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BRAT1 in Brain Function

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Abstract

BRAT1 (BRCA1-associated ATM activator-1) gene is involved in cell proliferation and migration, apoptosis, DNA repair, mitochondrial homeostasis, and mTOR signaling. This gene has been recently related to lethal neonatal rigidity and multifocal seizure syndrome (MIM# 614498). This syndrome is characterized by a progressive encephalopathy with refractory epilepsy, hypertonia, slow head growth, and dysautonomia. Brain MRIs and postmortem examinations have shown a severe and progressive cerebral and cerebellar atrophy secondary to a marked neuron depletion and gliosis in the white matter. The loss of BRAT1 expression and function due to homozygous or compound heterozygous BRAT1 mutations justifies this brain atrophy and its consequences. Further extended knowledge about BRAT1 functions might lead to new therapeutic options for this syndrome and perhaps for cancer treatment too.

Keywords: BRAT1; Lethal neonatal rigidity; Oncogene; Progressive encephalopathy; Sequencing

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Introduction

BRAT1 (BRCA1-associated ATM activator-1) gene was initially cloned by Aglipay et al. in 2006, which they called BAAT1 [1]. This ubiquitously expressed gene encodes a protein that interacts with the BRCA1 (breast cancer 1) and ATM (ataxia telangiectasia mutated) proteins. It is involved in DNA damage response, mitochondrial function, cell proliferation, and is necessary for protein stability of PIKKs.

As other oncogenes, the BRAT1 gene has been recently related to neuronal growth. Hartz mapped the BRAT1 gene to chromosome 7p22.3 in 2012 [2], and in the same year, the involvement of this gene in brain development was confirmed by exome sequencing in patients with lethal and progressive epileptic encephalopathy [3]. This syndrome is characterized by a progressive encephalopathy with intractable seizures, rigidity, progressive microcephaly, dysautonomia, and early lethality.

Next, we will describe the known BRAT1 functions, its role in brain development, the result of BRAT1 losses in childhood, and the advances supported by NGS in the knowledge of oncogene functions and neurodevelopmental disorders.

BRAT1 Functions

BRAT1 was initially isolated as a BRCA1 binding protein, interacting with the BRCT domain of BRCA1, a well-known oncogene related to tumorigenesis and DNA damage response (DDR) [1]. This BRAT1/BRCA1 interaction is necessary for BRCA1´s functions. Other studies have shown that BRAT1 also interacts with ATM and DNA-PKs, implicated in DNA repair and DDR in general [1,4,5]. BRAT1 is required for ATM Ser phosphorylation; indeed, phosphorylation of ATM, cardinal for activation of is catalytic function induced by DNA damage, is decreased in BRAT1 knockdown cells. The ATM protein is a member of PIKKs involved in DNA repair, cell growth, and neural stem cell differentiation. Numerous different mutations in the ATM gene have been identified in patients with immunodeficiency, leukemia, lymphoma or ataxia-telangiectasia [6,7].

BRAT1 is also involved in cell growth and apoptosis [1]. Apoptotic activity is increased BRAT1 knockdown mouse embryogenic fibroblasts and human osteosarcoma cells. BRAT1 is required for cellular proliferation; the loss of BRAT1 expression significantly reduces cell proliferation and migration in BRAT1 knockdown cancer cell lines. Akt/Erk activity regulates a wide variety of cellular processes like cell proliferation, differentiation, survival and cell transformation of tumor cells. Akt/Erk´s phosphorylation status and function are decreased in BRAT1 knockdown cancer

cell lines, suggesting the important function of BRAT1 in these cell processes.

In addition, BRAT1 plays an important role in cellular metabolism, particularly in regulating mitochondrial functions [8]. Glucose consumption, high levels of mitochondrial reactive oxygen species, worse mitochondrial membrane potential, reduced pyruvate dehydrogenase activity, and diminished production of ATP from mitochondria in BRAT1 knockdown cancer cells suggest it. These roles of BRAT1 in cell growth and metabolism could explain the clinical features of patients with homozygous or compound heterozygous BRAT1 mutations.

