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Report

Somatic Activation of *AKT3* Causes Hemispheric Developmental Brain Malformations

Annapurna Poduri,[1,4] Gilad D. Evrony,[2,5] Xuyu Cai,[2,5] Princess Christina Elhosary,[1] Rameen Beroukhim,[6,9,10,12,15] Maria K. Lehtinen,[2,3,7] L. Benjamin Hills,[2] Erin L. Heinzen,[16] Anthony Hill,[2] R. Sean Hill,[2,15] Brenda J. Barry,[2] Blaise F.D. Bourgeois,[1,4] James J. Riviello,[1,4,19] A. James Barkovich,[17] Peter M. Black,[13,18] Keith L. Ligon,[3,7,10,11,14] and Christopher A. Walsh[2,4,8,15,*]

[1]Department of Neurology
[2]Division of Genetics, Manton Center for Orphan Disease Research, and Howard Hughes Medical Institute
[3]Department of Pathology
Children's Hospital Boston, 300 Longwood Avenue, Boston, MA 02115, USA
[4]Department of Neurology
[5]Program in Biological and Biomedical Sciences
[6]Department of Medicine
[7]Department of Pathology
[8]Department of Pediatrics
Harvard Medical School, Boston, MA 02115, USA
[9]Department of Cancer Biology and Center for Cancer Genome Discovery
[10]Department of Medical Oncology
[11]Department of Pathology and Center for Molecular Oncologic Pathology
Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, USA
[12]Department of Medicine
[13]Department of Neurosurgery
[14]Department of Pathology
Brigham and Women's Hospital, 25 Francis Street and Harvard Medical School, Boston, MA 02115, USA
[15]Broad Institute, 7 Cambridge Center, Cambridge, MA 02142, USA
[16]Center for Human Genome Variation, Duke University School of Medicine, 308 Research Drive, Durham, NC 27708, USA
[17]Department of Radiology, University of California San Francisco, 505 Parnassus Street, San Francisco, CA 94143, USA
[18]World Federation of Neurosurgical Societies, 5 Rue du Marché, 1260 Nyon, Vaud, Switzerland
[19]Present address: Comprehensive Epilepsy Center, New York University Langone Medical Center, 550 1st Avenue, New York, NY 10016, USA
*Correspondence: christopher.walsh@childrens.harvard.edu
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SUMMARY

Hemimegalencephaly (HMG) is a developmental brain disorder characterized by an enlarged, malformed cerebral hemisphere, typically causing epilepsy that requires surgical resection. We studied resected HMG tissue to test whether the condition might reflect somatic mutations affecting genes critical to brain development. We found that two out of eight HMG samples showed trisomy of chromosome 1q, which encompasses many genes, including *AKT3*, a gene known to regulate brain size. A third case showed a known activating mutation in *AKT3* (c.49G→A, creating p.E17K) that was not present in the patient's blood cells. Remarkably, the E17K mutation in *AKT3* is exactly paralogous to E17K mutations in *AKT1* and *AKT2* recently discovered in somatic overgrowth syndromes. We show that *AKT3* is the most abundant AKT paralog in the brain during neurogenesis and that phosphorylated AKT is abundant in cortical progenitor cells. Our data suggest that somatic mutations limited to the brain could represent an important cause of complex neurogenetic disease.

INTRODUCTION

The role of somatic mutation in human brain development and disease is a source of intense interest to developmental neuroscientists and human geneticists. Somatic mutation, such as by the mobilization of retrotransposons during neurogenesis (Muotri and Gage, 2006; Singer et al., 2010) or by copy number variation in neurons (Rehen et al., 2005), has been proposed as a source of normal neuronal diversity. However, neurogenetic disease has also been attributed to somatic, postzygotic mutations in *TSC2*, *NF1*, and *DCX* that are detectable in some, but not all, blood cells and appear to be present in some, but not all, brain cells (Gleeson et al., 2000; Messiaen et al., 2011; Qin et al., 2010; Vogt et al., 2011). On the other hand, it has been essentially impossible to study potential roles of mutations that are limited to brain cells, because such mutations are by definition absent from blood and other tissues typically available for genetic study. Such somatic mutations could conceivably play important roles in complex neurogenetic disorders, such as epilepsy, intellectual disability, and psychiatric disease, for

