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Cell-Type Specificity of Mosaic Chromosome 1q Gain Resolved by snRNA-seq in a Case of Epilepsy With Hyaline Protoplasmic Astrocytopathy

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Abstract

Objectives

Mosaic gain of chromosome 1q (chr1q) has been associated with malformation of cortical development (MCD) and epilepsy. Hyaline protoplasmic astrocytopathy (HPA) is a rare neuropathologic finding seen in cases of epilepsy with MCD. The cell-type specificity of mosaic chr1q gain in the brain and the molecular signatures of HPA are unknown.

Methods

We present the case of a child with pharmacoresistant epilepsy who underwent epileptic focus resections at age 3 and 5 years and was found to have mosaic chr1q gain and HPA. We performed single-nuclei RNA sequencing (snRNA-seq) of brain tissue from the second resection.

Results

snRNA-seq showed increased expression of chr1q genes specifically in subsets of neurons and astrocytes. Differentially expressed genes associated with inferred chr1q gain included *AKT3* and genes associated with cell adhesion or migration. A subpopulation of astrocytes demonstrated marked enrichment for synapse-associated transcripts, possibly linked to the astrocytic inclusions observed in HPA.

Discussion

snRNA-seq may be used to infer the cell-type specificity of mosaic chromosomal copy number changes and identify associated gene expression alterations, which in the case of chr1q gain may involve aberrations in cell migration. Future studies using spatial profiling could yield further insights on the molecular signatures of HPA.

Introduction

Mosaic chromosome 1 q (chr1q) gain has been identified in several case reports and series as a genetic driver of pharmacoresistant epilepsy in the setting of malformation of cortical development (MCD),[1-5] an umbrella term that encompasses pathologies such as mild MCD (mMCD) with excessive heterotopic neurons,[6] focal cortical dysplasia (FCD), gray matter heterotopia, polymicrogyria, and hemimegalencephaly.