BRAT1 is required for protein stability of PIKKs, such as ATM, DNA-PK, mTOR, and mTOR-related proteins [9]. BRAT1 binds to mTORC1 complex and is required for protein stability and regulation of mTOR signaling. The mammalian target of the rapamycin (mTOR) pathway plays central roles in synaptic protein synthesis, and its dysregulation is linked to cancer, epilepsy, psychiatric disorders, and neurodevelopmental disorders [10,11]. The mTOR pathway is a central regulator of cell growth, proliferation, survival, and cap-dependent protein translation. In the brain, it plays a cardinal function in dendritic spine development and synaptogenesis [12]. mTOR complex regulates neuronal protein synthesis and actin cytoskeleton. It is involved in different cellular processes like cellular metabolism, oxidative stress, autophagy, cell proliferation, differentiation, and migration. In the brain, the dysfunction of the mTOR pathway is supposed to be associated with atypical neuronal morphology, dysfunctional autophagy, defective connectivity, cell death, mitochondrial stress, and abnormal metabolism. mTOR complex is regulated upstream by several proteins (TSC1, TSC2, PTEN…) and controls those processes through its downstream effectors (Rho GTPases, 4E-BPs, S6K1 and 2…).

Brain and BRAT1

Numerous cases of intellectual disability, autism and/or dysmorphic features, with deletions or duplications including BRAT1 gene, have been described in international databases. However, all these cases have wider genetic losses or gains encompassing other genes. This circumstance and the presence of different CNVs in BRATl in normal population suggest the low haploinsufficiency of this gene. Consequently, the direct and indirect functions of BRATl on cell growth, neural cell differentiation and migration, dendritic morphology, synaptogenesis, and mitochondrial metabolism could explain the important progressive characteristic of clinical features in patients with homozygous or compound heterozygous BRAT1 mutations [3,13-18].

In the last years, homozygous or compound heterozygous BRAT1 mutations have been described as a new cause of severe progressive encephalopathy with neonatal onset and high patient fatality [3,13-18] (**Table 1**). The pathogenicity of BRAT1 homozygous mutations has been related to the lethal neonatal rigidity and multifocal seizure syndrome (MIM# 614498).

Puffenberger et al. [3] described two unrelated Amish sibships with prenatal microcephaly, mild hypoplasia of the frontal lobes, multifocal seizures, hypertonia, apnea, and bradycardia [3]; postmortem examination revealed a marked neuronal loss and gliosis in frontal, occipital and temporal cortex, a reduced anterior hippocampus with neuronal loss and gliosis in CA-1 zone, and scarcity of neurons and abundant Alzheimer Type 2 astrocytes in the putamen. In the same year, Saunders et al. described a new case from Mexico with similar clinical features, including microcephaly at birth [16]; two MRI scans within the first three weeks of life were normal. This lethal neonatal rigidity and multifocal seizure syndrome was later described in Japanese siblings, born of unrelated parents, with compound heterozygous mutations in BRAT1; these authors described a postnatal microcephaly, secondary to cerebral and cerebellar atrophy in both cases [15]. The postmortem autopsy of one of them revealed a remarkable loss of neurons in the cortex and cerebellum, and moderate gliosis in the frontal lobe. Two years later, Srivastava et al. communicate another case with a compound heterozygous BRAT1 mutations in a patient with an intellectual disability that was alive at the age of 8 years; no more clinical features were included [19]. Straussberg et al. [17] described two siblings born to consanguineous Arab-Muslim parents who have mutations the BRAT1 gene, with hypertonia, seizures, progressive mild microcephaly, hypertonia, and dysautonomia [17]; however, although these two cases showed a postnatal microcephaly, brain MRI was normal in both of them. In the last months, five new cases have been reported. Mundy et al. described a patient with seizures, hypertonia, apneic episodes, and arrested head growth who remains alive at 6 years of age; brain MRI showed decreased myelination and thin corpus callosum at 3 months, and right temporal lobe encephalomalacia and cerebellar and vermis hypoplasia at 3 years [14]. Van de Pol et al. described three siblings, born to consanguineous parents, the lethal neonatal rigidity and multifocal seizure syndrome [18]; brain MRI was normal at the age of 2 months and showed severe generalized atrophy ten months later in one case; two MRI scans at the age of 2 and 3 months revealed mild hypoplasia of cerebellum and brainstem, and severe brain atrophy respectively in another one; postmortem examination demonstrated a moderate neuronal loss and strong reactive gliosis in frontal cortex, reactive astrocytes, numerous Alzheimer type 2 astrocytes in underlying white matter, and an intense loss of pyramidal neurons and gliosis in the CA1 area of the hippocampus. We identified a new case, without early lethality and seizures, but with hypertonia, severe psychomotor retardation, and postnatal microcephaly (**Figure 1**); two timed/spaced out brain MRIs, which were performed at the ages of 19 and 48 months, showed moderate progressive cerebellar atrophy (**Figures 2 and 3**) [13].

