a series of 97 cases. The characterization of the phenotype in large series is very useful to better know the clinical presentation of these rare diseases, to better help the patients and their relatives and to give an adapted genetic counselling to the whole extended family. Taken together, our results suggest that *BRAT1* biallelic variants are associated with two distinct clinical presentations such as RMFSL or NEDCAS. The most severe clinical presentation of this disorder, mainly seen in patients with two amorphic variations, is associated with severe encephalopathy, drug-resistant epilepsy, cerebral atrophy and early death. On the opposite, a phenotype of variable ID, cerebellar atrophy, ataxia, nystagmus and higher life expectancy is observed in patients with at least one missense variant. *BRAT1*-related neurodevelopmental disorders should therefore be considered from birth as a differential diagnosis of epileptic encephalopathy with rigidity due to *ATAD1*(34), *GRIA4*(35), *NALCN*(36) or *MAGEL2*(37) variations, but also to conditions associating developmental delay and ataxia linked to *WWOX*(38,39), *PNKP*(40) or *SCA21*(41) variations.

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FIGURES AND TABLES

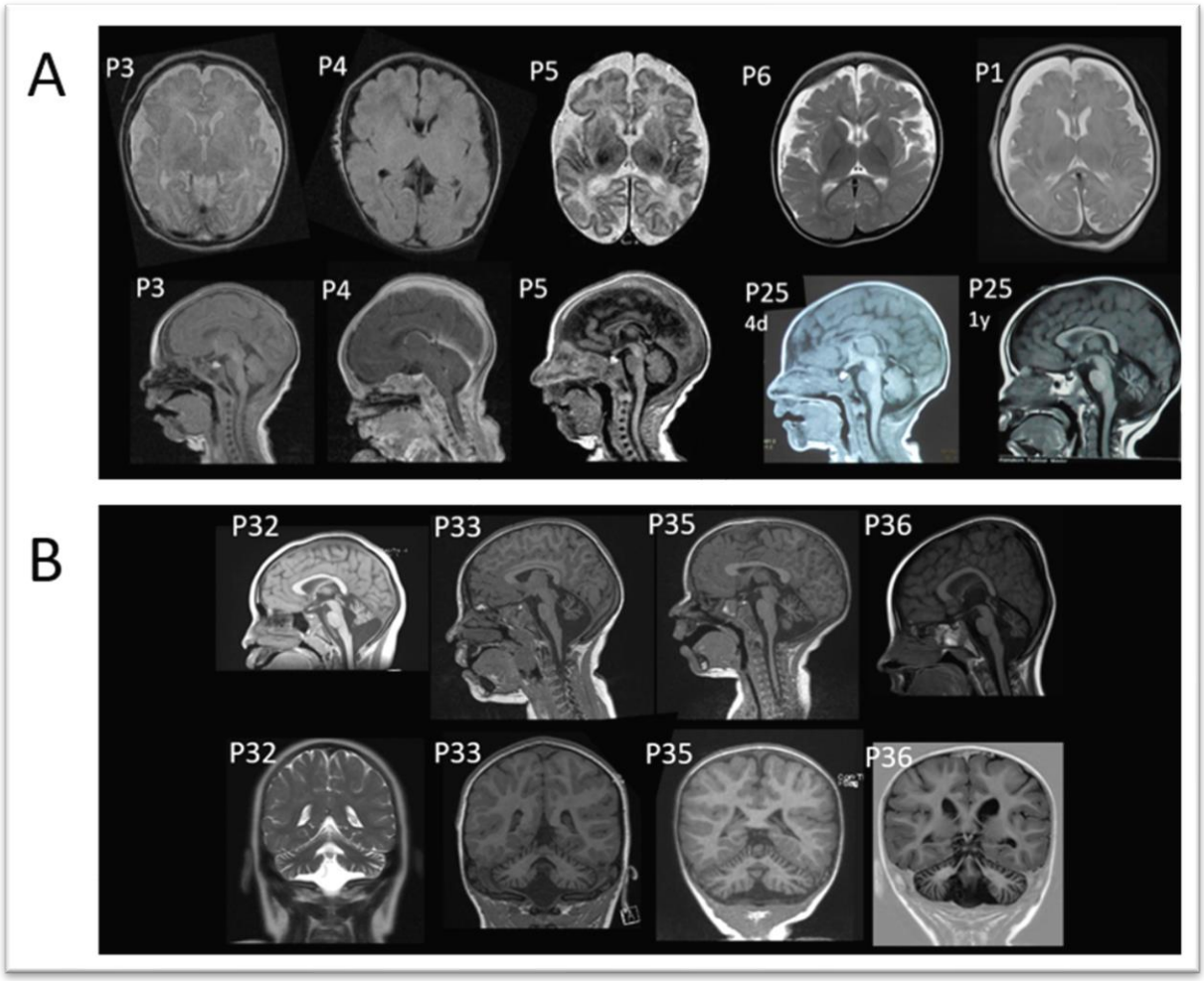


Figure 1: Brain magnetic resonance image (MRI) of individuals with biallelic *BRAT1* variations. A. Cerebral atrophy and enlarged subarachnoid spaces on axial and coronal planes from patients with epileptic encephalopathy (P1, P3, P4, P5, P25). **B**. Cerebellar atrophy on sagittal and coronal planes from patients with cerebellar ataxia (P32, P33, P35, P36)

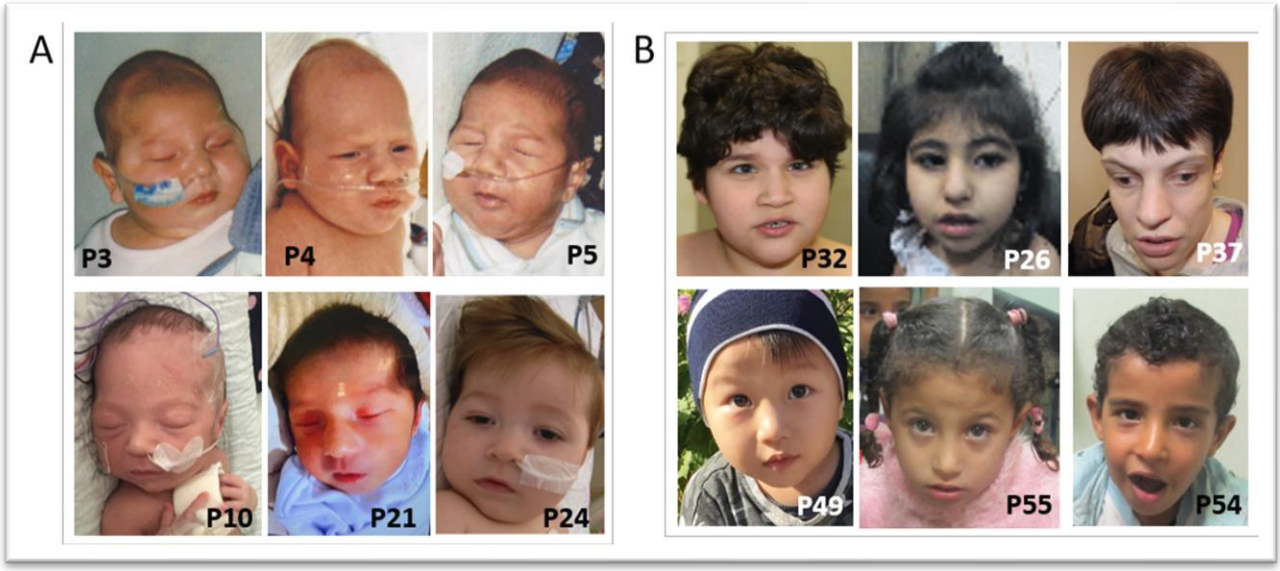


Figure 2: Photographs of individuals with *BRAT1*-related disorders. A. Patients from encephalopathy epileptic group. Coarse facies are noted without specific dysmorphic features. B. Patients from cerebellar ataxia group. Phenotype is not recognizable but triangular face, high nasal root and strabismus are noted in some patients.

