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Review article

Somatic mutation: The hidden genetics of brain malformations and focal epilepsies

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ABSTRACT

Over the past decade there has been a substantial increase in genetic studies of brain malformations, fueled by the availability of improved technologies to study surgical tissue to address the hypothesis that focal lesions arise from focal, post-zygotic genetic disruptions. Traditional genetic studies of patients with malformations utilized leukocyte-derived DNA to search for germline variants, which are inherited or arise *de novo* in parental gametes. Recent studies have demonstrated somatic variants that arise post-zygotically also underlie brain malformations, and that somatic mutation explains a larger proportion of focal malformations than previously thought. We now know from studies of non-diseased individuals that somatic variation occurs routinely during cell division, including during early brain development when the rapid proliferation of neuronal precursor cells provides the ideal environment for somatic mutation to occur and somatic variants to accumulate. When confined to brain, pathogenic variants contribute to the "hidden genetics" of neurological diseases. With burgeoning novel high-throughput genetic technologies, somatic genetic variations are increasingly being recognized. Here we discuss accumulating evidence for the presence of somatic variants in normal brain tissue, review our current understanding of somatic variants in brain malformations associated with lesional epilepsy, and provide strategies to identify the potential contribution of somatic mutation to non-lesional epilepsies. We also discuss technologies that may improve detection of somatic variants in the future in these and other neurological conditions.

1. Background: somatic mutation and mosaicism in the brain

Somatic mutation refers to an alteration in DNA that occurs at the post-zygotic stage. The processes leading to somatic mutation can happen any time a cell divides during development, resulting in variants present in only some but not all cells of the resulting organism. Somatic mutation thus leads to mosaicism, or a mixture of variant-positive and variant-negative cells, which may affect one or more tissue or cell type, depending on the timing and location of the mutational event. The majority of low-level mosaicism is difficult or even impossible to detect using conventional Sanger sequencing. Increasing evidence indicates that mosaicism has been under-recognized in the past (Acuna-Hidalgo et al., 2015; Campbell et al., 2014; Myers et al., 2018). In recent years, with the advent of more sensitive technologies, somatic variants have been more widely identified in association with several cancers and neurodevelopmental conditions.

Somatic mutation involving the brain can occur at any time in life. Traditionally, somatic mutation has been conceptualized as occurring in dividing cells. Only a small fraction of neurons (e.g., neurons in the dentate gyrus in the hippocampus) are still dividing post-natally and into adulthood (Eriksson et al., 1998; Nowakowski, 2006). The vast majority of neurons are produced prenatally and retained for the entire lifespan, and these neurons are terminally differentiated cells that no longer undergo mitosis. However, somatic variants may arise due to different mechanisms depending on whether mutation occurs during development in dividing cells vs. later in post-mitotic neurons, for example during aging.

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The effects of somatic mutation in early development have been recognized as most relevant to brain malformations and focal epilepsy (Iffland and Crino, 2017; Poduri et al., 2013; Thomas and Berkovic, 2014) The ability to detect somatic variants in the brain is largely dependent on the specific cell types affected and variant allele burden (proportion of the cells carrying the variant), which reflects the timing of mutagenesis (Fig. 1); further, as many variants are present only in brain tissue, detection requires access to brain tissue, most commonly from surgical tissues (e.g., tumor surgery or epilepsy surgery) or post-mortem tissue. In prenatal development, the zygote undergoes several cleavages to form a trilaminar structure called the gastrula, a process known as gastrulation. The gastrula comprises three germ layers: endoderm, mesoderm, and ectoderm (from which neuronal-glial progenitor cells derive). By the end of gastrulation, these three layers begin differentiation to establish distinct cell lineages, each tissue being derived from its own precursor cells in the process of organogenesis (Keller et al., 2003; Solnica-Krezel and Sepich, 2012). Somatic variants present in brain tissue that arise after fertilization and before gastrulation can be detected in a wide range of tissues, including blood (leukocytes), liver, lung, and pancreas (Bae et al., 2018; Lodato et al., 2015). Variants that arise after gastrulation but during early divisions of progenitor cells may be detected throughout the nervous system (e.g., prefrontal cortex, cerebellum, and spinal cord) (Lodato et al., 2015), while those occurring during late divisions of cortical progenitors may only be present in a restricted cortical area (Lodato et al., 2015; Leija-Salazar et al., 2018). Mature neurons can remain functionally active for decades, but the vast majority are post-mitotic. Somatic variants arise in the post-mitotic neurons in a replication-independent way (Lodato et al., 2015; Kosik, 2016). During aging, various factors (e.g., DNA damage and defects in DNA repair) could lead to the accumulation of somatic variants (Evans et al., 1995). These post-mitotic brain somatic variants are specific to single neurons, thus detection is confined to individual cells (Leija-Salazar et al., 2018).

2. Somatic variants present in normal brain tissue

Somatic mutation has been implicated as a common phenomenon in development and aging, and variants can accumulate in an individual at any stage from zygote formation until death (Forsberg et al., 2013; Blokzijl et al., 2016; Frank, 2010; Lodato et al., 2018; Martincorena and Campbell, 2015). Somatic variants arise spontaneously in normally developing brain tissue just as they may have in precursors of disease tissue, and vary by type and in size; examples include single nucleotide variants (SNVs), small insertions or deletions (indels), long interspersed nuclear element-1 (L1 or LINE-1) retrotranspositions, small (e.g., intragenic) to large copy number variants (CNVs), and even aneuploidy.