Although other genes different to BRAT1 could contribute to the pathogenesis of the lethal neonatal rigidity and multifocal seizure syndrome, the presence of this syndrome in three pairs of siblings with confirmed homozygous BRAT1 mutations, and the rapid description of new cases with compound heterozygous or homozygous mutations in the last years support its causality. All patients suffered from seizures, hypertonia, dysautonomia and/or psychomotor retardation. Despite to the presence of severe

Table 1 Clinical features, brain MRI findings and BRAT1 mutations.

	Puffenberg et al. N=2	Saunders et al. N=1	Saitsu et al. N=2	Srivastava et al. N=1	Straussberg et al. N=2	Mundy et al. N=1	Van de Pol et al. N=3	Fernández-Jaén et al. N=1
Clinical Features								
Dysmorphic features	-	+	+		-	+	+	-
Microcephaly	+ (at birth)	+ (at birth)	+ (postnatal)		+ (postnatal)	+	+ (postnatal)	+ (postnatal)
Rigidity/ Hypertonia	+	+	+		+	+	+	+
Seizures	+	+	+		+	+	+	-
Dysautonomia (apnea, bradycardia)	+	+	-		+	+	+	-
Brain MRI								
Cerebral atrophy	+ (mild hypoplasia of frontal lobes)	-	+		-	+	+ (2 cases)	-
Cerebellar atrophy	-	-	+		-	+	+ (2 cases)	+
Died	Before age 4 months	Age 5 months	Ages 1 year and 9 months (1st case) and 3 months (2nd case)	Alive at 8 years	Before age 6 months	Alive at 6 years		Alive at 52 months
BRAT1 mutation	Homozygous c.638_639insA	Homozygous c.453_454insATCTTCTC	Compound heterozygous c.176T>C and c.962_963del	Compound heterozygous c.638_639insA and c.803+1G>C	Homozygous c.1173delG	Compound heterozygous c.294dupA and c.1925C>A	Homozygous c.638dup	Compound heterozygous c.1564G>A and c.638dup
Postmortem examination	Neuronal loss and gliosis		1 case: neuron depletion and gliosis in the white matter				1 case: neuron loss and atrophy of the white matter	

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clinical features, cerebral MRIs only demonstrated partial or total atrophy in 8 of 12 cases, as in other progressive encephalopathies; in contrast, postmortem examination revealed marked neuron depletion and gliosis in the white matter in the 3 cases in which the autopsy was performed.

As we previously described, BRAT1 has important roles in cell proliferation processes, including cellular growth, differentiation, and tumorigenicity, and required for mitochondrial functions [8]. The BRAT1 suppression secondary to homozygous or compound heterozygous BRAT1 mutations may deteriorate cell growth and migration, influence mitochondrial homeostasis [8], and induce neuronal atrophy [3,16,18]. The presence of mild to severe arrested head growth in all cases with the neonatal rigidity and multifocal seizure syndrome and the presence of cerebral and/or cerebellar atrophy in brain MRI studies in 9 of 11 patients suggest the BRAT1 impact in cellular proliferation. The neuronal loss and gliosis of the cortex and white matter, the sparing of basal ganglia and the cells depletion of the cerebellum observed in the three cases with necropsia, confirm this hypothesis.