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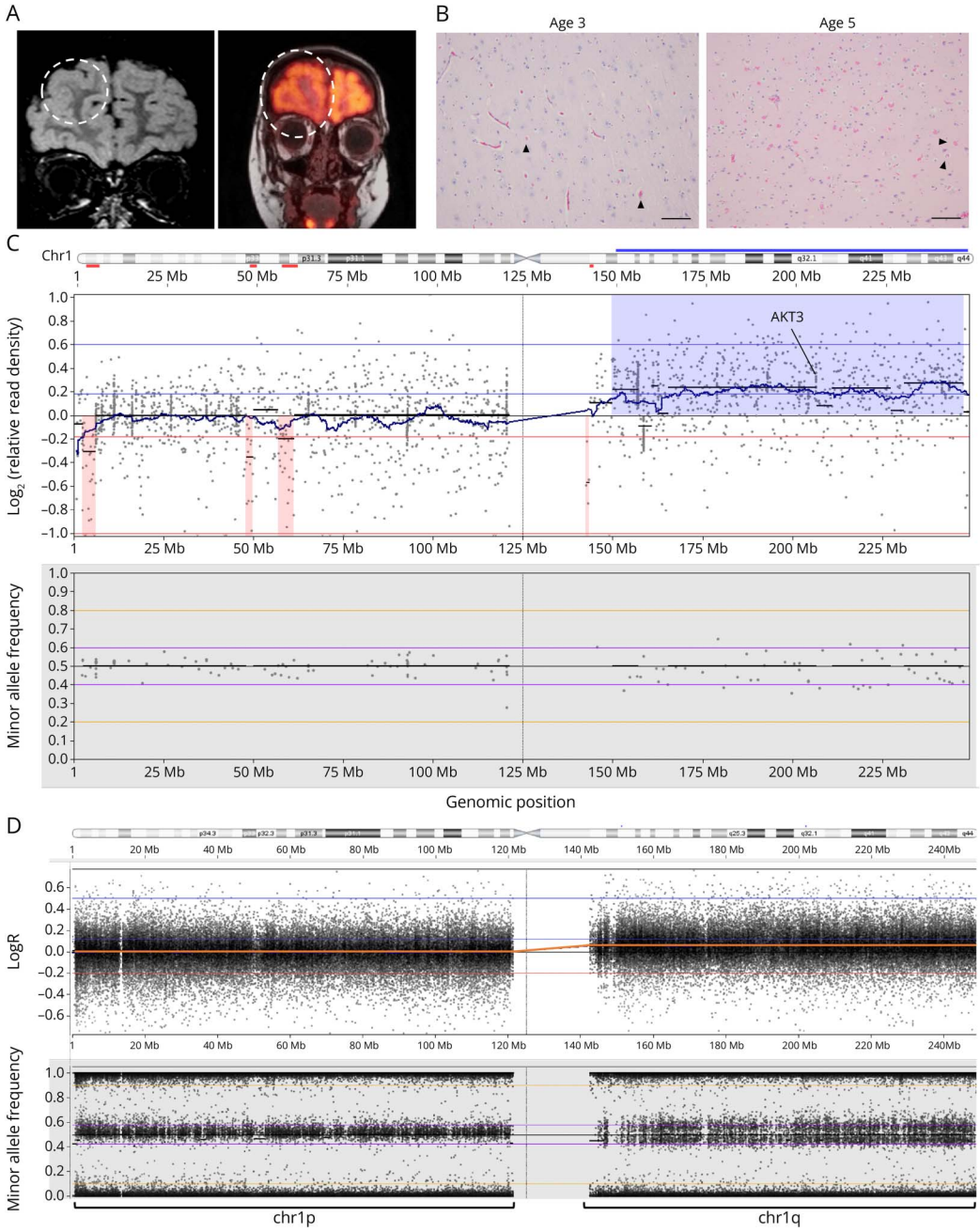
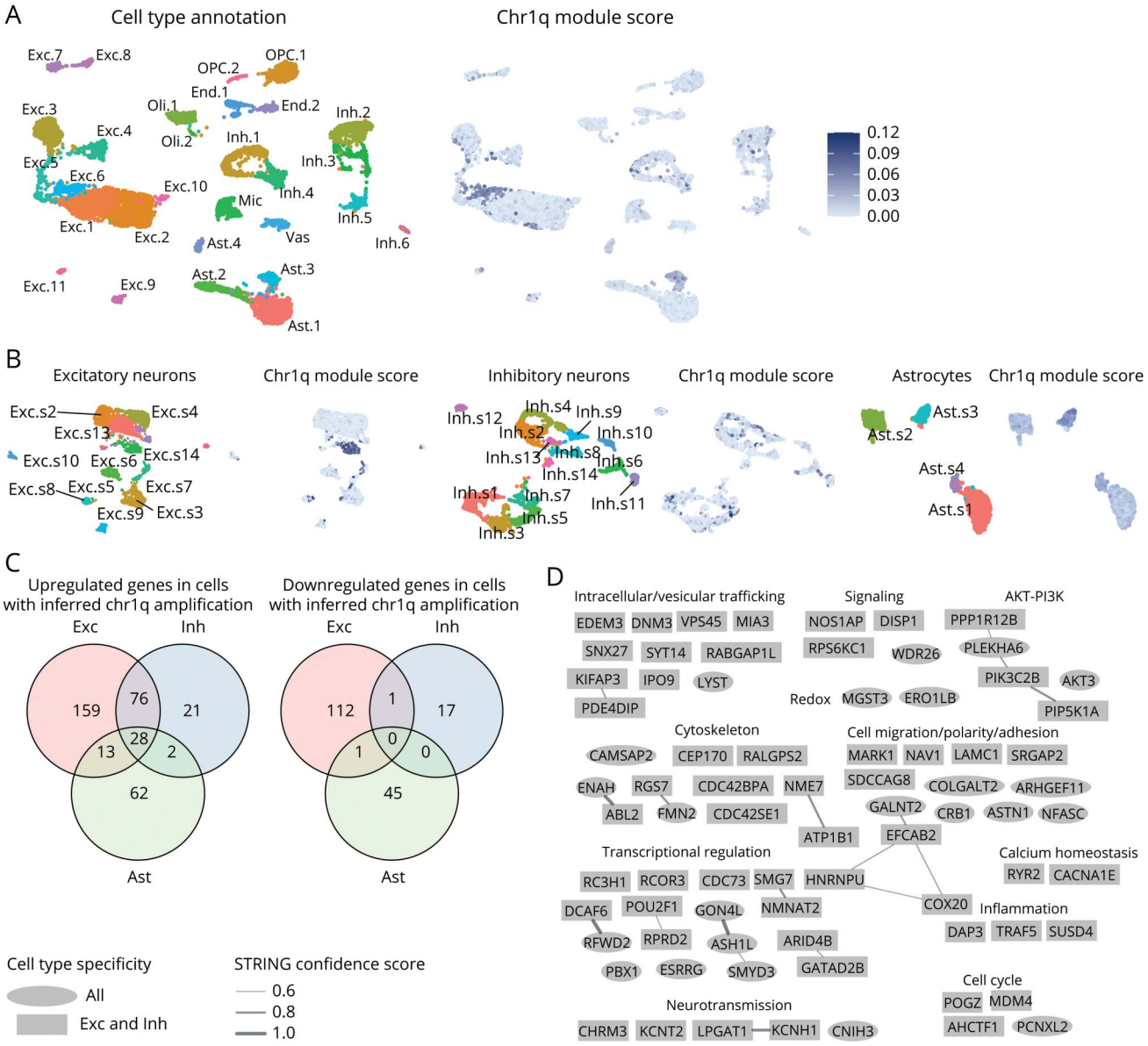
Figure 1 Right Frontal Lobe Abnormalities on Neuroimaging, Hyaline Protoplasmic Astrocytopathy on Neuropathology, and Evidence of Mosaic chr1q Gain on Genomic Analyses



