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NEPHROLOGY ROUNDS

Clinical Real-Time Genome Sequencing to Solve the Complex and Confounded Presentation of a Child With Focal Segmental Glomerulosclerosis and Multiple Malignancies

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Received 6 April 2022; revised 19 July 2022; accepted 25 July 2022; published online 7 August 2022

Kidney Int Rep (2022) **7,** 2312–2316; https://doi.org/10.1016/j.ekir.2022.07.174
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INTRODUCTION

The individualized clinical management and treatment of rare pediatric oncologic diseases often present multiple challenges due to the underlying severity of the primary neoplastic disease, additional complications related to surgical and medical treatment, and other confounding presentations that might or might not be related to the primary diagnosis.[1,2]

Here we present the case of 13-year-old male of Middle East ancestry, clinically diagnosed with tuberous sclerosis (TSC), who was treated at Columbia University Irving Medical Center/New York-Presbyterian Morgan Stanley Children's Hospital for glioblastoma multiforme (GBM). His clinical course was complicated and confounded by multiple comorbidities, including nephrotic syndrome with biopsy findings of focal segmental glomerulosclerosis (FSGS), and refractory ascites caused by gastrointestinal neoplasia, and whose diagnosis was clarified by real-time rapid clinical genome sequencing (GS) resulting in a unifying molecular diagnosis and individualized treatment.

CASE DESCRIPTION, GENETIC-DRIVEN DIAGNOSIS, AND MANAGEMENT

A 13-year-old boy of Middle Eastern ancestry with clinical diagnosis of TSC, presented to New York-Presbyterian Morgan Stanley Children's Hospital in August 2018 for a second opinion and treatment of GBM. The family history was negative for TSC or malignancy, but positive for unspecified kidney disease in a female maternal cousin.

In 2015, when he was 11, the patient was clinically diagnosed with TSC based on history of seizures and café-au-lait skin lesions. In January 2017, following recurrent generalized seizures, a brain magnetic resonance imaging (MRI) showed multiple lesions that, based on the clinical history, were considered as consistent with cortical tubers, and additional larger masses in the left hemisphere (left Monroe foramen region, left parietal lobe, and left temporal lobe). In April 2017, he underwent partial resection of the left parietal-occipital mass at an outside hospital and the pathology report showed GBM and subependymal

giant cell astrocytoma. After the surgery, he was started on everolimus, an mammalian target of rapamycin kinase inhibitor used in TSC; temozolomide, an alkylating chemotherapic agent; radiation therapy; and later, bevacizumab, an anti-VEGF monoclonal antibody. In April 2018, his course was complicated by nephrotic syndrome that prompted stopping bevacizumab.[3] He remained on everolimus and temozolomide and was then referred to our medical center for a second opinion.

In August 2018, when admitted to our institution, a new brain magnetic resonance imaging showed multiple brain masses, prompting 2 additional brain surgeries. Notably, both masses were consistent with residual glioblastoma and no lesions compatible with TSC were present (Figure 1a and 1b). The genetic testing performed on the resected mass was negative for microsatellite instability and oncogenic BRAF variant. One month later, shortly after being discharged, the patient presented to the New York-Presbyterian Morgan Stanley Children's Hospital Emergency Department with new onset abdominal pain and emesis. On physical exam at admission, he was noted to have bilaterally decreased breadth sounds at the bases with profound abdominal distension and no peri-orbital, facial, or peripheral edema. An abdominal