These features and the normal results of routine laboratory screening and extensive neurometabolic tests in the previously reported cases insinuate a more relevant role of BRAT1 in cellular proliferation and apoptosis compared with mitochondrial function. The clinical features of the lethal neonatal rigidity and multifocal seizure syndrome are shared with mitochondrial diseases [20]. Central nervous system, skeletal muscle, and heart are some of the most reliant on mitochondrial energy production and are the most symptomatic to mitochondrial defects. Intractable seizures, hypertonia, progressive encephalopathy, and dysautonomia may be observed in children with mitochondrial diseases. Besides, energetic ictal and electrical epileptogenic activity during brain development are believed to participate in the progressive cognitive deterioration or regression [21]. This suggests that the role of BRAT1 in mitochondrial function may also be important in the pathogenesis of this encephalopathic syndrome [8].

NGS, Oncogenes and Brain

NGS, including large gene panels, whole-exome sequencing (WES) and whole-genome sequencing (WGS), is rapidly advancing the precision of medical practice in diagnosis, medical management, systemic investigation and prognosis [22]. Whole exome sequencing was recently included in clinical practice and provides coverage of more than 95% of exons which contain approximately 85% of disease-causing or disease-predisposing mutations. Although NGS cannot replace a careful clinical evaluation, it is clearly changing the diagnostic algorithms.

Targeted NGS panels have been shown to increase the diagnostic yield in epilepsy, a suspected inherited ataxia, hereditary neuropathies, and myopathies [23-26]. However, in some patients, the neurological phenotype may mislead the clinician to select the wrong multi-gene panel. WES provides a potential solution to these problems. In neurodevelopmental disorders, particularly in intellectual disability and pervasive developmental disorders, diagnostic rates with WES have varied in part due to differences in the types of patients from one study to another, with rates of up to 50% [27-29].

In addition, WES has enabled the identification of previously undiagnosed diseases, novel presentations of known diseases, and specifically the discovery of new syndromes. NGS in health