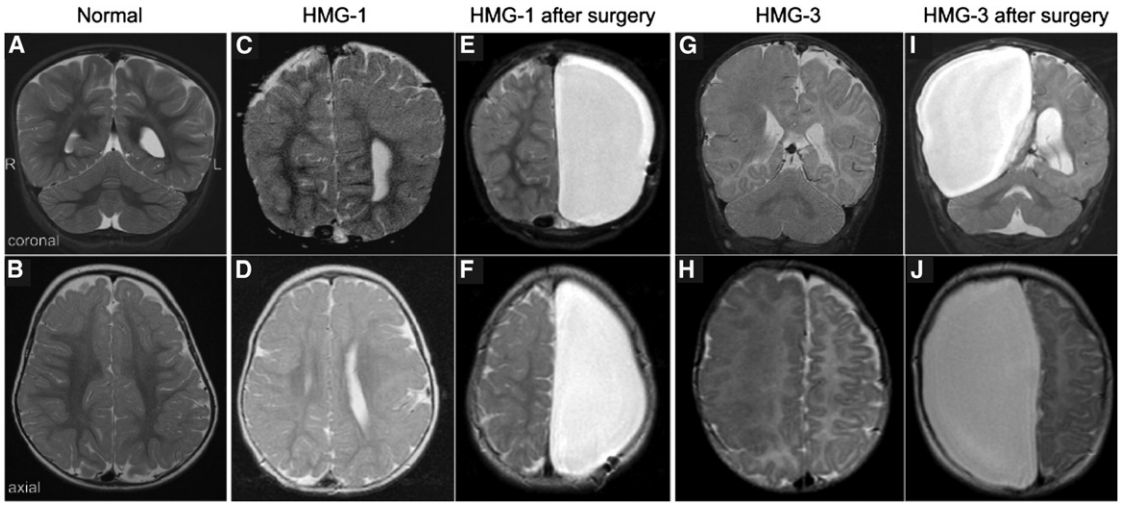

Figure 1. MRIs of Patients with Hemimegalencephaly Due to Somatic Mutations

(A and B) The first column shows an example of coronal T2-weighted and axial T2-weighted MRI images showing the brain of a normal 1 year old. Note the symmetric size of the right and left hemispheres, labeled R and L to denote standard MRI convention. (C–J) Representative images from the brain MRIs of two patients with HMG before and after surgical removal of the abnormal hemisphere are shown. (C and D) HMG-1 has somatic trisomy of chromosome 1q. MRI before surgery showed left-sided hemispheric enlargement, abnormal cortical thickness and configuration, and enlarged left lateral ventricle in the coronal T2-weighted and axial T2-weighted images. The right hemisphere is smaller and appears normal. (E and F) After left hemispherectomy surgery, there is cerebrospinal fluid (CSF) where the abnormal hemisphere had been, seen as bright signal in coronal and axial images taken at approximately the same plane as the preoperative images. (G and H) HMG-3 has a somatic mosaic mutation in *AKT3*. Coronal T2-weighted and axial T2-weighted MRI images show right-sided hemispheric enlargement, abnormal cortical thickness and signal, abnormal white matter signal, and an enlarged lateral ventricle. (I and J) After right hemispherectomy surgery, as in the previous case, CSF is visible as bright signal in place of the resected abnormal hemisphere.

which prominent roles for de novo mutations have been well documented (Awadalla et al., 2010; Poduri and Lowenstein, 2011; Ropers, 2008). Here we describe a highly epileptic disorder, hemimegalencephaly (HMG, literally, enlargement of one brain hemisphere), as a model to characterize the role of somatic mutation in the developing brain.

HMG is a developmental brain disorder characterized by an enlarged, malformed cerebral hemisphere (Flores-Sarnat et al., 2003). The clinical presentation typically includes intellectual disability and severe, intractable epilepsy, often necessitating surgical removal or disconnection of the abnormal hemisphere for seizure control (Gowda et al., 2010). Although no specific genetic causes have been identified for isolated HMG, HMG has been reported in association with Proteus syndrome (Griffiths et al., 1994)—another multisystem overgrowth disorder that has recently been associated with somatic activating mutations in the gene *AKT1* (Lindhurst et al., 2011)—as well as other rare neurocutaneous syndromes (Mochida et al., 2013). There are also rare reports of HMG associated with tuberous sclerosis complex (TSC) (Cartwright et al., 2005), a syndrome in which multiple organ systems display disordered and sometimes cancerous growths.

The striking asymmetry of the brain in individuals with HMG has long suggested that HMG reflects spontaneous, somatic, clonal mutation limited to the brain, analogous to cancer but without cellular transformation and ongoing proliferation. We hypothesized that the somatic mutations causing HMG might be essentially restricted to the brain and detectable by direct study of affected brain tissue. Here we show that three out of eight HMG samples studied showed somatic mutations involving *AKT3*: two with large duplications of chromosome 1q encompassing *AKT3*, as well as many other genes, and a third carrying a known activating mutation in *AKT3*. Moreover, we demonstrate that at least two out of three of these mutations are not detectable in blood of the same individuals, reflecting somatic mutations affecting the brain preferentially or exclusively.