(A) 3T T2-FLAIR MR image (left) at age 40 months demonstrating subtle increased hyperintensity in the right frontal white matter with minimal blurring of the gray-white border (dotted circle) suggestive of focal cortical dysplasia; PET image (right) at age 3 years demonstrating hypoperfusion in the right frontal lobe (dotted circle). (B) H&E sections of surgically resected cortical tissue from the surgeries at age 3 and 5 years demonstrating worsened hyaline protoplasmic astrocytopathy (representative inclusion-laden astrocytes labeled by black arrowheads) over time. Scale bar, 100 μm. (C) Visualization of relative read density (top) and minor allele frequency of common single-nucleotide polymorphisms (bottom) from the UCSF 500 Cancer Gene Panel performed on surgically resected brain tissue from the surgery at age 3 years. Regions highlighted in blue in the top subpanel reflect regions of chr1q with definitive copy number gain; the jagged appearing black line in the top subpanel corresponds to the running average of the relative read density, and the horizontal black lines in the top subpanel correspond to the average relative read density within corresponding genomic regions. (D) Visualization of logR values (see eMethods) and minor allele frequency derived from the Illumina CytoSNP 850 K array applied to genomic DNA extracted from neuronal nuclei isolated by fluorescence-activated nuclei sorting from surgically resected brain tissue at age 3 years. The orange line reflects the running average of the logR value along chr1. The remaining colored horizontal lines serve as visual guides.

Figure 2 Mosaic chr1q Gain in Neurons and Astrocytes and Associated Alterations in Gene Expression Identified by snRNA-seq



(A) Uniform manifold approximation projection (UMAP) of cells recovered from snRNA-seq, colored by cell cluster assignment (left) or chr1q module score (right). (B) UMAP of excitatory neuron, inhibitory neuron, or astrocyte subclusters colored by subcluster assignment or chr1q module score. (C) Venn diagrams of upregulated (left) or downregulated (right) differentially expressed genes (DEGs) associated with chr1q gain in excitatory neurons, inhibitory neurons, or astrocytes. (D) Network visualization of chr1q gain associated upregulated DEGs shared between excitatory and inhibitory neurons grouped by function. Lines connecting genes reflect evidence of regulatory relationships (see eMethods). Ast = astrocytes; End = endothelial cells; Exc = excitatory neurons; Inh = inhibitory neurons; Mic = microglia; Oli = oligodendrocytes; OPC = oligodendrocyte precursor cells; Vas = vascular cells.

Hyaline protoplasmic astrocytopathy (HPA) is a rare neuropathologic finding often associated with epilepsy and MCD[7] (see eAppendix 1) characterized by protoplasmic astrocytes laden with electron-dense, nonmembrane-bound hyaline inclusions reported to have filamin A, GLT-1 (SLC1A2), α-β-crystallin, and cytoglobin immunoreactivity.[8,9]