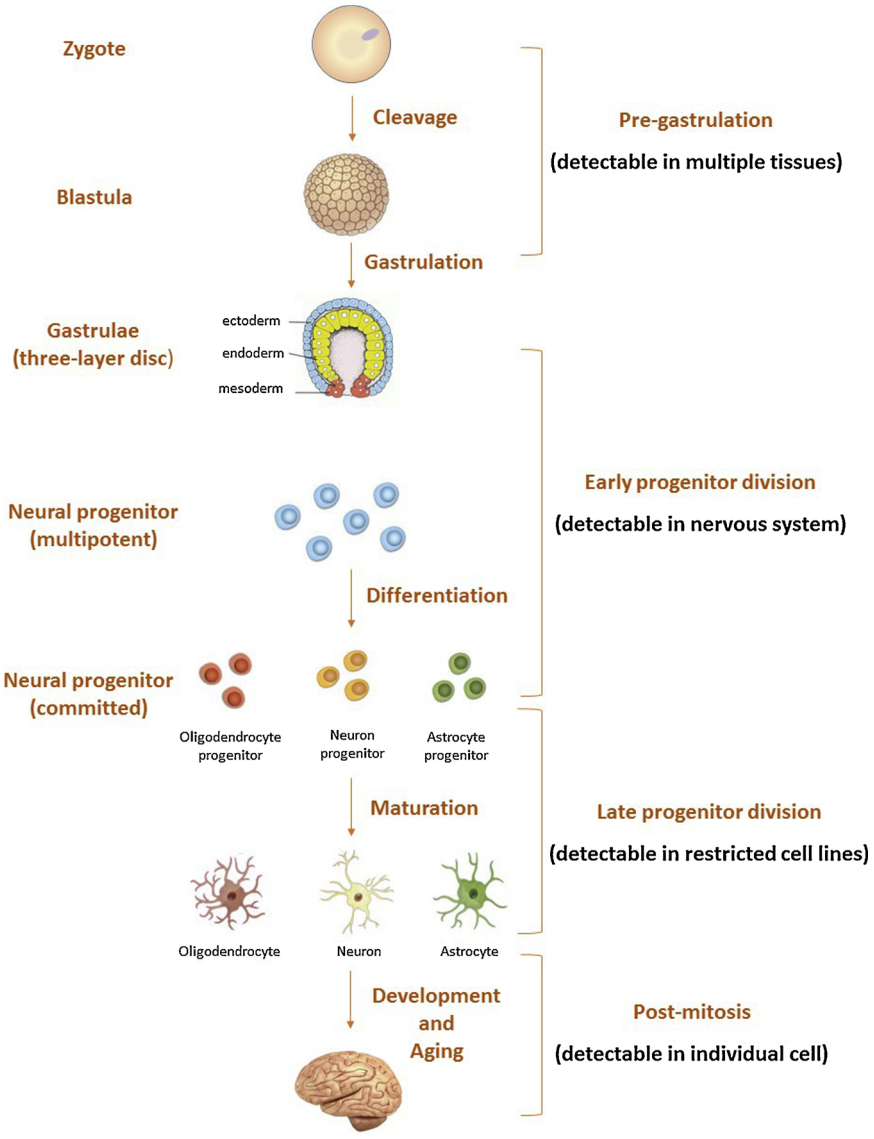

Fig. 1. The ablility to detect somatic variants in the brain depends on the timing of mutagenesis. Brain somatic variants that arise after fertilization and before gastrulation can be detected in a wide range of tissues; mutations that arise after gastrulation but during early divisions of progenitor cells may be detected throughout the nervous system; mutation occurring during late divisions of cortical progenitors may only be present in a restricted area; post-mitotic brain somatic variants are confined to individual cells. [Adapted from http://www.bio.miami.edu/dana/106/106F05_4.html., copyright by Pearson Education Inc; and from http://www.genetex.com/Web/News/NewsList.aspx?id=417., copyright 2013 by GeneTex Inc.].

Variants arising during development in progenitor cells reflect errors in DNA replication and structural rearrangements that occur during mitosis; given the very low percentage of dividing neurons post-natally, these mechanisms are most important for the generation of developmental disorders, such as structural brain malformations. In contrast, and somewhat surprisingly, there is evidence for a steady accumulation of variants in the brain during post-natal life, which is thought to reflect the effects of DNA damage and perhaps other mechanisms in post-mitotic neurons.

SNVs are the commonest somatic variants detected to date in normal brain tissue (Leija-Salazar et al., 2018; Abecasis et al., 2010; McConnell et al., 2017). Bae et al. reported 200–400 somatic SNVs per neuronal progenitor cell in the forebrains from phenotypically normal post-mortem human fetuses and inferred a rate of approximately 1.3 mutations per cell division (Bae et al., 2018). They also reported that 92% of somatic SNVs with a frequency > 2% in healthy brain were also present in normal spleens. Considering brain is of neuroectodermal origin and spleen is of mesodermal origin, this result indicates these shared SNVs likely occurred pre-gastrulation (Bae et al., 2018). Lodato et al. reported ˜1500 somatic SNVs in each neuron from the cerebral cortex of neurotypical adult individuals (Lodato et al., 2015), and SNVs found in more than 5%–10% of neurons were mostly detectable in other tissues including endodermal, mesodermal, and ectodermal derivatives (Lodato et al., 2015).

The human genome contains an estimated 500,000 copies of the L1 transposable element, accounting for ˜ 17% of the entire genome (Lander et al., 2001). Engineered human L1 can actively retrotranspose in neural progenitor cells derived from human embryonic stem cells *in vitro*, with the majority of events occurring at < 100 Kb from a gene and in some cases close to neuronally expressed genes (Coufal et al., 2009). In addition, increased L1 copies were observed in hippocampus when compared to other brain regions, heart, and liver from the same donor (Coufal et al., 2009). Engineered human L1 can also effectively retrotranspose in mature, non-dividing neuronal cells (Macia et al., 2017). The estimation of somatic L1 insertions is 0.04–13.7 per neuron (Evrony et al., 2012)., and L1 retrotransposition markers can be useful to trace cell lineage in the brain (Baillie et al., 2011; Evrony et al., 2015; Upton et al., 2015). Most recently, another study indicated that somatic L1-associated variants, including L1 retrotransposition-mediated and retrotransposition-independent L1 associated variants (which may cause deletion of the proximal genomic region), affect 44–63% of the cells in normal human brain (Erwin et al., 2016).

Large CNVs have also been detected in normal brain tissue. Single-cell sequencing was applied to analyze frontal cortex neurons from post-mortem individuals and human induced pluripotent stem cell (hiPSC) lines and revealed that 13–41% of neurons have at least one megabase-scale somatic CNV (McConnell et al., 2013). The presence of large CNVs was also detected with an average occurrence of 3.4 CNVs per neuron (Cai et al., 2014). Another study reported approximately 10% of brain cells harbor at least one megabase-scale CNV (Knouse et al., 2016). Furthermore, all three studies indicated somatic copy number losses were much more prevalent than gains (McConnell et al., 2013; Cai et al., 2014; Knouse et al., 2016).

Aneuploidy is the gain or loss of entire chromosomes as a result of chromosome mis-segregation during cell divisions (Hassold and Hunt, 2001). Most aneuploidies are incompatible with normal embryo development and survival, but they can exist as somatic aneuploidy in normal brains (albeit at an extremely low level) or in individuals with mosaic disease conditions (e.g., mosaic trisomy 13 or 18), and neurons with somatic aneuploidy can still be integrated into active brain circuitry (Bushman and Chun, 2013; Kingsbury et al., 2005; Westra et al., 2008). A mean aneusomy (aneuploidy rate/chromosome) of 0.6–3.0% in fetal brains and 0.1-0.8% in post-mortem adult brains has been reported (Yurov et al., 2005, 2007; Iourov et al., 2009). In addition to the molecular cytogenetic techniques used in these studies (e.g.fluorescence *in situ* hybridization), single-cell sequencing of neurons from frontal lobe grey matter also indicated a 2.2% aneusomy rate in normal brain (Knouse et al., 2014).