RESULTS AND DISCUSSION

We studied eight samples of brain tissue resected at the time of epilepsy surgery and identified two that showed trisomy of chromosome 1q. The first partial trisomy case (HMG-1) was a nondysmorphic boy requiring hemispherectomy at 15 months of age for treatment of epilepsy due to HMG. He had no clinical evidence of nonnervous system involvement. Magnetic resonance imaging (MRI) showed left-sided HMG, with the extent of the lesion reflected in the large amount of brain removed in order to control his seizures (Figures 1C and 1D show the left HMG before surgery, and Figures 1E and 1F show only the normal right hemisphere remaining after surgery). After surgery, seizures were dramatically reduced from approximately ten per day to one to four per month. At age 6, he had right-sided weakness but could walk independently; he had good language comprehension, though his speech production was limited to a few words, and he attended school with special services. Neuropathological analysis from the affected hemisphere revealed diffuse abnormalities of cortical development (cortical

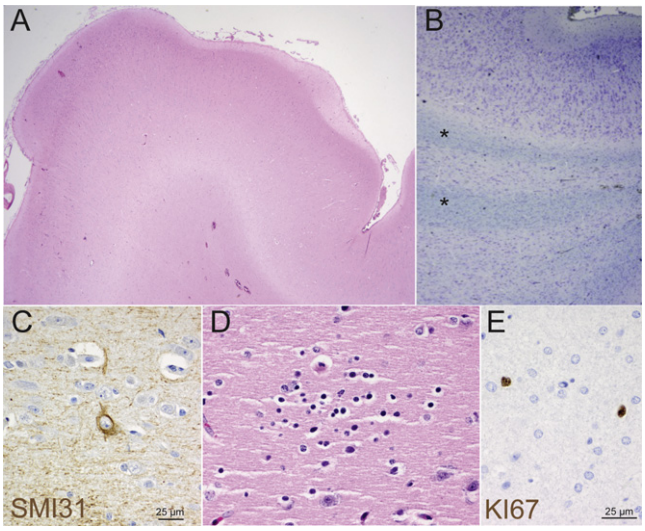

Figure 2. Abnormal Cortical Development in Hemimegalencephaly Case HMG-1 with Trisomy of Chromosome 1q

(A) Low-power view (20× magnification) of a gyrus from the cerebral cortex stained with hematoxylin and eosin (H&E) shows an abnormally contoured surface and variably thick cortical ribbon and molecular layer. (B) Analysis of subcortical white matter using cresyl violet and luxol fast blue highlights numerous subcortical bands and islands of ectopic gray matter that contain neurons and glia (asterisks). (C) Immunohistochemical staining for phosphorylated neurofilament SMI31 highlights scattered abnormal large neurons. (D) Rare small collections of neuroblast-like cells (microdysplasia) were present on H&E. (E) Immunohistochemical staining also demonstrated an abnormal number of proliferating Ki67-positive cells scattered throughout gray and white matter that had an atypical nuclear morphology. (A) has a view of 20× magnification, (B) has a view of 200× magnification, and (C)–(E) have a view of 600× magnification.

dysplasia) with irregular cortical architecture, ectopic bands of gray matter in the subcortical white matter, scattered proliferating cells, and abnormal neurons consistent with previous reports of HMG (Figure 2) (Flores-Sarnat et al., 2003). Copy number evaluation of single nucleotide polymorphism (SNP) data showed increased signal for the entire q arm of chromosome 1 in the brain sample (Figures 3A and 3B and Figure S1 available online), with an estimated copy number of 2.41 (SD 0.12). No other chromosomes displayed abnormal copy number (Figure 3A). Quantitative PCR (qPCR) confirmed the 1q trisomy, generating a calculated copy number of 2.39 (SD 0.30) from one brain sample; from a second sample, the calculated copy number was 2.68 (SD 0.16), 2.76 (SD 0.20), and 2.73 (SD 0.13) at 1q21.3, 1q31.1, and 1q42.2, respectively (Figure 3C). The intermediate copy number, between 2 and 3, suggests a mixture of normal and trisomic cells in the brain regions sampled, and together these results suggest that the ratio of normal and abnormal cells varied somewhat in different parts of the resected tissue. High-resolution karyotype and qPCR of peripheral blood cells in the patient did not reveal any evidence of trisomy 1q in these nonbrain cells (Figure 3C and data not shown).

We identified a second case of partial gain of chromosome 1, again involving the entire 1q arm, based on SNP data from the brain sample of an individual (HMG-2) reported to have isolated HMG on MRI, similar but somewhat milder neuropathological findings of mild dysplasia (manifest primarily as a thickened cortical ribbon), and no other medical problems (Figure S1). Copy number at 1q assessed by qPCR was 2.75 (SD 0.28), again consistent with mosaic partial trisomy. Leukocytes or other tissues were not available from this individual, so the somatic nature of the mutation could not be directly tested. Inspection of the published literature and the Database of Genomic Variants (http://projects.tcag.ca/variation), a large database of copy number variation, suggests that there are no known control individuals with large constitutional duplications of 1q (Iafrate et al., 2004). Wintle et al. (2011) recently conducted a sensitive copy number analysis on brain tissue from 52 individuals without HMG and reported no duplications of chromosome 1q larger than 1 Mb (whereas the 1q region spans nearly 250 Mb), demonstrating that our finding of two out of eight cases with trisomy of 1q is not a common variant.