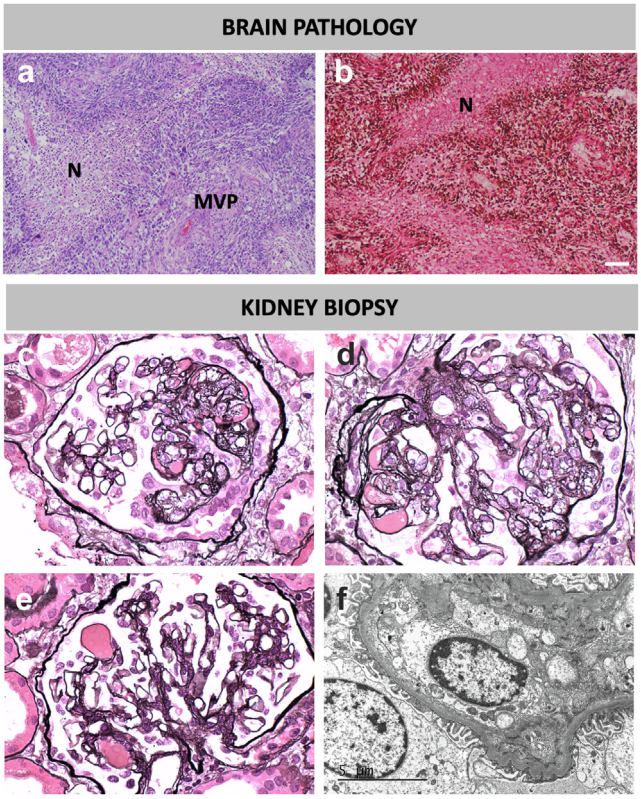

Figure 1. Histology findings from brain and kidney tissues. The brain pathology from resection of the left parietal mass showed GBM in absence of any lesion compatible with tuberous sclerosis-associated tubers. (a) The glioma is composed of highly atypical tumor cells and has typical features of a glioblastoma, including pseudopalisading necrosis and microvascular proliferation. H and E, hematoxylin and eosin. Scale bar = 100 μm. (b) The tumor cells are positive for glial fibrillary acidic protein and *SOX2*. Scale bar = 100 μm. The renal biopsy indicated a diagnosis of FSGS with focal collapsing features and focal features of subacute microangiopathy. Briefly, of the 45 glomeruli sampled for light microscopy, 3 were globally sclerotic and 5 contained discrete lesions of segmental sclerosis with hyalinosis, foam cells, visceral epithelial cell swelling, and tuft adhesions. One glomerulus exhibited global collapsing sclerosis with hyperplasia of the overlying glomerular epithelial cells. Some glomeruli also showed reticulated mesangial matrix suggestive of healing mesangiolysis, glomerular capillary microaneurysms containing hyaline material, and segmental narrow duplications of glomerular basement membrane. Immunofluorescence revealed focal segmental mesangial positivity for IgM (1–2+), with similar 1+ IgA, C3, C1q, kappa and lambda. Electron microscopy revealed focal endothelial cell swelling with loss of fenestrations and rare duplication of glomerular basement membrane. Podocyte foot process effacement involved approximately 30% of the glomerular capillary surface area. (c) A representative glomerulus contains a discrete lesion of segmental sclerosis and hyalinosis causing luminal obliteration, with hypertrophy of the overlying visceral epithelial cells (Jones methenamine silver, ×400). (d) A glomerulus contains segmental sclerosis and hyalinosis with tuft adhesion to Bowman's capsule, as well as mesangiolysis, reticulated mesangial matrix consistent with healing mesangiolysis, and focal narrow duplications of glomerular basement membrane (Jones methenamine silver, ×600). (e) There are focal glomerular capillary microaneurysms filled with eosinophilic hyaline material. Several glomerular basement membranes also have narrow duplications. (Jones methenamine silver, ×600). (f) Electron micrograph showing a swollen glomerular endothelial cell with loss of fenestrations and focal mild (30%) foot process effacement, (Jones methenamine silver, ×10,000).

ultrasound showed the presence of ascites, requiring large volume paracentesis. Laboratory testing showed significant proteinuria, with urine protein-to-creatinine ratio ranging from 1.2 to 5.5 mg/mg (normal <0.2 mg/mg), trace hematuria with dysmorphic red blood cells, low serum albumin (2.6 g/dl), increased serum creatinine (1.31 mg/dl) and serum cystatin C (1.1 mg/l), hyperlipidemia (total cholesterol 366 mg/dl, triglycerides 179 mg/dl, HDL 66 mg/dl, LDL 264 mg/dl), and normal or negative serologies. In light of the ascites associated to heavy proteinuria, low serum albumin, and abnormal kidney function, the patient underwent percutaneous left kidney biopsy.