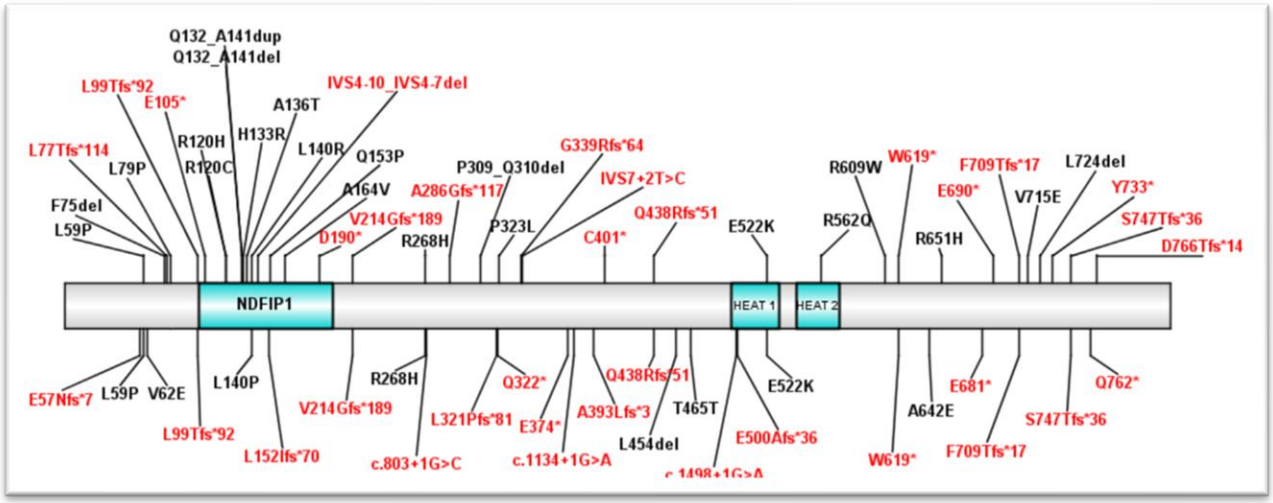


Figure 3 : Graphical representation of *BRAT1* variants. The variants above are those of our cohort, the variants below are those already reported in the literature. Amorphic variants are in red, hypomorphic variants in black.

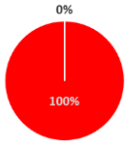
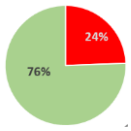
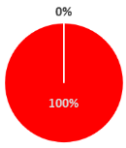
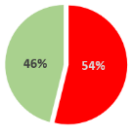
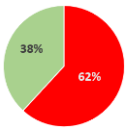
Table 1: Clinical data of the 97 patients from our cohort and the literature. IUGR intra uterine growth retardation; D, day; M, month; Y, year