Post-mitotic somatic variants have been found to accumulate with age: Lodato et al demonstrated that post-mitotic neurons had 300–900 SNVs within the first year of birth which then accumulated slowly with a rate of ˜23 somatic SNVs per year in prefrontal cortex and ˜40 per year in hippocampus (Lodato et al., 2018). This was consistent with an independent study (Hoang et al., 2016), which showed the mutation prevalence was $1.1 \pm 0.3 \times 10^{-7}$ per base pair (bp) in the frontal cortex of young children (< 10 years old), $2.2 \pm 1.1 \times 10^{-7}$ per bp in young adults (20–40 years old) and $6.3 \pm 2.3 \times 10^{-7}$ per bp in middle-aged and older adults (> 40 years old). The accumulation of somatic variants has been implicated as a common phenomenon of aging in healthy brain (Lodato et al., 2018; Hoang et al., 2016). Oxidative stress, high metabolic rate, epigenetic remodeling and neuronal activity-induced DNA breaks may lead to cumulative DNA damage in the post-mitotic neurons (Bae et al., 2018; Lodato et al., 2018; Lister et al., 2013; Madabhushi et al., 2015; Suberbielle et al., 2013; Watts et al., 2018). During aging, somatic mutation caused by DNA damage accumulate within the neurons, and might further lead to a certain cascade of events that ultimately result in the occurrence of epilepsy at random times in patients.

It is unclear why somatic variants in normal brain are not associated with structural brain abnormalities but spatial and temporal factors are likely to be important. It is clear that the somatic variants causally linked to malformations of cortical development (i) occur early during development, (ii) are located in cells that migrate during brain development, and (iii) affect genes critical for neuronal migration. Somatic variants that arise early in development without meeting these criteria are much less likely to lead to a pathology. Similar somatic variants that accumulate later in life are unlikely to have the same impact. It is possible that post-mitotic cells better compensate for deleterious variation compare to their developmental precursors.

3. Somatic mutation in brain malformations associated with focal epilepsy

Pathogenic somatic variants have been implicated in various brain malformations associated with epilepsy (Table 1). Some of these pathogenic variants were detected in blood (leukocytes, derived from the mesoderm), suggesting that mutation occurred before the differentiation of progenitor cells into ectoderm, mesoderm, and endoderm. Analysis of brain tissue from patients undergoing neurosurgery for refractory epilepsy has allowed identification of presumed 'brain-only' variants, undetectable in DNA from peripheral blood. Such somatic variants arise from mutation later in development, such that only neuroectodermal cell lineages are affected.

Somatic variants in malformations of cortical development have frequently been found in genes affecting the mammalian target of rapamycin (mTOR) pathway. The mTOR protein kinase forms part of the complexes mTORC1 and mTORC2. These complexes appear to have important roles in neuronal differentiation, migration, and myelination (Curatolo et al., 2018; Lipton and Sahin, 2014). mTORC1 in particular has a role in protein synthesis and the cell cycle (Lipton and Sahin, 2014). Gain-of-function (GoF) mutations in *MTOR* and other genes encoding mTOR pathway activators, and loss-of-function (LoF) mutations in genes encoding mTOR pathway suppressors would both result in MTOR hyperactivity, leading to abnormal brain cell growth, neuronal connectivity, and neuronal excitability (Curatolo et al., 2018; Baybis et al., 2004; Hodges et al., 2001; Tavazoie et al., 2005). While the first several years of gene discovery in focal brain malformations has been dominated by 'mTORopathies,' the mTOR pathway represents only one pathway known to be involved in the etiologies of epileptogenic brain malformations, as discussed below.

Table 1
Selected examples of single nucleotide somatic variants detected in epileptogenic focal brain malformations.

Brain malformation	*Gene*	Cellular Pathway	Somatic Mutation [second-hit variant]	Protein effect	Brain AAF	Blood AAF	Patients	Reference
Tuberous Sclerosis	*TSC1*	mTOR	c.3096 G > T, [AND germline c.2023delG]	p.Glu959X [p.Asp675ThrfsX49]	NA	Not detected	1	(Crino et al., 2010)
	TSC2	mTOR	c. 337 G > A, [AND germline c.1839 + 1G > T]	p. Glu114Lys	NA	Not detected	1	(Crino et al., 2010)
			c. 1564C > T, [AND germline del exon1-15]	p. His522Tyr	NA	Not detected	1	(Crino et al., 2010)
			c. 3412C > T, [AND germline c.3498_ 3499 in. T]	p.Arg1138X [p. Glu1167X]	NA	Not detected	1	(Crino et al., 2010)
			c. 4079C > T, [AND germline c.4397C > A]	p.Pro1358Leu [p.Ser1466X]	NA	Not detected	1	(Crino et al., 2010)
			c.1864C > T, [AND germline c.4375 C > T]	p. Arg622Trp [p. Arg1459X]	0.7-7.2%	NA	1	(Qin et al., 2010)
HME	*MTOR*	mTOR	c.4448C > T	p.Cys1483Tyr	8.1-9.7%	Not detected	1	(Lee et al., 2012)
			c.5930C > A	p.Thr1977Lys	9.0-10.4%	Not detected	1	(D'Gama et al., 2017)
			c.6644C > T	p.Ser2215Phe	18.3-20.6%	Not detected	1	(D'Gama et al., 2017)
			c.6644C > A	p.Ser2215Tyr	7.1-8.3%	NA	1	(D'Gama et al., 2017)
	PIK3CA	mTOR	c.1624 G > A	p.Glu542Lys	15.9-31%	Not detected in (D'Gama et al., 2017), NA in (Jansen et al., 2015)	2	(D'Gama et al., 2017; Jansen et al., 2015)
			c.1633 G > A	p.Glu545Lys	16-36.6%	All Not detected	4	(Lee et al., 2012)
	AKT3	mTOR	c.49 G > A	p.Glu17Lys	3.4-40.4%	Not detected in (D'Gama et al., 2017; Alcantara et al., 2017; Lee et al., 2012; Poduri et al., 2012), NA in (Jansen et al., 2015)	5	(D'Gama et al., 2017; Jansen et al., 2015; Alcantara et al., 2017; Lee et al., 2012; Poduri et al., 2012)
	AKT1	mTOR	c.49 G > A	p.Glu17Lys	8.9-9.3%	NA	1	(D'Gama et al., 2017)
	TSC2	mTOR	c.4672 G > A [AND germline c.1892 T > C]	p.Glu1558Lys [p.Leu631Pro]	7.5-11.6%	NA	1	(D'Gama et al., 2017)
	DEPDC5	mTOR	c.4187delC	p.Ala1396ValfsX78 [with suggested LOH in brain]	28-35%	NA	1	(Mirzaa et al., 2016)
FCD IIa	*MTOR*	mTOR	c.1871 G > A	p.Arg624His	1.8-4.41%	NA	1	(Lim et al., 2015)
			c.4379 T > C	p.Leu1460Pro	6%	NA	1	(Mirzaa et al., 2016)
			c.5126 G > A	p.Arg1709His	1.52-1.63%	NA	1	(Lim et al., 2015)
			c.6577C > T	P.Arg2193Cys	1.26-2.99%	NA	1	(Lim et al., 2015)
			c.6644C > T	p.Ser2215Phe	0.93-8.6%	NA in 2 of (Mirzaa et al., 2016); 0.63% in (Moller et al., 2016)	3	(Mirzaa et al., 2016; Moller et al., 2016)
			c.6644C > A	p.Ser2215Tyr	1.06-3.5%	NA in (Mirzaa et al., 2016), 0.82% in (Moller et al., 2016)	2	(Mirzaa et al., 2016; Moller et al., 2016)
			c.7280 T > C	p.Leu2427Pro	4.11-12.63%	0-0.43% and 0-0.32%	2	(Lim et al., 2015)
	TSC1	mTOR	c.64C > T	p.Arg22Trp	2.52-2.81%	NA	2	(Lim et al., 2017)
			c. 610C > T	p. Arg204Cys	1-1.75%	NA	1	(Lim et al., 2017)
	TSC2	mTOR	c.4639 G > A	p.Val1547Ile	1.19-1.55%	NA	1	(Lim et al., 2017)
	PIK3CA	mTOR	c.3140A > G	p.His1047Arg	4.7%	NA	1	(Jansen et al., 2015)
	DEPDC5	mTOR	c.865C > T [AND germline c.856C > T]	p.Gln289X [p.Arg286X]	10%	Not detected	1	(Ribierre et al., 2018)
			c. 1264C > T [AND germline c.715C > T]	p. Arg422X (p. Arg239X)	NA	NA	1	(Baulac et al., 2015)