The biopsy revealed FSGS with focal collapsing features and focal features of subacute microangiopathy, including glomerular basement membrane duplications, endothelial cell swelling, mesangiolysis, and capillary loop microaneurysms (Figure 1c-1f). Because of the positive family history for kidney disease and the renal biopsy findings suggesting an FSGS pattern secondary to an underlying Mendelian genetic mutation, in October 2018 the patient was enrolled in our IRB-approved clinical-grade GS study "Rapid Genome Sequencing to Guide Clinical Management of Children with Nephrotic Syndrome." Following pretest counseling and explanation of the study by the investigational team, signed consent was obtained by the patient's guardian, and a blood sample was collected and sent to the clinical laboratory at the New York Genome Center as part of our study. The GS variant analysis was performed using the New York Genome Center's clinical pipeline, which follows the GATK best practices guidelines to extract single nucleotide and copy number variants.[4–6] Using an in-house designed variant annotation pipeline, we prioritized variants that occurred in a manually curated list of 678 nephropathy-associated genes, which included 126 genes that are associated with Mendelian forms of nephrotic syndrome/FSGS or phenocopies of disease, and additional 552 genes associated with Mendelian forms of non-NS/FSGS nephropathies.[7,8] We next extended our analysis to Online Mendelian Inheritance in Man annotated, Mendelian disease–associated genes.[9] Variants were classified as "pathogenic" or "likely pathogenic" according to the American College of Medical Genetics and Genomics guidelines for the interpretation of DNA sequence variants in clinical setting.[S1] The initial genetic analysis, reported to the nephrologist in November 2018, with total turnaround time of 29 days, excluded causal variants in any of the known FSGS genes or other genes that when mutated can cause a kidney disease that can phenocopy FSGS.[7,S2] Simultaneously, upon return of the kidney-related genetic results, pathology studies from a peritoneal surgical resection showed disseminated signet cell carcinoma. At that moment, the presence of multiorgan malignancies in a young individual questioned the diagnosis of TSC as the predisposing factor to glioblastoma and suggested an inherited multiple neoplasia syndrome. We therefore extended the GS analysis beyond the kidney-associated genes and indeed excluded causal point mutations or copy number variations at both chromosomes 9q34.13 (*TSC1*) and 16p13.3 (*TSC2*) loci, thus rectifying a wrong clinical diagnosis that acted as a confounding predisposing factor to GBM. Remarkably, GS identified a likely pathogenic homozygous variant in *MSH6* (c.263delG, p.Cys88LeufsTer61), which was diagnostic for constitutional mismatch repair deficiency syndrome (CMMRD; MIM# 276300). The same mutation was subsequently confirmed by exome sequencing as part of the IRB-approved Diagseq study conducted at the Institute of Genomic Medicine, the genetic analysis later performed on the peritoneal resected tissue tested negative for microsatellite instability whereas immunohistochemistry showed loss of MSH2 and MSH6 proteins.[S3–S6] Following the positive *MSH6* finding diagnostic for CMMRD, the patient's treatment was immediately switched from palliative FOLFOX4 (Leucovrin, Oxaliplatin, and 5-Flurourcil) chemotherapy, started following the diagnosis of signet cell carcinoma, to immunotherapy with nivolumab, an immune checkpoint inhibitor that is indicated in mismatch repair deficient cancers.[S7,S8]

The presence of a homozygous *MSH6* mutation also prompted cascade genetic testing in the family, that was performed in January 2019 as part of our protocol. As expected, both the patient's mother (32-year-old and healthy) and the father (40-year-old and healthy), were heterozygous for the p.Cys88LeufsTer61 mutation, as well as the 12-year-old sister. This heterozygous carrier status poses individuals at risk for Lynch syndrome and cancer screening was therefore recommended. The 9-year-old brother was negative for the *MSH6* mutation.

The patient showed initial response to nivolumab, and in January 2019 was discharged and able to return home to United Arab Emirates with his family, although he subsequently experienced a gastrointestinal perforation and expired secondary to septic shock in February 2019.

DISCUSSION

We describe the case of a child with multiple malignancies, FSGS, and confounding clinical diagnoses, where the real-time use of genomic sequencing was instrumental to identify a unifying molecular etiology of disease, to disprove the muddling clinical diagnosis of TSC, and to support the medication-induced origin

of FSGS from the anti-VEGF bevacizumab and the kinase inhibitor everolimus treatment.[3,S9] This resulted in the correct diagnosis of CMMRD and initiation of personalized treatment, as well as cascade family genetic testing (Figure 2).

The diagnostic and clinical course was complex, having been delayed by several confounding factors. First, multiple central nervous system malignancies and brain masses in the pediatric setting can be a feature of various syndromes, including TSC, where the presence of tubers has been associated with predisposition to brain masses, including GBM and astrocytoma,[S10,S11] as well as primary genetic syndromes such as the CMMRD.[S12] Whereas the diagnosis of TSC is often based on clinical features and imaging work-up, this can lead to misdiagnosis and delay in identification of the cause of brain cancer and in the initiation of appropriate treatment. Conversely, only genetic testing enables the definitive diagnosis of CMMRD. CMMRD is an autosomal recessive cancer predisposition syndrome caused by recessive (homozygous or compound heterozygous) variants in the mismatch repair genes *MLH1, MSH2, MSH6,* or *PMS2*, and is characterized by brain tumors, colon cancer, hematologic cancer, and café-au-lait macules.[S13,S14] CMMRD tumors have been shown to respond well to therapy with checkpoint inhibitors, such as nivolumab.[S7,S8] TSC is a rare autosomal dominant genetic disorder due to point mutations or deletions in *TSC1* or *TSC2*, and characterized by the growth of numerous benign tumors (tubers) in many parts of the body, including brain, skin and kidneys. Its cutaneous manifestations also include café-au-lait macules that, together with seizures and multiple brain lesions at imaging studies lead to the wrong clinical diagnosis of TSC as the