		Patients with epileptic encephalopathy						Patients with cerebellar ataxia						Total					
		Cohort		Literature		Total		Cohort		Literature		Total		Cohort		Literature		Total	
Epidemiological data	Number of families	25/45	56%	20/29	69%	45/74	61%	10/45	44%	9/29	31%	29/74	39%	45/74	61%	29/74	39%	74 families	
	Number of patients	31/57	54%	28/40	70%	59/97	61%	26/57	46%	12/40	30%	38/97	39%	57/97	61%	40/97	39%	97 patients	
Prenatal features	Prenatal features	15/30	50%	7/14	50%	22/44	50%	1/21	5%	0/9	0%	1/30	3%	16/51	31%	7/23	30%	23/74	31%
	Hydramnios	2/30	7%	0/14	0%	2/44	5%	0/21	0%	0/9	0%	0/30	0%	2/51	4%	0/23	0%	2/74	3%
	Oligohydramnios	1/30	3%	1/14	7%	2/44	5%	1/21	5%	0/9	0%	1/30	3%	2/51	4%	1/23	4%	3/74	4%
	Abnormal movements	8/30	27%	6/14	43%	14/44	32%	0/21	0%	0/9	0%	0/30	0%	8/51	16%	6/23	26%	14/74	19%
	IUGR	9/30	30%	0/14	0%	9/44	20%	0/21	0%	0/9	0%	0/30	0%	9/51	18%	0/23	0%	9/74	12%
Developmental stages	No developmental	30/30	100%	27/28	96%	57/58	98%	1/26	4%	0/12	0%	1/38	3%	31/56	55%	27/40	68%	58/96	60%
	Walk acquisition	-		-		0/57	0%	21/26	81%	8/12	67%	29/38	76%	21/56	38%	8/39	21%	29/95	31%
	Only few steps							8/21	38%	0/8	0%	8/29	28%	8/21	38%	0/8	0%	8/29	28%
	Only with support							11/21	52%	4/8	50%	15/29	52%	11/21	52%	4/8	50%	15/29	52%
	Ataxic gait							17/21	81%	3/4	75%	20/25	80%	17/21	81%	3/4	75%	20/25	80%
	Language	-		-		0/56	0%	17/26	65%	9/12	75%	26/38	68%	17/55	31%	9/39	23%	26/94	28%
	Use of few words							6/14	43%	5/6	83%	11/20	55%	6/14	43%	5/6	83%	11/20	55%
	Use of short sentences							6/14	43%	1/6	17%	7/20	35%	6/14	43%	1/6	17%	7/20	35%
	Dysarthria							8/10	80%	6/6	100%	14/16	88%	8/10	80%	6/6	100%	14/16	88%
	Intellectual disability	2/2	100%	1/1	100%	3/3	100%	26/26	100%	11/12	0%	37/38	97%	28/28	100%	12/13	92%	40/41	98%
Epilepsy	Seizures	31/31	100%	28/28	100%	59/59	100%	2/26	8%	5/12	42%	7/38	18%	33/57	58%	33/40	83%	66/97	68%
	Age of onset (median)	3 days (D0-M15)		1 day (D0-M8)		1 day (D0-M8)		4 years (Y3-Y5)		2years (M3-Y13)		3 years (M3-Y13)		10 days (D0-Y5)		1 day (D0-Y13)		3,5 days (D0-Y13)	
	Pharmacoresistance	25/28	89%	27/27	100%	52/55	95%	1/2	50%	3/5	60%	4/7	57%	26/30	87%	30/32	94%	56/62	90%
Physical examination	Age at last examination	80 days		5 months		90 days		7 years 7 months		6 years 1/2		7 years 3 months		3 years 1/2		1 year 1 month		1 year 8 months	
	Microcephaly	20/23	87%	22/23	96%	42/46	91%	6/22	27%	5/11	45%	11/33	33%	26/45	58%	27/34	79%	53/79	67%
	Dysmorphism	14/26	54%	17/22	77%	31/48	65%	7/20	35%	4/9	44%	11/29	38%	21/46	46%	21/31	68%	42/77	55%
	Hypotonia	15/30	50%	14/26	54%	29/56	52%	17/20	85%	6/9	67%	23/29	79%	32/50	64%	20/35	57%	52/85	61%
	Spasticity/ hypertonia	27/30	90%	26/27	96%	53/57	93%	6/26	23%	5/10	50%	11/36	31%	33/56	59%	31/37	84%	64/93	69%
Ophtalmological features	Optic atrophy	3/12	25%	3/4	75%	6/16	38%	3/22	14%	3/7	43%	6/29	21%	6/34	18%	6/11	55%	12/45	27%
	Retinopathy	1/12	8%	0/4	0%	1/16	6%	6/22	27%	1/7	14%	7/29	24%	7/34	21%	1/11	9%	8/45	18%
	Nystagmus	2/7	29%	0/6	0%	2/13	15%	15/19	79%	8/9	89%	23/28	82%	17/26	65%	8/15	53%	25/41	61%
Brain MRI	Normal	7/29	24%	15/25	60%	22/54	41%	0/26	0%	0/11	0%	0/37	0%	7/55	13%	15/36	42%	22/91	24%
	Cerebellar atrophy	8/29	28%	6/25	24%	14/54	26%	26/26	100%	11/11	100%	37/37	100%	34/55	62%	17/36	47%	51/91	56%
	Cerebral atrophy	16/29	55%	15/25	60%	31/54	57%	0/26	0%	0/11	0%	0/37	0%	16/55	29%	15/36	42%	31/91	34%
	Delayed myelinisation	6/29	21%	6/25	24%	12/54	22%	0/26	0%	3/11	27%	3/37	8%	6/55	11%	9/36	25%	15/91	16%
	Corpus callosum anomalies	7/29	24%	6/25	24%	13/54	24%	5/26	19%	1/11	9%	6/37	16%	12/55	22%	7/36	19%	19/91	21%
Survival	Death	27/31	87%	26/28	93%	53/59	90%	0/26	0%	0/12	0%	0/38	0%	27/57	47%	26/40	65%	53/97	55%
	Age of death (median)	105 days (D11-Y23)		150 days (D6-Y5M9)		113 days (D6-Y23)		-		-		-		105 days (D11-Y23)		150 days (D6-Y5M9)		113 days (D6-Y23)	