Chromosome 1q contains many genes, but among them *AKT3* is a particularly strong candidate for HMG, because deletions including *AKT3* are associated with microcephaly, suggesting a role for *AKT3* in control of brain size (Ballif et al., 2012; Boland et al., 2007; Hill et al., 2007). Furthermore, somatic-activating mutations in *AKT1* cause Proteus syndrome, and somatic-activating mutations in *AKT2* have been reported to cause hypoglycemia and asymmetrical somatic growth (Hussain et al., 2011; Lindhurst et al., 2011). Earlier screening for candidate mutations in cancer-associated genes did not reveal any mutations in our cases (data not shown), but *AKT3* was not included among the genes screened. We sequenced *AKT3* as a candidate gene in the six remaining nontrisomy cases of HMG and identified one out of six with a somatic point mutation in *AKT3*. This case (HMG-3) was a nondysmorphic boy requiring hemispherectomy at 5 months of age for seizures beginning in the first week of life due to right-sided HMG (MRI before surgery is shown in Figures 1G and 1H and after surgery in Figures 1I and 1J). After surgery, he had two periods of breakthrough seizures but has been seizure free for 6 years at 9 years of age. He has left-sided weakness but walks independently, speaks fluently, is able to read, and attends school with special education services. DNA sequencing revealed the mutation *AKT3* c.49G→A, p.E17K in the DNA derived from the brain; this mutation was not detectable in DNA derived from the patient's leukocytes (Figure 3D). To confirm the presence of the mutation in brain cells, we cloned the PCR product from the brain and resequenced multiple clones (Figure 3D). Forty-six individual clones showed either the mutant sequence only (8/46, or 17.4%) or the normal sequence only (38/46, or 82.6%) (examples are shown in Figure 3D), suggesting that the mutation exists in the heterozygous state in ≈35% of the cells.

The activating nature of the *AKT3* E17K mutation has been shown previously biochemically (Davies et al., 2008). Evaluation of data from the Exome Variant Server revealed that the *AKT3* c.49G→A point mutation is not present in >5,000 control individuals (http://evs.gs.washington.edu). Published estimates suggest a somatic mutation frequency on the order of 10^{-9} per cell division (Lynch, 2010b); published mutation rates from exome sequencing in humans, coupled with extrapolation of