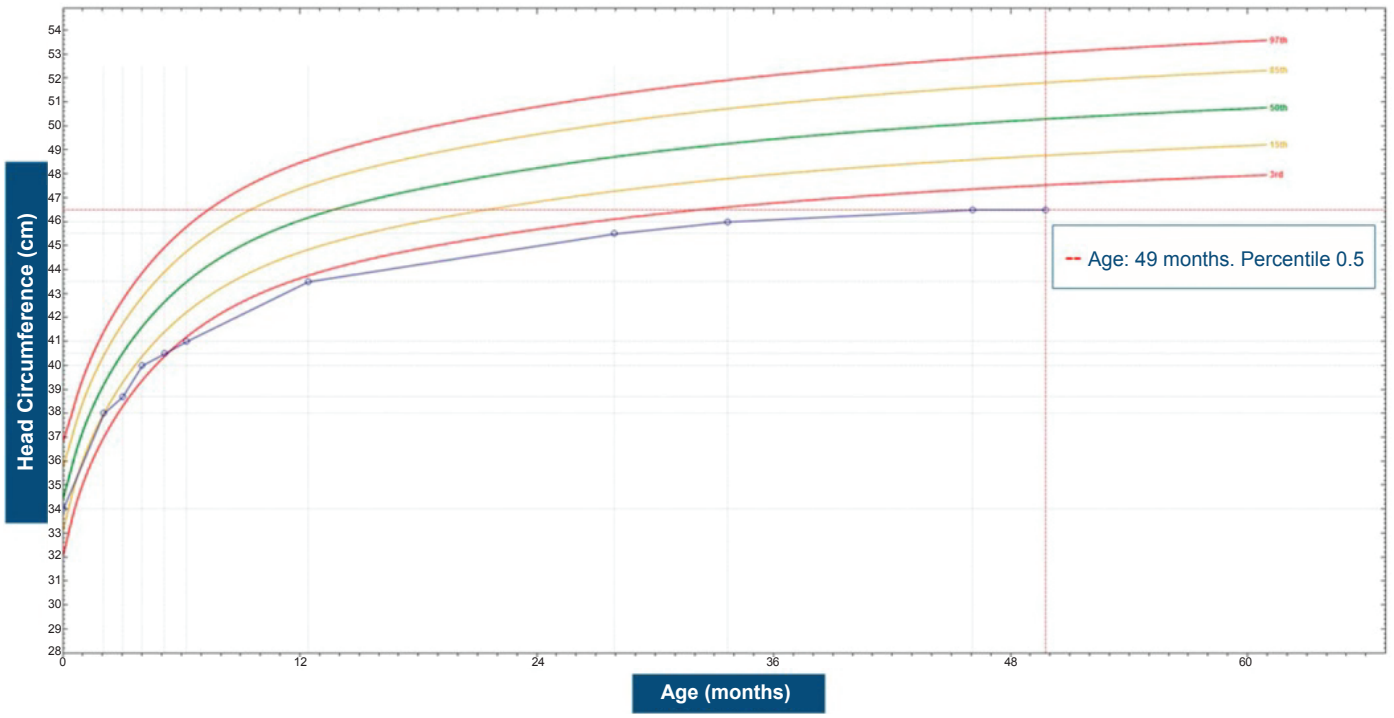

Figure 1 Plot showing the pattern of head growth (in centimeters) from birth to 49 months old (blue line). The other colored lines represent head circumference-for-age standards (from the 3rd to 97th percentile).

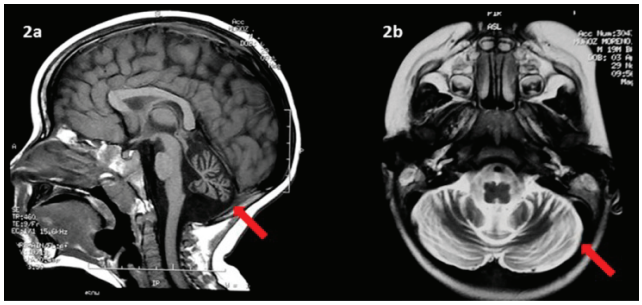

Figure 2 On the upper side, MRI study performed on a 19-month-old boy (2a-2b). **2a:** Midsagittal T1-weighted image exhibits a severe vermian diffuse atrophy (arrow) with fourth ventricle enlargement. The corpus callosum is normal, and the brainstem has a normal size. **2b:** Axial T2-weighted image shows enlarged interfolia spaces (arrow) and moderately reduced volume of both cerebellar hemispheres.

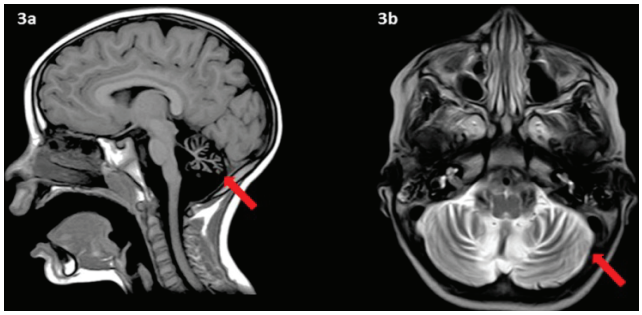

Figure 3 On the down side, MRI study performed at 48 months old (3a-3b). **3a:** Midsagittal T1-weighted image evidences a more severe vermian diffuse atrophy (arrow) with enlargement of the supravermian cistern and a wide fourth ventricle. The corpus callosum and the brainstem remain normal. **3b:** Axial T2-weighted image shows severe symmetric atrophy of the cerebellar hemispheres (arrow).