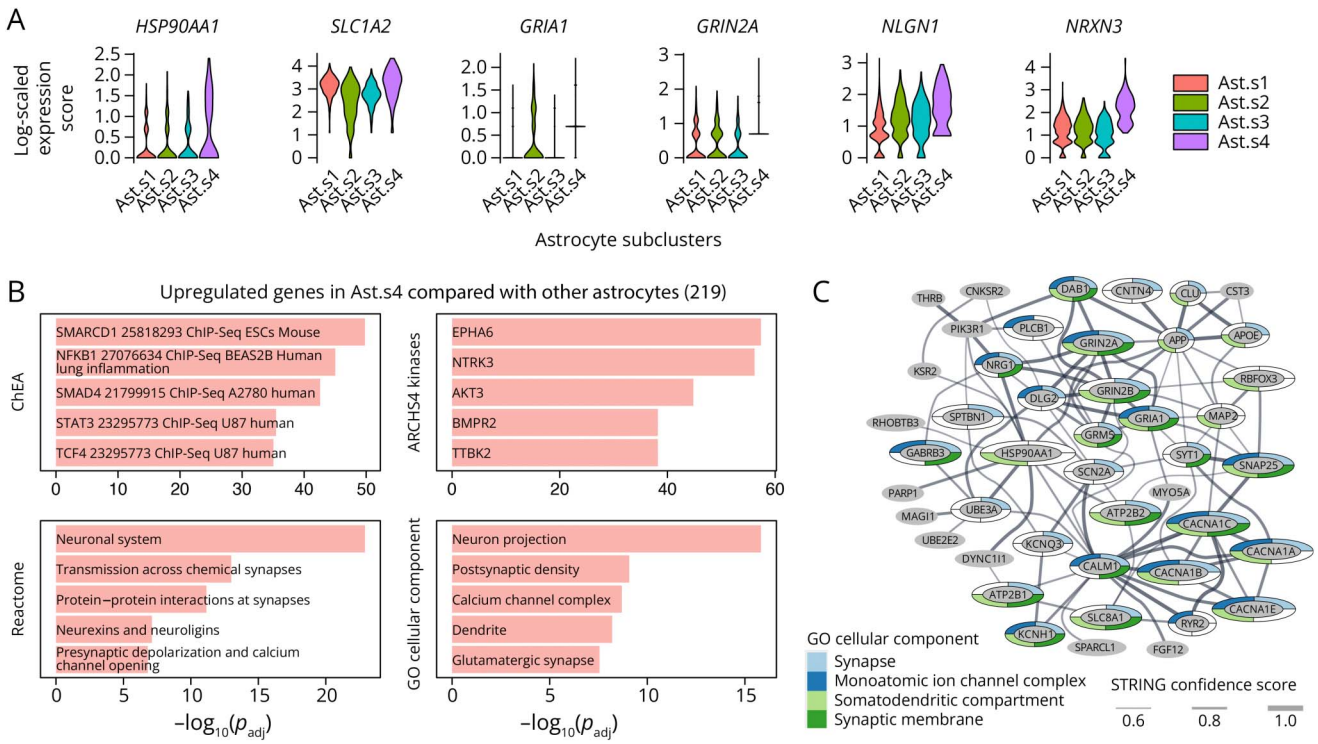
In this study, we present the case of a child with pharmacoresistant epilepsy who was found to have mosaic chr1q gain as well as HPA from 2 sequential epileptic focus resection surgeries. We performed single-nuclei RNA sequencing (snRNA-seq) of brain tissue from the second resection to gain insights into the cell-type specificity of his mosaic chr1q gain and the molecular signatures associated with HPA.

Methods

Research Ethics and Informed Consent

Tissue was acquired and deidentified by the Neurosurgery Tissue Bank at the University of California, San Francisco,

Figure 3 A Subpopulation of Astrocytes Enriched in Synapse-Associated Transcripts Identified on Subclustering Analysis



(A) Log-scaled expression of selected synapse-associated transcripts in astrocyte subclusters. (B) Enriched terms from the ChEA, ARCHS4, Reactome, and GO Cellular Component gene set libraries (see eMethods) for genes upregulated in Ast.s4 compared with other astrocytes. (C) Network visualization of upregulated genes in Ast.s4 that have a known regulatory relationship with Hsp90 (HSP90AA1) or with other genes that have a known regulatory relationship with Hsp90.

with patient consent in strict observance of the legal and institutional ethical regulations under the UCSF Committee on Human Research (IRB # 10–01318). The tissue was snap frozen in liquid nitrogen in less than 30 minutes after surgical resection.

Single-Nuclei RNA Sequencing (snRNA-seq)

snRNA-seq was performed on fresh frozen brain tissue from the patient's second epileptic focus resection. 128 mg of frozen brain tissue was cut at −20°C and sent on dry ice to SingulOmics Corporation (New York) for nuclei extraction and snRNA-seq using the 10x Genomics v3 platform, targeting recovery of 10,000 nuclei and 25,000 reads per cell. Approximately 200 million PE150 reads were sequenced on an Illumina NovaSeq 6000 sequencer.