(*continued on next page*)

Table 1 (*continued*)

Brain malformation	*Gene*	Cellular Pathway	Somatic Mutation [second-hit variant]	Protein effect	Brain AAF	Blood AAF	Patients	Reference
FCD IIb	*MTOR*	mTOR	c.4348 T > G	p.Tyr1450Asp	2.64-3.76%	NA	1	(Lim et al., 2015)
			c.4376C > A	p.Ala1459Asp	1.34-1.65%	0.05-0.06%	1	(Nakashima et al., 2015)
			c.4379 T > C	p.Leu1460Pro	1.45-4.87%	NA in (D'Gama et al., 2017); Not detected in (Avansini et al., 2018); 1.3% in (Moller et al., 2016), 0.05-0.06% in (Nakashima et al., 2015)	4	(Avansini et al., 2018; D'Gama et al., 2017; Moller et al., 2016; Nakashima et al., 2015)
			c.4447 T > C	p.Cys1483Arg	6.38-10.6%	NA	2	(D'Gama et al., 2017; Lim et al., 2015)
			c.5930C > A	p.Thr1977Lys	1.46-3.25%	NA	2	(Lim et al., 2015)
			c.6644C > T	p.Ser2215Phe	1.88-6.80%	NA in 2 of (Lim et al., 2015); 1.45% and 3.16% in (Moller et al., 2016); 0-0.01% (Nakashima et al., 2015)	5	(Lim et al., 2015; Moller et al., 2016; Nakashima et al., 2015)
			c.6644C > A	p.Ser2215Tyr	1.11-3.67%	0.13% in (Moller et al., 2016); 0-0.03% in (Nakashima et al., 2015)	2	(Moller et al., 2016; Nakashima et al., 2015)
			c.7280 T > A	p.Leu2427Gln	2.86-5.11%	NA	1	(Lim et al., 2015)
	TSC1	mTOR	c.64C > T	p.Arg22Trp	1.37-2.21%	NA	1	(Lim et al., 2017)
			c. 163C > T	p.Q55*	5.1-6.7%	NA	1	(D'Gama et al., 2017)
	TSC2	mTOR	c.3781 G > A [AND somatic *AKT1* c.349_351del in the same patient]	p.Ala1261Thr [p.Glu117del]	1.7% [2.2%]	Not detected	1	(Avansini et al., 2018)
	PTEN	mTOR	c.834C > G	p.Phe278Leu	NA	NA	1	(Schick et al., 2006)
Subcortical Band Heterotopia	*LIS1*	Dynein	c.190A > T	p.Lys64X	~5%	13%	1	(Damiano et al., 2017; Jamuar et al., 2014)
			c.722 G > C	p.Arg241Pro	NA	18%	1	(Sicca et al., 2003)
	DCX	Dynein	c. 555C > T	p.Arg186Cys	NA	5%	1	(Jamuar et al., 2014)
			c.233 G > A	p.Arg78Leu	NA	9%	1	(Jamuar et al., 2014)
Hypothalamic Hamartoma	*GLI3*	Sonic Hedgehog	c.2071C > T	p.Gln691X	37%	0.8%	1	(Hildebrand et al., 2016)
			c.2776C > T	p.Gln926X	NA	Not detected	1	(Wallace et al., 2008)
			c.3172C > T	p.Arg1058X	18%-40.5%	Not detected in (Hildebrand et al., 2016); 0.05-0.4% in (Saitsu et al., 2016a)	2	(Hildebrand et al., 2016; Saitsu et al., 2016a)
			c.3442C > T	p.Gln1148X	24%	Not detected	1	(Hildebrand et al., 2016)
	OFD1	Sonic Hedgehog	c.1183 G > T	p.Glu395*	7.62-8.82%	0.04-0.06%	1	(Saitsu et al., 2016a)
Leptomeningeal Angioma	*GNAQ*	MAPK/ERK	c.548 G > A	p.Arg183Gln	0.42-18.1%	0.01-0.44% in 15 of (Nakashima et al., 2014); NA in 12 of (Shirley et al., 2013b); Not detected in 4 of (Hildebrand et al., 2018)	31	(Nakashima et al., 2014; Shirley et al., 2013b; Hildebrand et al., 2018)

AAF: alternate allele fraction, Ref: references, HME: hemimegalencephaly, FCD: focal cortical dysplasia, NA: not available, LOH: loss of heterozygosity. For HME and FCD II, all the reported SNVs were included; for other malformations, selected examples were included.

3.1. Tuberous sclerosis complex

Tuberous sclerosis complex (TSC) is a syndrome involving multi-organ hamartomas and tumors. Brain manifestations include cortical tubers, white matter migration abnormalities, subependymal nodules, and subependymal giant cell astrocytomas (Northrup and Krueger, 2013). Neurological and neuropsychiatric features of tuberous sclerosis include epilepsy, intellectual disability, and autism spectrum disorder (Curatolo et al., 2015). Seizures in tuberous sclerosis appear to originate within cortical tubers (Kannan et al., 2016). Histopathologically, cortical tubers show dysmorphic neurons, eosinophilic giant cells, reactive astrogliosis, and abnormal cortical organization.