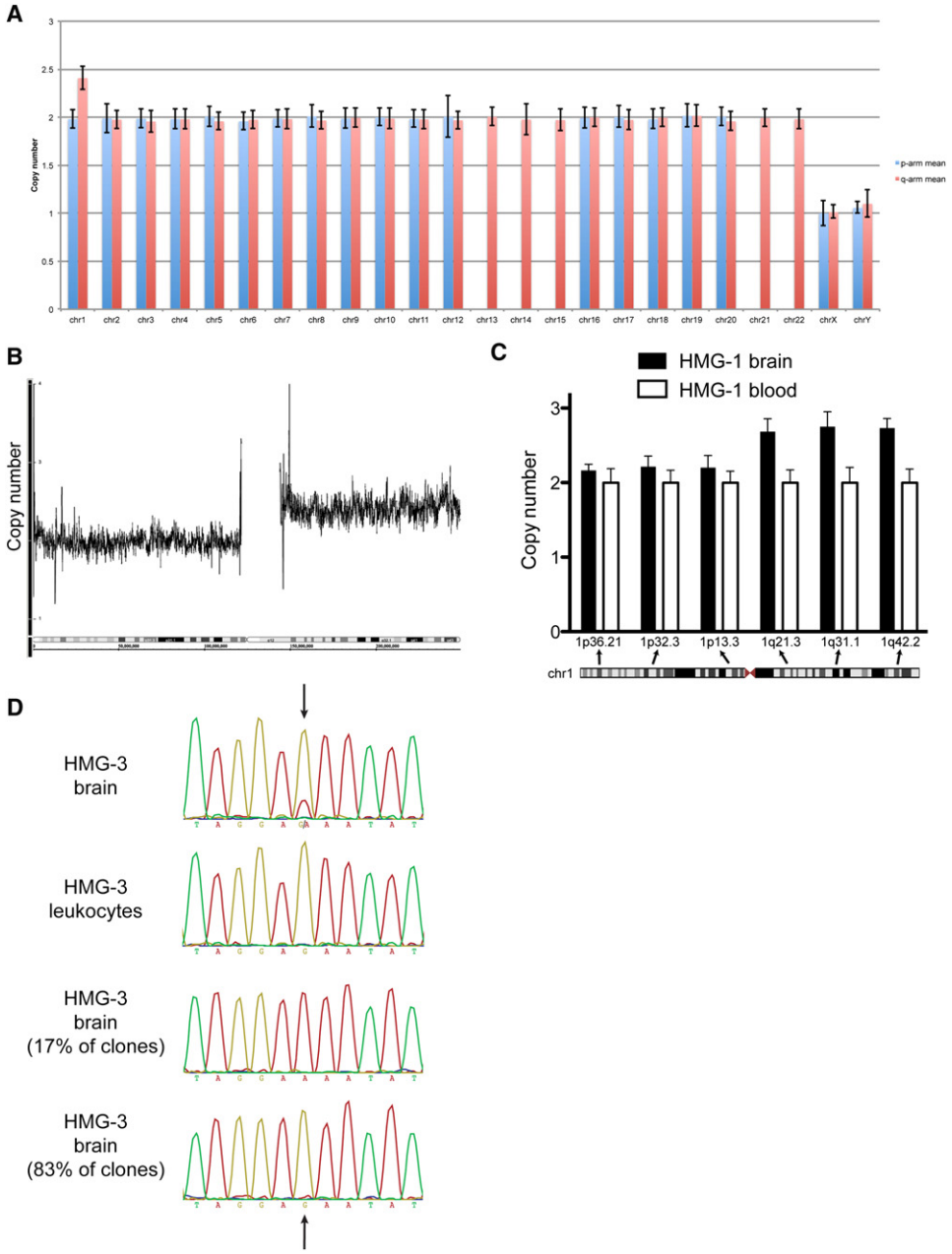

Figure 3. Mosaic Mutations in Hemimegalencephaly: Trisomy of Chromosome 1q and an Activating Point Mutation in *AKT3*

(A) Copy number for all of the chromosomes is shown for HMG-1; the estimated copy number for 1q is 2.41 (SD 0.12), consistent with mosaic trisomy 1q. Chromosome 1p, as well as the other autosomes, has normal copy number of 2, and chromosomes X and Y each show copy number of 1. (B) Copy number evaluation of Affymetrix 6.0 data shows the gain in copy number at chromosome 1q for HMG-1, with the x axis representing nucleotide position along chromosome 1 and the y axis denoting copy number. (C) Assuming a copy number of 2 for all regions in the DNA derived from leukocytes (white columns), the calculated copy number from the brain tissue (black columns) was 2.68 (SD 0.16) at 1q21.3, 2.76 (SD 0.20) at 1q31.1, and 2.73 (SD 0.13) at 1q42.2. (D) The *AKT3* c.49G→A, p.E17K heterozygous mutation is present in the sequencing traces from brain-derived DNA (first row) and absent in the traces from leukocyte-derived DNA from HMG-3 (second row). The arrows point to *AKT3* nucleotide position 49. Cloning results indicate that the mutation is present in 8/46 (17.4%) of the DNA reads from a brain tissue sample, suggesting that the mutation exists in the heterozygous state in 35% of the cells; traces from two clones are shown in the third and fourth rows; the trace in the third row shows the results of sequencing from a clone with the *AKT3* c.49G→A mutation present (A), and the bottom row shows the results from a clone without the mutation but rather with the reference allele present (G).

somatic mutation rates in mouse, suggest a $<1 \times 10^{-7}$ chance that the specific *AKT3* c.49G→A mutation would occur by chance (Awadalla et al., 2010; Lynch, 2010a).

Somatic mutations in *AKT3*, which encodes the serine-threonine kinase protein kinase B-gamma, have been reported in cancers, including a p.G171R substitution mutation in a glioma (Bamford et al., 2004). The *AKT3* c.49G→A E17K mutation itself has been observed in melanoma and lung cancer, and melanoma cell lines overexpressing this exact missense mutation have been demonstrated to show increased AKT phosphorylation (Davies et al., 2008; Do et al., 2010). Most remarkably though, the somatic *AKT3* mutation we report is precisely paralogous to the recurrent E17K mutations in *AKT1* associated with Proteus syndrome and recurrent E17K mutations in *AKT2* associated with hypoglycemia and left-sided overgrowth, each also with varying degrees of mosaicism (Hussain et al., 2011; Lindhurst et al., 2011). Interestingly, despite prior reports of Proteus-associated HMG (Griffiths et al., 1994), no brain malformations are reported in the patients with *AKT1* and *AKT2* mutations, consistent with the observation in mice that *AKT3* may be the predominant functional member of the AKT family in the human brain (Easton et al., 2005).

AKT3 expression in the human fetal brain is higher than *AKT3* expression in any other tissue sampled (Wu et al., 2009), suggesting that its primary role is in brain development. In contrast, *AKT1* and *AKT2* show levels of fetal brain expression comparable to or lower than those seen in other tissues (Wu et al., 2009). We compared the expression levels of *AKT1*, *AKT2*, and *AKT3* by RNaseq analysis of the perisylvian cortex of the human brain at 9 weeks' gestation, during active neurogenesis, and found that *AKT3* is expressed at higher levels than *AKT1* and *AKT2* (normalized read depth, reads per kilobase-exon per million mapped reads: *AKT1* = 51.90, *AKT2* = 18.50, *AKT3* = 90.52). Examination of published data sets reveals that *AKT3* is expressed at a higher level than *AKT1*, and both are expressed at higher levels than *AKT2*, starting at 8 weeks and for the duration of human embryonic cortical development (Kang et al., 2011). To determine the cell types in the brain that would likely be affected by activation of *AKT3*, we performed immunohistochemistry in sections of mouse brain by using an antiserum that recognizes all three phosphorylated forms of AKT (P-Akt). We observed widespread P-Akt localization in the developing cortex, with notable enrichment in apical progenitor cells in the ventricular zone. For example, a subset of cells marked by P-Akt also showed the presence of P-Vimentin 4A4, illustrating the presence of Akt activity in dividing radial glial cells (Figure 4). Similarly, P-Akt colocalized with phospho-Histone H3, a marker of M phase, in dividing apical progenitors as well as GLAST, a marker of radial glial cells (data not shown). Because these apical progenitor cells give rise to both neurons and glia, this localization is consistent with activation of *AKT3* in both neurons and glia. Abnormal AKT function would be consistent with the MRI patterns and neuropathological studies (Figures 1 and 2), which show abnormal organization of neurons in the cortex and abnormal MRI signal characteristics of white matter.