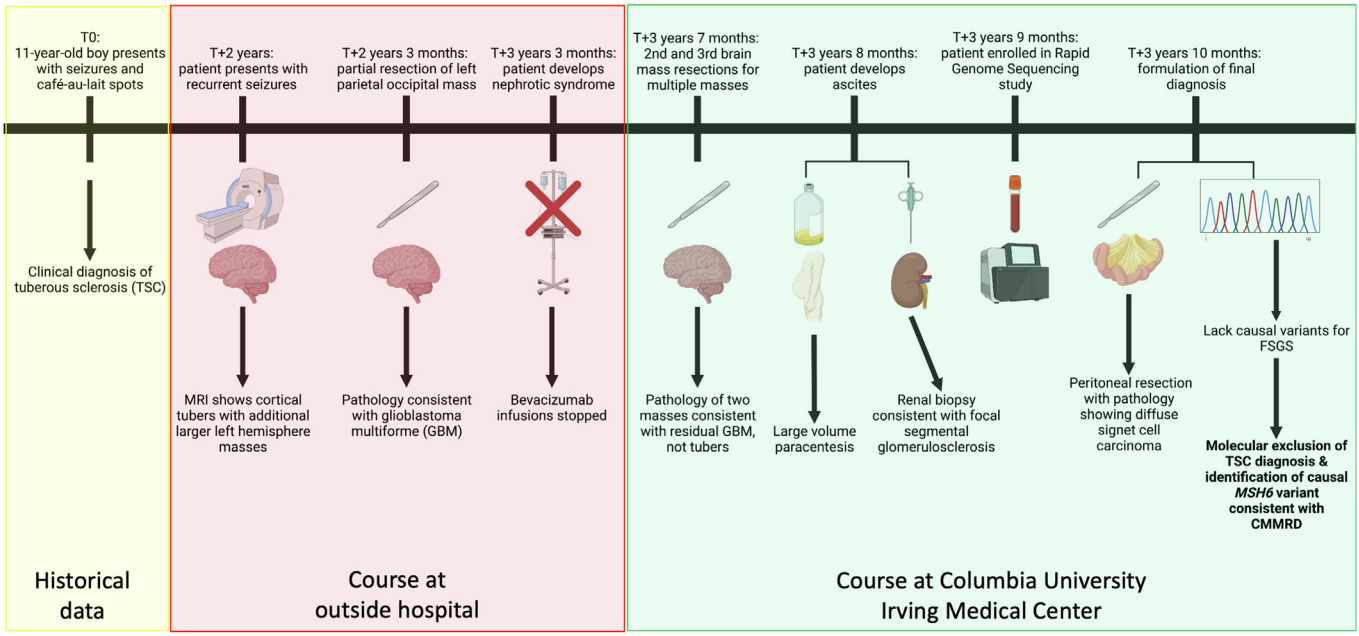

Figure 2. Timeline of clinical course, diagnostic testing, and procedures T0: clinically diagnosis of TSC based on history of seizures and café-au-lait skin lesions at the age of 11 years. T+2 years: the brain magnetic resonance imaging performed for recurrent generalized seizures showed multiple lesions, considered as consistent with cortical tubers based and with the clinical diagnosis of TSC, and additional larger masses in the left hemisphere. T+2 years 3 months: resection of the left parietal-occipital mass. The pathology was consistent with GBM and subependymal giant cell astrocytoma. After the surgery, the patient was started on everolimus, temozolomide, and, bevacizumab. T+3 years 3 months: the onset of nephrotic syndrome prompted the interruption of bevacizumab treatment. T+3 years 7 months: a new brain magnetic resonance imaging showed multiple masses requiring 2 additional brain surgeries. The pathology of both masses was consistent with residual glioblastoma. No lesions compatible with TSC were present. T+3 years 8 months: admission for ascites, requiring large volume paracentesis, and nephrotic syndrome with abnormal kidney function and normal or negative serologies. A percutaneous kidney biopsy showed FSGS with focal collapsing features and focal features of subacute microangiopathy, including glomerular basement membrane duplications, endothelial cell swelling, mesangiolysis, and capillary loop microaneurysms. T+3 years 9 months: patient was enrolled in the IRB-approved clinical-grade GS study "Rapid Genome Sequencing to Guide Clinical Management of Children with Nephrotic Syndrome." T+3 years 10 months: pathology from peritoneal surgical resection showed disseminated signet cell carcinoma. Simultaneously, the initial genetic analysis excluded causal variants in any of the known FSGS genes or other genes that when mutated can cause a kidney disease that can phenocopy FSGS. Given the presence of multiorgan malignancies in a young individual, the GS analysis was extended beyond the kidney-associated genes, excluding causal variants in *TSC* genes (*TSC1* and *TSC2*), and identifing a likely pathogenic homozygous variant in *MSH6* diagnostic for CMMRD thus prompting the switch from palliative chemotherapy to immunotherapy with the immune checkpoint inhibitor nivolumab. Cascade genetic testing in the family followed. CMMRD, constitutional mismatch repair deficiency; FSGS, focal segmental glomerulosclerosis; GBM, glioblastoma multiforme; MRI, magnetic resonance imaging; TSC, tuberous sclerosis. This figure was created with BioRender at BioRender.com.

Table 1. Teaching points

Number	Teaching point
1	Anti-VEGF agents and kinase inhibitors can cause nephrotic syndrome with a biopsy pattern of focal segmental glomerulosclerosis
2	The occurrence of glioblastoma is possible but rare in patients with tuberous sclerosis
3	CMMRD syndrome caused by mutations in *MSH6* is associated to multiple cancers, including astrocytoma, glioblastoma, and gastrointestinal carcinomas