TSC is associated with loss-of-function variants in *TSC1* or *TSC2* (Sampson and Harris, 1994; van Slegtenhorst et al., 1997), encoding negative regulators of the mTOR pathway (Gao et al., 2002). TSC provides an example of the "two-hit" genetic model for focal brain malformations, based on the two-hit model of cancer genetics (Knudson, 1971). In TSC, somatic mutation may be required in addition to germline loss-of-function mutation in the same gene (on opposite alleles) to cause a lesion. This model may also apply when a somatic variant is present in a different gene of the same cellular pathway as a germline variant. The two-hit mechanism in TSC was supported by recent studies in hiPSCs and hiPSC-derived neurons, which suggest biallelic inactivation of *TSC1/TSC2* was required to cause the formation of TSC tubers (Blair et al., 2018; Goswami and Hsieh, 2019; Bongaarts et al., 2017). However, second-hit mutations have not been consistently identified in TSC patients: in one study using single-cell sequencing, five out of six tuberous sclerosis patients with a known germline *TSC1* or *TSC2* variant had a second (somatic) variant in the same gene in isolated tuber giant cells (Crino et al., 2010). However, in a second study, a 'second-hit' somatic variant in tuber tissue was detected in only one patient out of 34 (Qin et al., 2010), and in a third study a second variant was found in 35% of tubers (Martin et al., 2017). One possible reason is that the second (Qin et al., 2010) and third (Martin et al., 2017) studies sequenced bulk DNA from entire tuber samples rather than from specific tuber cell types, which may not be sensitive enough to detect low-level second variants present in only small fractions of cells. Another possible reason is that the second-hit mutation might be an intronic mutation, large structure variations, or large CNVs that were somehow missed by the conventional pipelines.

3.2. Focal cortical dysplasia and hemimegalencephaly

3.2.1. Somatic mutation within the mTOR pathway in focal malformations

Focal cortical dysplasia (FCD) and hemimegalencephaly (HME) are related malformations of cortical development frequently associated with severe epilepsy. Type II focal cortical dysplasias (FCD II) are characterized by dyslaminated cortex with dysmorphic neurons, without (FCD IIa) or with (FCD IIb) large balloon cells (Blumcke et al., 2011). Histopathologically, FCD II lesions (and FCD IIb lesions in particular) have similar features to cortical tubers (Najm et al., 2018). Accordingly, somatic mosaic variants in *TSC1* and *TSC2* have been identified in FCD II brain tissue. Somatic variants in *MTOR* have also been implicated in FCD II lesions (Avansini et al., 2018; D'Gama et al., 2017; Leventer et al., 2015; Lim et al., 2015; Mirzaa et al., 2016; Moller et al., 2016; Nakashima et al., 2015). Further upstream in the mTOR pathway, FCD II has been linked to somatic variants in *PIK3CA* (encoding part of PI3K, a positive regulator of the mTOR pathway) (Jansen et al., 2015), *PTEN* (Schick et al., 2006) and *DEPDC5* (Baulac et al., 2015; Ribierre et al., 2018) (encoding negative regulators of the mTOR pathway).

HME involves similar molecular and histopathological abnormalities to FCD II, but across much or all of one whole brain hemisphere (Najm et al., 2018; D'Gama et al., 2017; Jansen et al., 2015). HME has been associated with somatic activating variants in mTOR pathway genes *AKT1/3*, *MTOR* and *PIK3CA* (D'Gama et al., 2017; Jansen et al., 2015; Alcantara et al., 2017; D'Gama et al., 2015; Lee et al., 2012; Poduri et al., 2012), and somatic repressing variants in *DEPDC5* (Mirzaa et al., 2016) and *TSC2* (D'Gama et al., 2017).

A large proportion of FCD II and HME cases still remain genetically unsolved. The majority of solved cases have somatic SNVs in the mTOR pathway; unsurprising given the screening bias for this pathway. We have summarized all the reported somatic SNVs in the mTOR pathway in FCD II and HME in Table 1 and Fig. 2. Surprisingly, many of these SNVs are recurrent (reported in more than 1 patient). It is not clear why certain somatic mutations are recurrent in sporadic cases; it is possible these sites are highly mutable and pathogenic. In FCD II, 29 patients had somatic SNVs in *MTOR*, accounting for 72.5% of total cases with detected somatic mutations (29 out of a total of 40 patients with reported SNVs). Among them, 24 have recurrent SNVs in *MTOR*, with the most recurrent SNVs located at amino acid 2215 (p.S2215 F, n = 8 and p.S2215Y, n = 4, total n = 12). Other recurrent *MTOR* variants include p.L1460 P (n = 5), p.L2427 P (n = 3), p.C1483R (n = 2), p.T1977 K (n = 2). A *TSC1* recurrent SNV p.R22W was identified in 3 patients with FCD II. In HME, only 22% of the reported cases (4 out of a total of 18 patients) have somatic SNVs in *MTOR*, while the majority (11 out of a total of 18 patients) have recurrent somatic SNVs in *PIK3CA* (p.E542 K, n = 2; p.E545 K, n = 4) or *AKT3* (p.E17 K, n = 5). Notably, 3 recurrent somatic *MTOR* SNVs reported in FCD II (p.S2215 F, p.S2215Y, p.T1977 K) were also detected in HME, and 1 *MTOR* SNV (p.C1483Y) in HME affects the same residue as another reported in FCD II (p.C1483R). Moreover, in general, the variant allele frequency (VAF) is higher in HME compared to FCD II, including that of the overlapped SNVs in *MTOR*. The higher VAF and hemispheric involvement in HME may represent somatic mutations occurring earlier in development than for FCD lesions (D'Gama et al., 2017).

There is emerging interest in the "two-hit" genetic model for FCD and HME. For mTOR pathway activator genes (e.g. *MTOR; PIK3CA; AKT3*) a somatic mutation appears sufficient to cause a brain malformation. In contrast, for mTOR pathway inhibitor genes (e.g. *DEPDC5*, *TSC1, TSC2*) germline mutation may be tolerated requiring a second-hit somatic mutation in brain tissue to cause malformation (Baulac et al., 2015; Ribierre et al., 2018), and reviewed in (Poduri et al., 2013; Anderson, 2018; D'Gama and Walsh, 2018)). The two-hit model is supported by observations in FCD and HME cases. Two recent studies found a second-hit somatic *DEPDC5* nonsense variant in FCD brain tissue with a germline *DEPDC5* nonsense variant (Baulac et al., 2015; Ribierre et al., 2018). Second-hit brain somatic variants in the *TSC2* gene have been found in addition to germline *TSC2* variants in two patients with HME without features of tuberous sclerosis (D'Gama et al., 2017). There are, however, numerous cases who have a germline mutation in *TSC1/2* or *DEPDC5* where a second-hit somatic mutation was not detected with very deep sequencing (D'Gama et al., 2017; Lim et al., 2015, 2017).

Genetic analysis of the same somatic mutation in multiple affected brain tissue samples from FCD II patients revealed a gradient distribution of VAF, with higher VAF observed in epicenter of the most epileptogenic area, and lower VAF in the surrounding epileptogenic border (Mirzaa et al., 2016; Ribierre et al., 2018). These findings have potential implications for predicting surgical outcome based on the extent of the resection.