Our data suggest that activation of *AKT3*, either by duplication or by point mutation, contributes to hemispheric brain overgrowth. Two of our cases (the point mutation and one partial trisomy) are confirmed to be de novo, somatic mutations, undetectable in blood, and although nonbrain tissues were not available from the other partial trisomy case, this is likely to be a somatic mutation as well, because individuals reported with constitutional trisomy 1q, even a portion of 1q, show dysmorphic features and, in nearly all cases, early lethality (Mark et al., 2005; Mefford et al., 2008; Patel et al., 2009). We postulate that increasing *AKT3* dosage and activation of *AKT3* would have the same effect in the setting of a somatic mutation. Interestingly, HMG has not been reported in the constitutional trisomy cases, even those that have partial trisomy including *AKT3*. It is possible that HMG might not be present in the cases with early lethality; perhaps more important, because all of the constitutional trisomy 1q cases were de novo, the trisomy may not be present in all tissues. Though we have not sampled other tissues, there was no clinical evidence of extracerebral involvement phenotypically in any of the three cases, suggesting that either the mutation was limited to the brain or activation of *AKT3* in other tissues does not have phenotypic consequences. Increased rates of brain cancer are not reported in the setting of isolated HMG. In the cases we report here, which have not shown any form of cancer, it is likely that activation of *AKT3* disrupts normal cortical development but does not result in continued dysregulated growth outside the setting of cortical progenitor cells.

Further support for the role of *AKT3* in controlling brain size comes from animal studies. A mouse *Akt3* knockout model shows selective reduction in brain size due to decreased neuronal number and size (Easton et al., 2005), whereas mice with an activating mutation in the kinase domain of *Akt3* show larger hippocampal size and abnormal Ki67-positive ectopic neurons in the hippocampus (Tokuda et al., 2011). Additionally, in zebrafish, overexpression of wild-type *akt3* produces increased embryonic brain thickness (Chen et al., 2011). All of these results strongly suggest that *AKT3* activity dynamically regulates brain size and that increased dosage of *AKT3* might increase brain size in humans.

Somatic mosaicism refers to the presence of more than one genetically distinct population of cells in an individual. Somatic mutations are thought to arise not infrequently during development (Youssoufian and Pyeritz, 2002), and some chromosomal rearrangements and mutations that may be lethal if present in the entire embryo could be sustained in clonal populations of cells and produce localized abnormalities. The size and architecture of HMG may be determined in part by the stage at which the mutation occurs relative to the period of neurogenesis, which is when *AKT3* normally becomes the predominant AKT form in brain. As better techniques emerge for copy number and whole-exome or genome sequencing on smaller and smaller amounts of DNA, somatic mutations in other genes might emerge as causes of other neurogenetic disorders not associated with obvious morphological phenotypes like HMG. For example, de novo copy number variations are an important cause of autism spectrum disorders and schizophrenia (Sanders et al., 2011), and hence may also occur somatically. In epilepsy, at least one third of individuals with imaging-negative, refractory, focal seizures