4	CMMRD can present with café-au-lait spots, thus potentially confounding the clinical diagnosis of tuberous sclerosis, which should always be confirmed by genetic testing
5	Real-time clinical genome sequencing can improve diagnosis and treatment in children with complex and confounded presentations

CMMRD, constitutional mismatch repair deficiency; VEGF, vascular endothelial growth factor.

predisposing factor to GBM, ultimately leading to a delay in the recognition of CMMRD in our patient. Finally, also the occurrence of FSGS contributed to complicate this clinical picture. FSGS is a kidney histopathological pattern characterized by the presence of segmental sclerotic lesions in at least one glomerulus revealed by kidney biopsy. It can be idiopathic; secondary to several processes, including congenital or acquired reduced renal mass, to a monogenic disorder; or medication-induced such as in the case of anti-VEGF agents or everolimus.[3,S9,S15] In this case, the presence of drug-induced heavy proteinuria confounded the recognition of malignant ascites due to the yet undiagnosed signet cell carcinoma as if it was part of the nephrotic syndrome picture, further complicating the diagnostic work-up.

The real-time use of GS prompted us to rapidly exclude a genetic form of FSGS, re-evaluation of the kidney biopsy to support the iatrogenic origin of the kidney disease, the rectification and exclusion of the clinical diagnosis of TSC as the predisposing factor to the GBM, and, finally, the identification of a homozygous *MSH6* pathogenic variant resulting in the final diagnosis of CMMRD (Table 1). This resulted in the initiation, although delayed by all the above-mentioned confounders, of nivolumab with initial positive response, that allowed the patient to be discharged home to his family. The discovery of CMMRD also affected the other members of the family because heterozygous *MSH6* (found in the parents and one sibling at cascade testing) are associated with Lynch syndrome.[S16] The mutations carriers are now subjected to strict imaging and endoscopic surveillance for the predisposition to different cancers, including colorectal, endometrial, ovarian, gastric, hepatic, biliary, urinary, and neurologic. Because sequencing is becoming faster, cheaper and reimbursable by third-party payers, and clinicians become more comfortable with ordering and interpreting genetic tests, the early use of GS will facilitate diagnosis and treatment in children with complex clinical presentations.

DISCLOSURE

This publication was supported by the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant Numbers UL1TR001873 and RC2 DK122397. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

All the authors declared no conflicts of interests.

PATIENT CONSENT

The authors declare that they have obtained consent from the parents of the patient discussed in the report.

ACKNOWLEDGMENTS

We want to thank the patient and family members who participated to the study.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary References.

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