3.2.2. Somatic mutation beyond the mTOR pathway in focal malformations

The majority of reported somatic mutations in FCD and HME are in mTOR pathway genes because most studies have intentionally targeted genes in this pathway. However, recent studies indicate somatic mutations in non-mTOR pathway genes can also contribute to the etiology of FCD and HME, suggesting that these genes should also be interrogated in targeted or genome-wide screens. One study described a patient with early infantile onset developmental and epileptic encephalopathy, developmental delay, autism spectrum disorder, and FCDI, with a known germline heterozygous *STXBP1* partial deletion

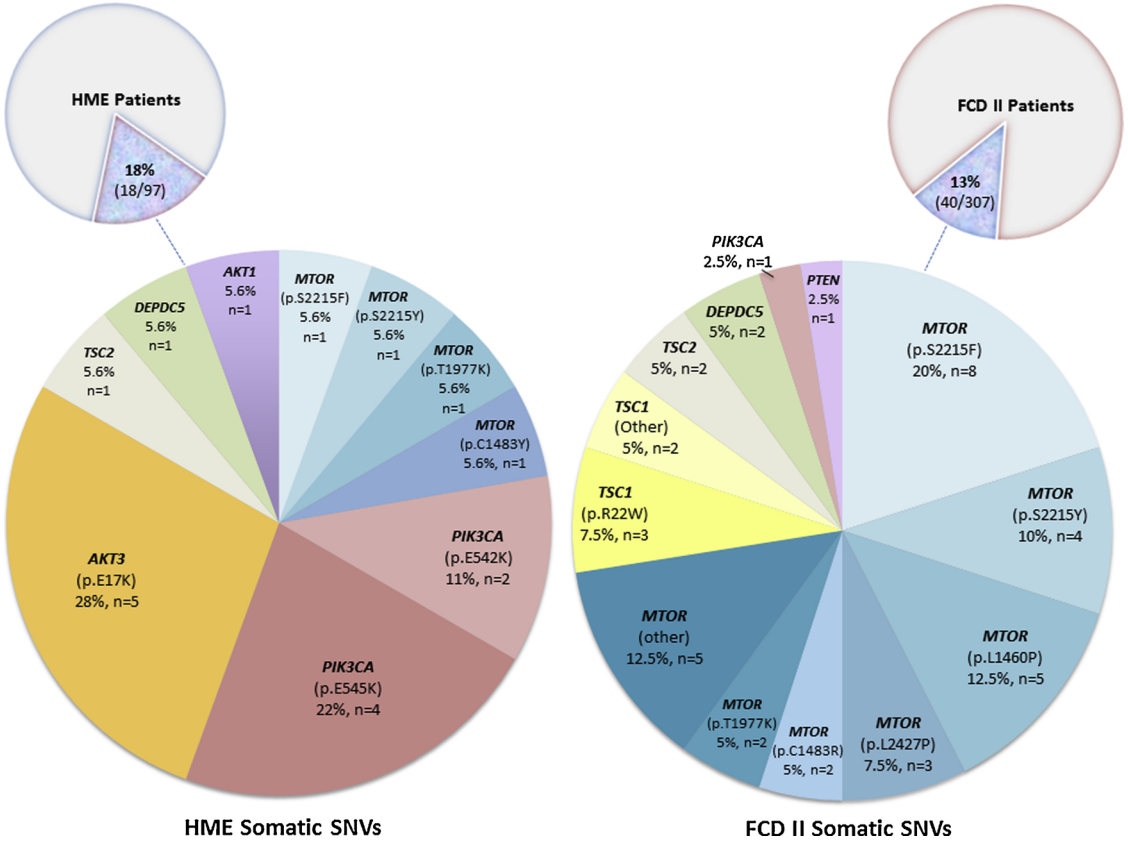

Fig. 2. mTOR pathway somatic single nucleotide variants (SNV) in hemimegalencephaly (HME) and Focal Cortical dysplasia Type II (FCD II). Small pie charts show the proportion of reported cases with somatic SNVs in the mTOR pathway. (18/97 HME patients and 40/307 FCD II patients whose brain samples were tested). Large pie charts show reported variants. Different variants in the same gene are shown in different shades of the same color (blue, *MTOR*; red, *PIK3CA*; yellow, *TSC1*). For recurrent mutations the amino acid change is indicated below the gene. Patients reported in multiple studies were only counted for once. Recurrent somatic SNVs in *PIK3CA* and *AKT3* account for about 60% of the HME cases with SNVs; while recurrent mutations in *MTOR* account for 60% of the FCD II cases with SNVs. All references for these variants are cited in Table 1.

(Uddin et al., 2017). The *STXBP1* gene encodes syntaxin-binding protein 1, a protein involved in neurotransmitter release. A second-hit somatic deletion involving *STXBP1* was found in lesional brain tissue but not in adjacent brain tissue or blood. This mechanism may well be responsible for other cases of apparently focal epilepsy in patients with germline variants in established epilepsy genes; while these patients do not often undergo surgery, for the few who do, study of their brain tissue for additional variants will be invaluable in addressing this hypothesis.

Another reported cause of FCD is mutation of the X-linked *SLC35A2* gene. This gene encodes a UDP galactose transporter in the Golgi apparatus and had previously been associated with a severe congenital disorder of glycosylation and developmental and epileptic encephalopathy, predominantly in girls (Kodera et al., 2013; Vals et al., 2019; Kimizu et al., 2017). A recent study examined paired brain tissue and leukocyte DNA from patients with non-lesional focal epilepsy or with FCD based on pre-operative MRI studies (Winawer et al., 2018). Three of 18 studied MRI-non-lesional cases had somatic variants in the *SLC35A2* gene, two of which had FCDI features on histology. In the MRI-lesional cases, two of 38 cases studied also had variants in *SLC35A2*. Another study investigated patients with non-lesional epilepsy and mild MCD without variants in mTOR pathway genes based on a previous assessment (Sim et al., 2018). Six of 31 cases had brain-only somatic variants in the *SLC35A2* gene, confirming the role of *SLC35A2*. Both studies (Winawer et al., 2018; Sim et al., 2018) support the presence of FCD pathogenesis independent of the mTOR pathway, which has important implications for possible targeted treatments for epilepsy in patients with focal epilepsy.