Table 3: Genotypes of the patients with *BRAT1* variations.

	Patients with epileptic encephalopathy						Patients with cerebellar ataxia						Total					
	Cohort		Literature		Total		Cohort		Literature		Total		Cohort		Literature		Total	
Homozygosity of the variation identified																		
Homozygous variations	16/31	52%	19/28	68%	35/59	59%	14/26	54%	2/12	17%	16/38	42%	30/57	53%	21/40	53%	51/97	53%
Heterozygous variations	15/31	48%	9/28	32%	24/59	41%	12/26	46%	10/12	83%	22/38	58%	27/57	47%	19/40	48%	46/97	47%
Type of the variation identified																		
2 amorphic variations (nonsense or frameshift)	16/31	52%	20/28	71%	36/59	61%	0/26	0%	0/12	0%	0/38	0%	16/57	28%	20/40	50%	36/97	37%
p.(Gln132_Ala141del)/p.(Gln132_Ala141dup) variation (homozygous or in trans with 1 amorphic variation)	6/31	19%	0/28	0%	6/59	10%	0/26	0%	0/12	0%	0/38	0%	6/57	11%	0/40	0%	6/97	6%
Splice variation (homozygous or in trans with 1 amorphic variation)	2/31	6%	4/28	14%	6/59	10%	1/26	4%	6/12	50%	7/38	18%	3/57	5%	10/40	25%	13/97	13%
1 amorphic + 1 hypomorphic variation	4/31	13%	3/28	11%	7/59	12%	9/26	35%	4/12	33%	13/38	34%	13/57	23%	7/40	18%	20/97	21%
2 hypomorphic variations (missense or inframe deletion)	3/31	10%	0/28	0%	3/59	5%	16/26	62%	2/12	17%	18/38	47%	19/57	33%	2/40	5%	21/97	22%

Table 4: Genotype-phenotype correlation. Phenotypes associated with different *BRAT1* genotypes: in red, phenotype of epileptic encephalopathy; in green, phenotype of cerebellar ataxia.

	2 nonsense or frameshift variations		At least 1 missense or in-frame deletion[#]		p.(Gln132_Ala141dup) / p.(Gln132_Ala141del)*		Splice variation*		Total	
Number of families	27/74	36%	32/74	43%	5/74	7%	10/74	14%	74 families	
Number of patients	37/94	39%	41/97	42%	6/97	6%	13/97	13%	97 patients	
Epileptic encephalopathy	37/37	100%	10/41	24%	6/6	100%	7/13	54%	60/97	62%
Cerebellar ataxia	0/37	0%	31/41	76%	0/6	0%	6/13	46%	37/97	38%
Graphical representation										

[#]except the recurrent p.(Gln132_Ala141dup) / p.(Gln132_Ala141del) variations ; *homozygous or in trans with an amorphic variation (nonsense or frameshift))

Supplementary data S1: Clinical, radiological and genetic data of patients in our cohort with pathogenic *BRAT1* variations

Supplementary data S2: Clinical, radiological and genetic data of patients with pathogenic *BRAT1* variations currently described in the literature

Supplementary data S3: *In silico* prediction scores of *BRAT1* variants found in our cohort

Supplementary data S4: Detailed clinical data of the 97 patients from our cohort and the literature