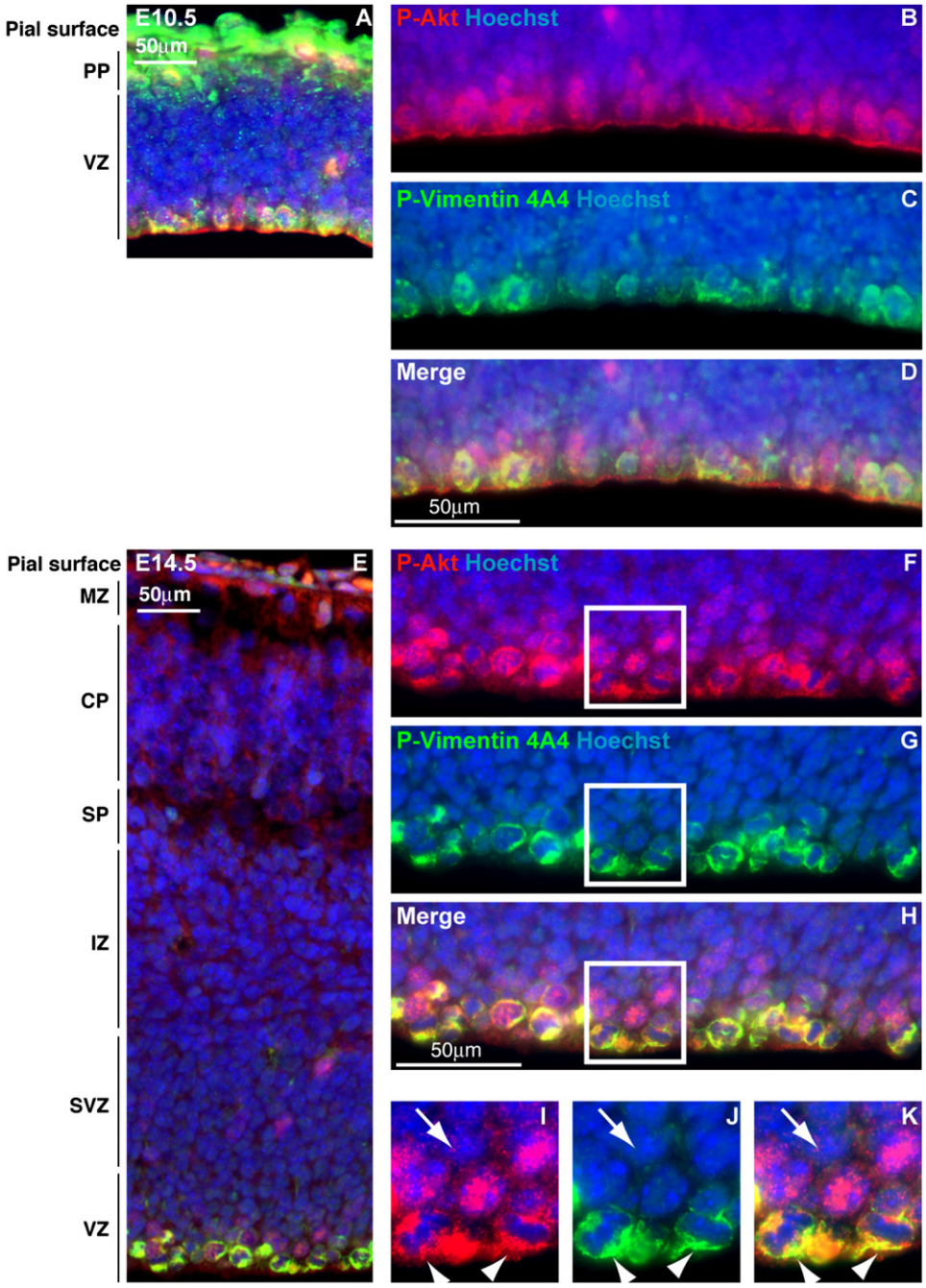

Figure 4. Active Akt Signaling in the Developing Cortex Is Enriched in Apical Progenitor Cells and the Cortical Plate

(A) Immunohistochemistry of cortical sections at embryonic day 10.5 (E10.5) reveals Akt activity as assessed by pan-phospho(P)-Akt immunostaining (red) in the cortical plate and ventricular zone. (B–D) Higher-magnification images of ventricular zone at E10.5 are shown. Overlay of P-Akt with P-Vimentin 4A4 (green) shows that dividing radial glial cells, which generate cortical pyramidal neurons and glial cells, are P-Akt positive. (E–H) At E14.5, dividing radial glial cells show a similar pattern of immunostaining for P-Akt and P-Vimentin 4A4. (I–K) High-magnification images of the areas delineated by white boxes in (F)–(H) demonstrate that P-Akt activity (marked by arrowheads) is not restricted to the P-Vimentin 4A4-positive-staining, M phase cells in the ventricular zone. The arrows indicate an example of a P-Akt-positive, P-Vimentin 4A4-negative cell. Nuclei are labeled with Hoechst. Scale bars represent 50 μm. MZ, marginal zone; CP, cortical plate; SP, subplate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone.

show pathological evidence of dysplasia (Porter et al., 2003) that may also be due to somatic mutations. Therefore, more detailed exploration of somatic mosaicism may allow for better genetic understanding of many neurogenetic disorders, especially those for which de novo mutations are known to play a role.

EXPERIMENTAL PROCEDURES

Brain Sample Ascertainment

Tissue samples for molecular analysis were available through two sources: (1) patients enrolled in clinical research in accordance with requirements of the Institutional Review Boards of Children's Hospital Boston (CHB) and Beth Israel Deaconess Medical Center (six cases, including HMG-1 and HMG-3) and (2) excess tissue obtained from the Brigham and Women's Hospital Department of Neurosurgery Tissue Bank, along with limited clinical information (two cases, including HMG-2).

Phenotypic Assessment

Detailed clinical information and leukocyte-derived DNA were available for six cases enrolled in human subjects research, including HMG-1 and HMG-3. We reviewed the history and examination of each case reported (A.P., B.F.D.B., and J.J.R.) and the MRI (A.P., A.J.B., and C.A.W.). Table S1 summarizes the imaging and neuropathological findings of the three cases with mutations.