Data Analysis

Processing and mapping of raw reads from snRNA-seq and cell calling were performed with Cell Ranger (v7.1.0) using GRCh38-2020-A with inclusion of intronic regions as the reference genome. Analysis of snRNA-seq data was performed in R (v4.3) using Seurat (v5). See eMethods in Supplemental Information.

Data Availability

Raw reads and the filtered feature-barcode matrix from snRNA-seq are available on GEO (GSE241521).

Results

Clinical Course

Our patient presented with seizures at 6 months of age. For details of his initial clinical presentation and workup, see eAppendix 2. 3T MRI at age 40 months demonstrated very subtle increased T2-FLAIR signal throughout the white matter of the anterior right frontal lobe with minimal blurring of the gray-white border, raising suspicion for FCD (Figure 1A), which was not visible on 3T MRIs performed at age 6 months and 23 months due to incomplete myelination of the frontal lobes. PET scan at age 40 months showed right frontal lobe hypoperfusion (Figure 1A).

An intraoperative electrocorticography (ECOG)–guided right frontal lobe resection was performed at age 40 months to remove the epileptogenic focus (eFigure 1A). On neuropathology, HPA was seen (Figure 1B; eFigure 1B). In addition, there appeared to be an abnormal number of heterotopic neurons (>30/mm^2) in the subcortical white matter (eFigure 1C) without definite cortical dysplasia (eFigure 1B), consistent with the new ILAE classification of mMCD with excessive heterotopic neurons.[6] Genomic analysis of the resected brain tissue using the UCSF500 Cancer Gene Panel with a buccal swab sample for comparison revealed an approximate 1.2-fold gain in copy number of chr1q in the resected brain tissue (Figure 1C, eFigure 2A)

but not in the buccal sample, indicative of mosaic chr1q gain in the brain. Due to continued seizures and failure of medical management, a motor-sparing right frontal lobectomy was performed at age 5 years. The resected tissue from the second surgery demonstrated an approximate 1.05- to 1.1-fold gain in chr1q copy number, which was lower compared with prior but with more extensive HPA (Figure 1B). Electron microscopy performed on tissue from the second surgery showed numerous large inclusions composed of electron-dense, nonmembrane-bound granular material (eFigure 1D). The inclusions appeared to be predominantly in cortical astrocytes and were frequently juxtanuclear in location, consistent with prior ultrastructural studies of HPA.[8,9]

Transcriptomic Profiling of Surgically Resected Brain Tissue

We performed snRNA-seq of fresh frozen brain tissue from the second surgery of our patient, recovering nuclear transcriptomes corresponding to 10,764 cells at ~23,000 mean reads per cell and 1,880 median genes detected per cell. After initial clustering and quality control (see eMethods), 10,179 cells were retained for further analysis. Uniform manifold approximation projection of abovementioned cells showed well-separated clusters (Figure 2A) of excitatory and inhibitory neurons, astrocytes, oligodendrocytes, oligodendrocyte precursor cells, microglia, endothelial cells, and vascular cells, which were assigned their respective cell types based on alignment to a gold standard reference data set (see eMethods). All clusters demonstrated expression of appropriate cell-type specific genes (eFigure 3A) and acceptable quality metrics (eFigure 3B).

To infer the cell-type specificity of mosaic chr1q gain, the expression of genes on chr1q was compared with that of all other genes to generate a chr1q module score (Figures 2A-B, eFigures 4–5). In parallel, we also used inferCNV to conduct an unbiased assessment of chromosomal copy number (eFigure 6, see eMethods). Both methods revealed chr1q gain to be restricted to neurons and astrocytes (eFigures 4–6). See eAppendix 3 for a detailed analysis. Next, we experimentally confirmed mosaic chr1q copy number gain in neurons using fluorescence-activated nuclei sorting followed by RT-PCR (see eMethods and eFigure 7) and high-density SNP array analysis (Figure 1D, eFigure 1B) on genomic DNA extracted from sorted nuclei.