3.3. Lissencephaly and double cortex syndrome

Lissencephaly is a severe malformation of cortical development involving abnormal neuronal migration, thickened cerebral cortex and abnormal cortical folding (Barkovich et al., 2015). The disorder involves regions of pachygyria (wider, fewer cerebral gyri) and agyria (lack of gyri) (Barkovich et al., 2015). Double cortex syndrome, or subcortical band heterotopia (SBH), is a milder related disorder of neuronal migration involving a band of grey matter deep to the cortex, but without the same degree of gyri abnormalities (Barkovich et al., 2015). Pathogenic variants in *DCX* and *LIS1* are implicated in lissencephaly and SBH. *DCX* encodes a neuronal microtubule-binding protein that directs neuronal migration in neurodevelopment (reviewed in (Liu, 2011)), while *LIS1* encodes a protein necessary for neurogenesis, neuroepithelial stem cell generation and nuclear translocation during neuronal migration (reviewed in (Wynshaw-Boris, 2007)). Loss-of-function mutations in *DCX* and *LIS1* result in disruption of normal neuronal migration thus cause lissencephaly and SBH (Yap et al., 2016; Moore et al., 2012). *DCX* mutations typically involve anterior abnormalities while *LIS1* mutations involve posterior changes (Dobyns et al., 1999; Forman et al., 2005). Germline *DCX* and *LIS1* mutations lead to lissencephaly. Somatic mosaicism in *DCX* and *LIS1* (variant allele frequency ranging from 5 to 26%) was associated with milder SBH with these mosaic mutations detectable in tissues other than brain, including blood, saliva, and hair roots (Gonzalez-Moron et al., 2017; Damiano et al., 2017; Jamuar et al., 2014; Sicca et al., 2003).

3.4. Hypothalamic hamartoma

Mutations affecting a distinct cellular pathway have been implicated in somatic cells in patients with hypothalamic hamartomata resected for treatment of refractory gelastic epilepsy. Somatic variants involving *GLI3*, part of the sonic hedgehog pathway, were reported in hypothalamic hamartoma specimens a decade ago (Craig et al., 2008; Wallace et al., 2008). More recently, 14 somatic variants were reported in this pathway in hamartoma tissue; over a third of patients studied had variants in genes in this pathway, including multiple variants in the *PRKACA* and *GLI3* genes detected between 18–57% in hamartoma tissue through WES (Hildebrand et al., 2016). This has been replicated in a Japanese cohort where *GLI3* and *OFD1* somatic variants were detected between 7–55% through deep-sequencing of resected tissue in two hypothalamic hamartoma (HH) patients (Saitsu et al., 2016a). The sonic hedgehog pathway is critical throughout development for tissue patterning in the brain (Ruiz i Altaba et al., 2002). Multiple genes identified in sporadic HH are associated with the regulation of the sonic-hedgehog signalling pathway indicating that its disruption underlies disease pathogenesis (Saitsu et al., 2016b).

3.5. Vascular lesions

Sturge-Weber syndrome (SWS) is a neurocutaneous syndrome that involves leptomeningeal angiomatosis, port-wine stain, and vascular glaucoma. A recurrent somatic p.R183Q variant in the *GNAQ* gene has been found at variable levels in the affected regions of brain tissue of over 80% of SWS patients (Shirley et al., 2013a; Nakashima et al., 2014; Shirley et al., 2013b). The same brain somatic variant has four patients with leptomeningeal angiomatsosis without port-wine stain or ocular features (*forme fruste* SWS) (Hildebrand et al., 2018).

3.6. Non-lesional focal epilepsy

While focal somatic mutation has been hypothesized to play a role in non-lesional focal epilepsy for many years (Poduri et al., 2013; Lindhout, 2008), evidence in the form of somatic variants in cases of non-focal epilepsy has been reported only recently. As mentioned above, there are two recent reports of somatic *SLC35A2* variants in MRI-non-lesional cases; interestingly, features of FCD were found on pathological examination (Winawer et al., 2018; Sim et al., 2018), as has been previously reported in other series of non-lesional focal epilepsy (Porter et al., 2003).

Limitations related to the distinction between lesional and non-lesional focal epilepsy on MRI should be noted, as the distinction reflects a *pre*-operative designation that does not reflect what is ultimately identified on post-operative neuropathological analysis of resected tissue. Certainly, MR imaging quality and variability of neuroradiologists' interpretation may play a role in the identification of lesions pre-operatively. As above, in some operated cases of non-lesional focal epilepsy, pathological findings such as evidence of FCD are identified upon examination of surgical tissue (Winawer et al., 2018; Porter et al., 2003). Another example involves the gene *DEPDC5*, initially associated with apparently non-lesional familial focal epilepsy until some family members were noted to have subtle but MRI-evident dysplasia (Dibbens et al., 2013; Ishida et al., 2013; Poduri, 2014; Scheffer et al., 2014). However, a *DEPDC5* somatic variant was detected in the brain tissue from a patient with MRI-negative focal epilepsy, who was subsequently diagnosed with FCD II based on histopathological analysis (Baulac et al., 2015). Where resected tissue shows no detectable neuropathological abnormality, it may represent a limitation in sampling of the tissue, which varies from center to center based on surgical technique, or an epileptogenic focus without detectable microscopic changes.

4. Techniques and strategies for somatic mutation discovery

4.1. Techniques for detecting somatic variants in brain

Techniques for the detection of somatic variants can be classified as unbiased screens or targeted assays. The former are more comprehensive, as they permit hypothesis-free genome- or exome-wide analysis and identification of novel genes associated with a given condition, but they are prone to the identification of false-positive findings and variants of uncertain significance. The latter are more specific, only interrogating a single gene or relatively small number of genes, allowing higher-depth and specificity but addressing only a limited scope of genes.

4.1.1. Unbiased screening tools

Next-generation sequencing (NGS) technologies, especially whole-genome sequencing (WGS) and whole-exome sequencing (WES), have dramatically accelerated gene discovery. Conventional NGS approaches typically target a population of cells from a bulk tissue. Newer single-cell sequencing enables genome-wide screening of single cells at high resolution (Chi, 2014). Single-neuron sequencing has been rapidly developed and applied to reveal somatic variants, and it has great potential to revolutionize future studies of brain somatic mutation. Another emerging technology is third-generation sequencing (TGS), which is a long-read sequencing method at the single molecule level. The average read-length of TGS is more than 1000 bp (Schadt et al., 2010; Lee et al., 2016). The longer read-length renders TGS a superior tool to interrogate complex and large DNA structural variants and repeat elements (Liu et al., 2017; Loomis et al., 2013; Sanchis-Juan et al., 2018). TGS has not yet been widely exploited for the study of somatic mutations in brain tissue, but has potential for this purpose.

4.1.2. Targeted methods

Targeted NGS investigates particular genomic regions of interest, and is cost-effective for screening large patient cohorts (Dilliott et al., 2018). One state-of-the-art method for effective target-enrichment by selective circulation is single molecule molecular inversion probes (smMIPs)-based capture (Hiatt et al., 2013). smMIPs have been successfully applied in targeted sequencing panels to identify germline and somatic variants in patients with developmental and epileptic encephalopathy, epileptic spasms, and focal epilepsies (Myers et al., 2018; D'Gama et al., 2015; Hildebrand et al., 2016; Tsai et al., 2016; Carvill et al., 2015, 2013). Another targeted method is ddPCR, which enables absolute quantification of DNA copies and alleles. ddPCR can achieve independent and direct quantification of DNA molecules without standard curves, with a detection limit as low as 0.005-0.01% (Taylor et al., 2017; Hindson et al., 2011). ddPCR can be reliably used to validate somatic mutations identified by NGS, and screen for recurrent hotspot somatic mutations.