care has been directly involved in a rapid increase in the number of new monogenic diseases reported to OMIM. Indeed, in the last years, hundreds of new de novo and familial risk genes have been recently identified in neurodevelopmental disorders because of NGS. Dixon-Salazar et al. demonstrated the presence of twenty-two probably causative genes not previously associated with disease in the study of 118 probands with a diagnosis of a pediatric-onset neurodevelopmental disease in which most known causes had been excluded [30]. The diagnostic rate of WES in epileptic encephalopathies (EE) is also important. Veeramah et al. described the presence of de novo mutations in genes of known or plausible clinical causality in seven of ten children with refractory epilepsy and other neurological problems (autism, intellectual disability, EE) [31]; as in other neurological diseases, WES has allowed the knowledge of other causative genes in EE. Although the diagnostic yields of NGS in progressive neonatal-onset encephalopathies have not been established yet, some authors have suggested that NGS will replace the current biochemical method of newborn screening or recommended the use of WGS in neonatal intensive care units, for the diagnostic approach of complex cases [16].

NGS has also been successfully applied to evaluate susceptibility, diagnosis, treatment, and prognosis of cancer [32-34]. Targeted gene panels have been more commonly used in clinical practice. Indeed, different guidelines (American Society of Clinical Oncology, National Institute for Health and Care Excellence) have recommended the use of different panels or genetic studies according to the type of cancer and the presence of family history [35]. Although the diagnostic rates of these panels range from 20 to 50%, comparable to that of WES or WGS according to some authors, WES has allowed defining previously undescribed mutations in cancer as in neurological disorders [36-38].

The advances in the knowledge of the genetic architecture that underlies neurologic and oncologic problems have shown the extensive overlap between risk genes for autism and cancer [39,40]. Chromatin remodeling and DNA repair factors (CHD family, ARID1B…), proteins involved in histone methylation (EHMT1, EHTM2, KMT2C…) or ubiquitination (UBE3A, CUL3, TBL1XR1…) have been related to cancer, neurodevelopmental disorders and even known syndromes (ARID1B in Coffin-Siris syndrome, EHMT1 in Kleefstra syndrome, UBE3A in Angelman syndrome, TBL1XR1 in Pierpont syndrome…) [41-43]. Transcription factors (FOXP family, ADNP…) are also involved in both cancer, neuronal development, autism and intellectual disability (ADNP is related with Helsmoortel-Van Der AA syndrome) [44]. Other genes involved in signal transduction pathways regulating nuclear changes (PTEN, mTOR, RAS oncogene family, AKT…) are implicated in cancer, brain development, and autism; they are the cause of known syndromes too (PTEN in Bannayan-Riley-Ruvalcaba syndrome, HRAS in Costello syndrome, AKT1 in Proteus syndrome...) [45-47].

These genetic mutations associated with cellular proliferation could affect prenatal and postnatal brain development, resulting in progressive encephalopathies, intellectual disabilities, pervasive developmental disorders or brain malformations. These aberrations could contribute to a greater susceptibility to tumors during adult life [40]. However, although some studies have demonstrated a higher tumor risk in patients with autism, other studies have paradoxically shown a decreased cancer rate in these cases [48-50].

Conclusions

NGS studies are supporting the advances in knowledge of underlying causes of tumorigenesis, normal brain development, and neurodevelopmental disorders. It is particularly interesting how some oncogenes are simultaneously involved in those processes. Genetic mutations of oncogenes linked to cell growth could modify prenatal and postnatal cerebral development, causing autism, psychomotor retardation, progressive encephalopathies or cortical dysplasias.

BRAT1 is directly or indirectly involved in cell growth, apoptosis, DNA repair, mitochondrial metabolism, and regulation of