Differentially expressed genes (DEGs) in cells with inferred chr1q gain compared with those without (see eMethods) showed cell-type specificity, with 28 upregulated genes shared among all 3 cell types (Figure 2C, eFigure 8, eTable 1). Among the shared DEGs were *LYST*, a regulator of lysosomal and endosomal trafficking, and *AKT3*, an upstream activator of the mTOR pathway; both genes are located on chr1q. Activating somatic mutations in *AKT3* have been associated with epilepsy and MCD and may drive pathology through overactivation of the mTOR pathway.[10,11] However, we did not recover a significant enrichment for the mTOR pathway in chr1q gain–associated DEGs, which could be attributed to the fact that mTOR acts mainly through its kinase activity and does not directly affect transcription. Chr1q gain–associated DEGs demonstrated enrichment for genes involved in cellular adhesion and intercellular junctions (eFigures 9–12, eTable 2), processes that are important for cell migration. Given that cortical development requires the appropriate migration of neuronal progenitors and young migratory neurons, we suspected that chr1q gain–associated DEGs in neurons may be involved in cellular migration. Indeed, examination of NCBI and UniProtKB gene summaries of upregulated DEGs shared between excitatory and inhibitory neurons revealed annotations for processes such as cytoskeletal regulation (e.g., *FMN2*), vesicular trafficking, cellular adhesion, and cellular migration (e.g., *ASTN1*); furthermore, there was evidence of regulatory interactions among many of these genes (Figure 2D).

We next turned our attention to evaluate whether chr1q gain exhibited enrichment in cell-type subpopulations by subcluster analysis excitatory neurons, inhibitory neurons, and astrocytes. We found subclusters with marked enrichment of chr1q-gained cells in excitatory neurons (Exc.s6) and astrocytes (Ast.s3, Ast.s4), but not inhibitory neurons, where chr1q-gained cells were distributed more evenly across subclusters (Figure 2B). In excitatory neurons, Exc.s6 compared with all other excitatory neurons demonstrated differential expression of genes involved in neuronal projections (eFigure 13, eTables 3–4). In astrocytes, Ast.s3, compared with all other astrocytes, demonstrated downregulation of genes involved in glutamate uptake, such as *SLC1A2*, and potassium transport, such as *KCNIP4* (eFigure 14, eTable 3–4). Notably, Ast.s4, compared with all other astrocytes, demonstrated a striking enrichment of synapse-associated transcripts such as *SHISA9*, *GRIA1*, *GRIN2A*, *GRIN2B*, *NGLN1*, and *NRXN3*[12] in conjunction with upregulation of *HSP90AA1* (Figure 3, A–C, eFigure 15, eTables 3–4), which encodes the Hsp90 chaperone. We confirmed the presence of this astrocyte subpopulation in a recently published snRNA-seq data set of additional patients with mosaic chr1q gain and HPA[13] (eFigure 16).

Discussion

The enrichment of chr1q gain–associated DEGs for genes involved in cell adhesion and migration, which was also seen in an independent data set[13] suggests that chr1q gain disrupts the developmental migration of neuronal and glial progenitor cells. See eAppendix 4 for further discussion.

In astrocytes, chr1q gain may disrupt homeostatic astrocyte functions such as glutamate uptake and potassium buffering (as seen in Ast.s3), which may contribute to epilepsy. Furthermore, we discovered a subpopulation of astrocytes with overrepresentation of chr1q-gained cells and marked

enrichment of synapse-associated transcripts (Ast.s4), which was also present in the data from the study conducted by Miller et al.[13] (eFigure 16). We hypothesize that the enrichment of synapse-associated transcripts in these astrocytes stems from phagocytosis of synaptic debris.[14] This hypothesis is supported by the fact these astrocytes have increased expression of *ADGRB1* (eFigure 16F), which encodes BAI1, an adhesion GPCR that recognizes phosphatidylserine.[15] We speculate that with cytoskeletal, vesicular trafficking, and lysosomal abnormalities secondary to chr1q gain, these astrocytes may have exceeded their degradative or intracellular trafficking capacity, which may contribute to the inclusions observed in protoplasmic astrocytes on neuropathology and electron microscopy. Please see eAppendix 5 for additional discussion.

Last, please see eAppendix 6 for additional discussion of the clinical findings and eAppendix 7 for additional discussion of the methodologic merits and limitations of our snRNA-seq results. In summary, we identified cell-type specific changes in gene expression associated with chr1q gain and possibly HPA in our patient concordant with findings in similar patients, which pointed toward cellular pathways potentially affected by these pathologies. Ultimately, spatial transcriptomics and proteomics will further elucidate the molecular signatures and dysregulated cellular functions associated with chr1q gain and HPA.

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Disclosure

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Orit A. Glenn, MD	Department of Radiology and Biomedical Imaging, University of California, San Francisco	Drafting/revision of the article for content, including medical writing for content; analysis or interpretation of data
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