4.2. Alternative strategies for detection of brain somatic variants

At present, the route to discovery of somatic variants in brain is via the privileged situation of brain tissue from surgical resection or autopsy, which is not generally applicable to common epilepsies. Here we propose two potential strategies to detect brain-only somatic mosaicism in the absence of brain tissue: (i) examination of cell-free DNA (cfDNA) in cerebrospinal fluid (CSF), and (ii) isolation of DNA from neuro-epithelium obtained by outpatient nasal biopsy (Fig. 3).

cfDNA are short DNA fragments generated following cell death that are externalized and circulate in the bodily fluids. CSF is a readily accessible body fluid that can reflect the underlying pathological state of the central nervous system, and cfDNA shed from dying brain cells into CSF is an established "liquid biopsy" for brain tumor variant detection (Fontanilles et al., 2018; De Mattos-Arruda et al., 2015; Martinez-Ricarte et al., 2018; Pan et al., 2015). Thus CSF "liquid biopsy" could be

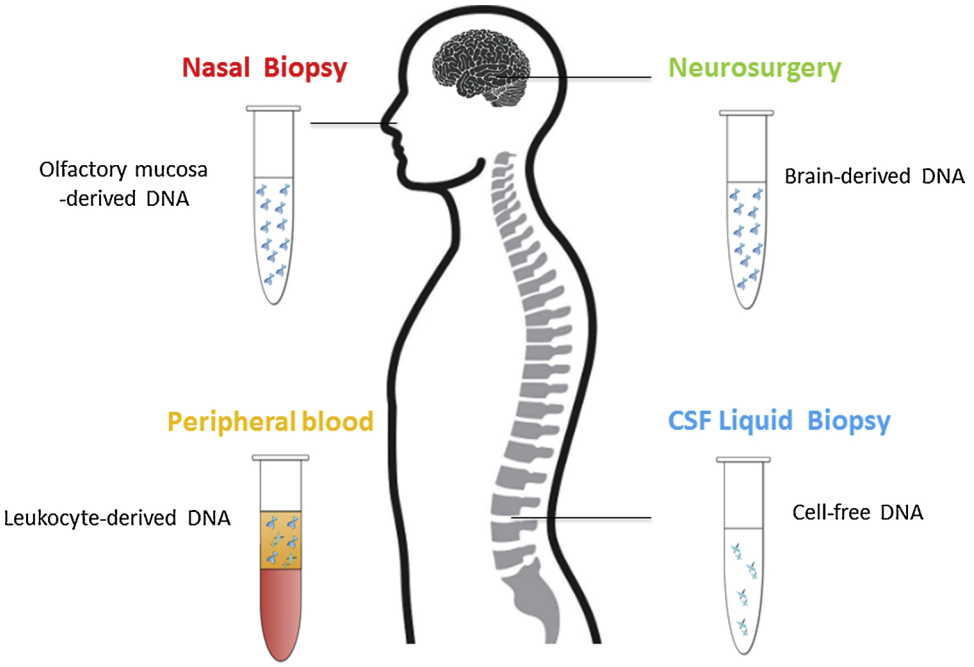

Fig. 3. Potential strategies for detection of somatic mutations in the brain. Potential strategies include genetic examination of cell-free DNA (cfDNA) in cerebrospinal fluid (CSF), and DNA obtained from neurosurgery, nasal biopsy, and peripheral blood. [Adapted from https://www.istockphoto.com/au/vector/spine-gm523577159-51981582., copyright 2018 by iStockphoto LP.].

a potential surrogate for brain tissue to provide an alternative route for detection of somatic mosaicism in epilepsy.

The olfactory mucosa is the only CNS tissue exposed to the external environment (Gizurarson, 2012). Nasal biopsy provides minimally invasive access to olfactory mucosa (Feron et al., 2013, 1998; Tanos et al., 2017), and have been applied to study several neurological diseases (Abrams et al., 1999; Arnold et al., 2001, 2010; Cook et al., 2011; Matigian et al., 2010; Ronnett et al., 2003; Wolozin and A.E. Bennett Research Award, 1993). Epilepsy-associated somatic variants can occur relatively early in embryogenesis, and they are likely to also be present in the olfactory mucosa which is embryologically derived from the neural crest cells and olfactory placode. Thus, human olfactory mucosa is an accessible neuroectodermal tissue to study neurogenesis, and is a potential tool to diagnose and functionally interrogate neurological disorders like epilepsy. While sampling may represent a relatively low-risk procedure, it would only be justified in the clinical environment if the potential findings might lead to treatment changes that would benefit the patient. As pathways emerge with potential treatments (e.g., mTOR pathway potentially modifiable with mTOR inhibitors, *SLC35A2*-related glycosylation defects potentially treatable with oral galactose supplementation), the minimal risk of nasal biopsy may be balanced with the potential for precision diagnosis leading to targeted treatment.

5. Future directions

Detecting pathogenic somatic variants ends the diagnostic odyssey for patients, and informs genetic counseling, clinical management and the development of future targeted therapies, particularly if variants affect specific molecular pathways. The mTOR inhibitor everolimus has been approved by the European Commission for use as an add-on treatment for pharmaco-resistant focal-onset seizures in tuberous sclerosis patients 2 years of age or older (European Medicines Agency and Votubia everolimus, 2017). Pathway-based precision medicine strategies (including mTOR inhibition) theoretically could prove effective in epileptogenic malformations where brain-only somatic variants are implicated once the gene discoveries from the lab can be effectively and reliably translated to the clinical arena. There is a substantial gap in this area currently, and it will require a major paradigm shift in the field of epilepsy and a prioritization of diagnostic assessment alongside on-going empiric treatment of focal epilepsies.

Genetic changes occur during development and accumulate in a variety of somatic cells during the life of an individual. The brain is of particular interest because the vast majority of neurons persist without replacement once formed during early development, yet post-mitotic mutation may alter brain function and contribute to normal aging processes or neurological disease. While the landscape of somatic mutation in the brain is complex, cutting-edge technologies are allowing us to decipher their contribution to development, aging, and disease. The establishment of the Brain Somatic Mosaicism Network (BSMN) is a prime example of this progress (McConnell et al., 2017); this multi-site network seeks to exploit advances in genomic technologies to study the patterns of somatic variants in brain tissue from individuals with and without neurological or psychiatric disorders. Further larger-scale international collaborative projects will accelerate the role of somatic mutation during and beyond brain development and the pathogenesis of neurological diseases. These efforts hold not only scientific promise but also the promise of clinical genetic diagnoses and potential for targeted therapies for a large proportion of patients with epilepsy.

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