Neuropathological Analysis

Formalin-fixed paraffin-embedded sections from the clinical resection specimens were obtained from the CHB pathology archives for pathological review by a board-certified neuropathologist (K.L.L.). Slides were stained with hematoxylin and eosin (H&E) and cresyl violet and luxol fast blue according to standard methods.

Immunohistochemistry was performed by using phosphorylated neurofilament (SMI31, Covance) and Ki67 (DAKO, Clone MIB1) using DAKO Envision Plus and diaminobenzidine development.

Copy Number Assessment

We obtained eight samples of flash-frozen brain tissue resected during focal epilepsy surgery for HMG. DNA was extracted by using standard methods and was then digested, amplified, and hybridized to Affymetrix 100K SNP arrays for six of the samples (Affymetrix). In the original arrays (e.g., 100K), copy number was assessed based on intensity of signal from each SNP. For the Affymetrix 6.0 arrays, copy number probes are included in addition to the full array of SNPs, and both are used for quantitation. The Gaussian-smoothed signal log2-ratio of all probe intensities normalized to a reference of 270 normal HapMap samples was calculated by Affymetrix Genotyping Console with standard settings. Additional DNA from HMG-1 and two other samples was assessed by using the Affymetrix 6.0 SNP array. The software dChipSNP was used for analysis.

For HMG-1 and HMG-2, we performed qPCR in cases in which copy number change was detected. Primers were designed to 1q44 and 1p21.1. DNA from two control individuals (Promega) was used for comparison. We repeated qPCR in an additional specimen from HMG-1 for confirmation by using primers targeting 1p (1p13.3, 1p32.3, and 1p36.2) and 1q (1q21.3, 1q31.1, and 1q42.2).

Leukocytes were obtained from six of the cases; DNA was extracted by using standard methods and was used for SNP analysis as above. For HMG-1, we performed SNP analysis and clinical karyotype to assess for the presence of the trisomy 1q in peripheral blood leukocytes (evaluating 50 cells to detect even a low level of mosaicism).

Screening for Candidate Mutations in Oncogenes

Based on the hypothesis that our cases harbor somatic mutations in genes that result in dysregulated growth, we screened the DNA from the brain samples for a panel of known point mutations in cancer-associated genes (OncoMap Project, Dana Farber Cancer Institute) (MacConaill et al., 2009). This panel did not include *AKT3*; genes included in the 1q region were *ABL2*, *DDR2*, and *NTRK1*.

Evaluation for AKT3 c.49G→A-Activating Mutations

We designed primers by using Primer 3 software (http://primer3.sourceforge.net) for the second exon of *AKT1*, *AKT2*, and *AKT3* in order to evaluate nucleotide position 49. In cases without trisomy 1q, we sequenced DNA from brain tissue (six HMG cases) and leukocytes from the same cases (five cases).

Evaluation for Mosaicism of the AKT3 Mutation in HMG-3

To determine the degree of mosaicism in the brain tissue specimen of HMG-3, we performed TOPO TA cloning by using standard methods (Invitrogen), successfully analyzing 46 clones for the *AKT3* c.49G→A mutation.

Estimation of the Likelihood that the AKT3 Mutation Would Occur by Chance

Published sequencing data indicate that each individual has approximately one to two de novo nonsynonymous variants per diploid genome generation (Awadalla et al., 2010). The likelihood that this would affect this one base pair in all of the 6×10^7 base pairs of the diploid genome is therefore 2×10^{-8}–3×10^{-8}. Correcting by a factor of 10 to reflect the increased somatic versus germline mutation rate (Lynch, 2010a) and accounting for three potential mutations at a given nucleotide position, the estimated likelihood that our mutation would occur by chance is at most 1×10^{-7}.

Localization of P-AKT in the Developing Cortex

We labeled embryonic mouse cortex at embryonic day 10.5 (E10.5), E12.5, E14.5, E16.5, and E18.5 with the following antibodies: rabbit anti-phospho-Akt 1:50 (4060S, Cell Signaling), mouse anti-phospho-Vimentin 4A4 1:100 (Assay Designs), rabbit anti-phospho-Histone H3 1:400 (Upstate), and anti-GLAST 1:5,000 (Chemicon).

RNA-Seq Analysis of AKT Isoforms in the Developing Human Cortex

We obtained snap-frozen brain tissue from a human fetus at roughly 9 weeks' gestation from the Institute of Human Genetics at Newcastle University. RNA was isolated from several regions of the cortex, including the perisylvian region, and purified by using standard methods. We purified polyA-tailed mRNA by using an Oligotex mRNA minikit (QIAGEN) and prepared a barcoded sequencing library by using the SOLiD Whole Transcriptome Analysis Kit (Applied Biosystems). We sequenced the library on the SOLiD v3 Plus system (read depth: 105 million reads), mapped the reads with Bioscope v1.2 (Applied Biosystems) to the hg18 human genome reference, and normalized coverage of uniquely mapping reads to the number of million mapped reads.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one table and one figure and can be found with this article online at doi:10.1016/j.neuron.2012.03.010.

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