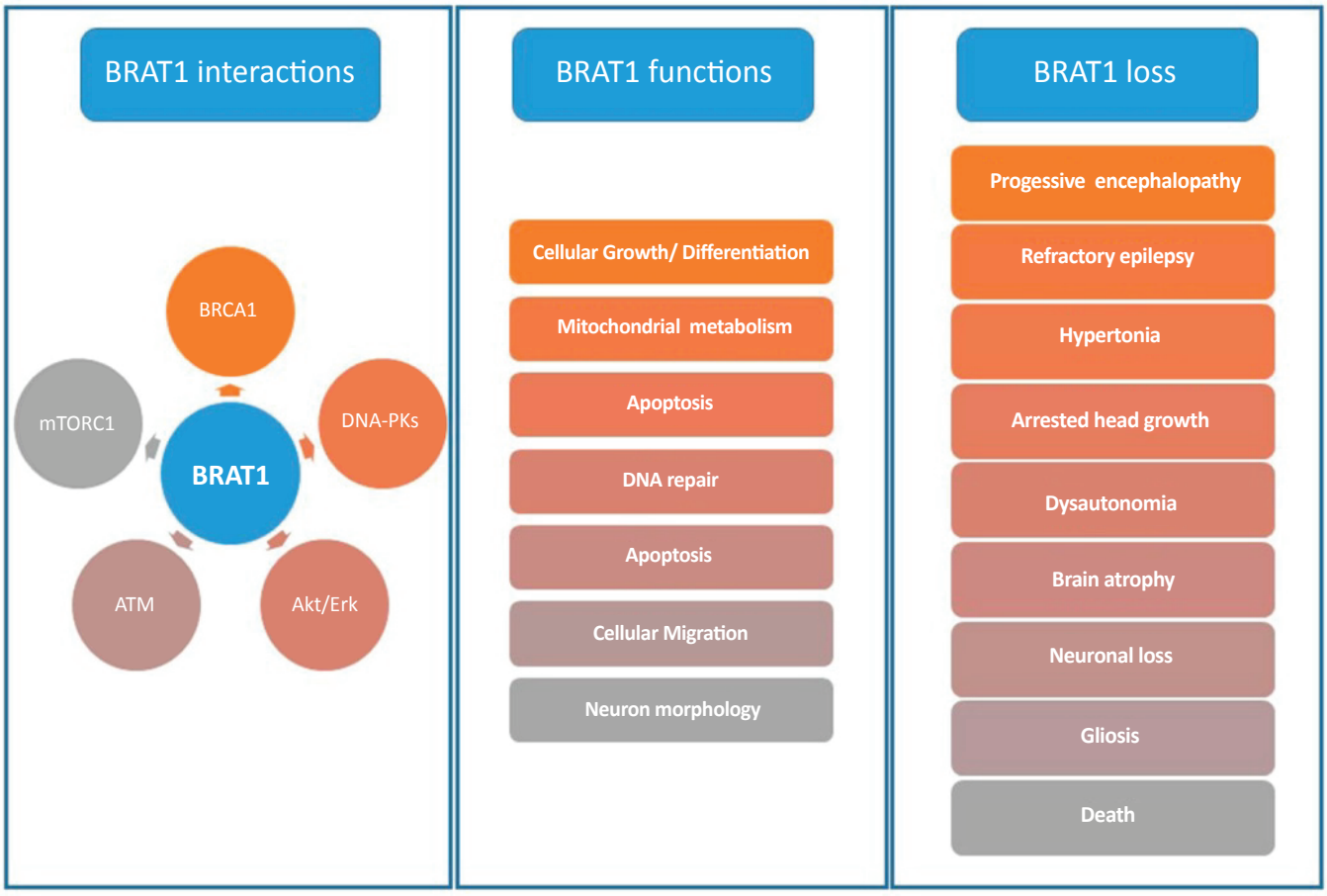

Figure 4 BRAT1 interactions, BRAT1 direct and indirect functions, and the consequences of the loss of these functions.

mTOR signaling (**Figure 4**). BRAT1 mutations are the cause of a severe progressive encephalopathy characterized by intractable epilepsy, hypertonia, arrested head growth, dysautonomia, and death. The loss of BRAT1 expression due to homozygous or compound heterozygous BRAT1 mutations leads to a deficient neuronal growth and migration, mitochondrial dysfunction, and cause neuronal atrophy. Defects in cell growth and differentiation might influence both brain development and neoplasm; some drugs targeting oncogenic pathways might also contribute to the treatment of these problems.

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