Molecular Genetics & Genomic Medicine

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CLINICAL REPORT OPEN ACCESS

A Novel *EP300* Variant in an African American Girl With Global Developmental Delay and Leukemia

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Received: 18 December 2024 | **Revised:** 9 April 2025 | **Accepted:** 15 April 2025

Funding: The authors received no specific funding for this work.

Keywords: chromosomal microarray | EP300 | Menke–Hennekam syndrome-2 | whole exome

ABSTRACT

Background: Pathogenic germline missense and *in-frame* indel variants in exons 30 or 31 of the *EP300* gene are associated with Menke–Hennekam syndrome-2 (MKHK2). The phenotypic spectrum associated with MKHK2 is variable, including neurodevelopmental, respiratory, skeletal, and immunological impairments. Based on their genetic, clinical, and DNA methylation profiles, a recent study proposed three domain-specific subtypes of MKHK: MKHK-ZZ, MKHK-TAZ2, and MKHK-ID4. In somatic cells, *EP300* variants have been reported in lymphoma, leukemia, and various solid tumors. We present an African American girl with global developmental delay, failure to thrive, microcephaly, seizure, osteopenia, and T-cell acute lymphoblastic leukemia (T-ALL).

Method: We performed karyotype, FISH, chromosomal microarray, and exome sequencing with probands bone marrow, blood, and buccal swab.

Result: Comprehensive genetic studies using multiple tissues detected somatic complex cytogenomic changes in blood cells and a *de novo* germline missense variant (NM_001429.4: c.5258G>A, p.Cys1753Tyr) in the TAZ2 domain of *EP300* from her buccal swab, which is consistent with a diagnosis of MKHK2. While in our patient we observed phenotypic overlaps with affected individuals harboring variants in the TAZ2 domain, some phenotypes such as osteopenia and alopecia have not been reported previously. The hematolymphoid malignancy of our patient also raises the question of whether germline *EP300* variants are associated with a genetic predisposition to cancer.

Conclusion: Together, this case expands the growing body of knowledge regarding the clinical and genetic spectrum of MKHK2. This is the first MKHK individual reported in the literature in an underrepresented population of African American ancestry.

1 | Introduction

The *EP300* gene (OMIM#: 602700) encodes p300, a protein initially discovered as a nuclear binding target of the adenovirus E1A cancer protein with a molecular weight of 300 kDa (Eckner et al. 1994; Whyte et al. 1989). It functions as a histone acetyltransferase and transcription coactivator that regulates transcription via chromatin remodeling. EP300 plays an important role in various fundamental biological processes including cell proliferation, differentiation, apoptosis, and DNA repair (Chan and La Thangue 2001; Goodman and Smolik 2000). The functionally important domains of EP300 protein structure include the histone acetyltransferase (HAT) domain, bromodomain (BD), plant homeodomain (PHD), interesting new gene

(RING) domain, and three zinc finger domains (TAZ1, ZZ, and TAZ2) (Delvecchio et al. 2013).

Germline monoallelic loss-of-function variants in *EP300* and its closely related paralog *CREBBP* cause Rubinstein–Taybi syndrome (RSTS1 for *CREBBP* and RSTS2 for *EP300*, OMIM#: 180849 and 613684 respectively), a well-known disorder characterized by distinctive facial features, broad thumbs and halluces, short stature, and intellectual disability (Stevens 1993). In addition, pathogenic missense and in-frame indel variants in exons 30 and 31 of these two genes have been reported in some individuals who do not share the classic RSTS phenotype, such as facial characteristics or broad thumbs/halluces (Menke et al. 2016, 2018). The main characteristics among those patients include developmental delay, intellectual disability, behavioral problems including autism spectrum disorder, epilepsy, cerebral anomalies, feeding difficulties, recurrent upper airway infections, vision and hearing impairments, short stature, and microcephaly, which were recently described as Menke–Hennekam syndrome (MKHK1 for *CREBBP* and MKHK2 for *EP300*, OMIM#: 618332 and 618333 respectively). In somatic cells, *EP300* and *CREBBP* can function as either oncogenes or tumor suppressor genes depending on the cellular context, microenvironment, and tumor type, and a growing number of both loss- and gain-of-function genetic alterations in *EP300* and *CREBBP* have been reported in solid tumors and hematological malignancies (Attar and Kurdistani 2017).

Here we report a non-dysmorphic African American girl with global developmental delay, failure to thrive, microcephaly, seizure, osteopenia, and T-cell acute lymphoblastic leukemia (T-ALL). Comprehensive genetic studies using multiple tissues identify a *de novo* missense variant in the TAZ2 zinc finger domain of the *EP300* gene, which indicates a diagnosis of MKHK2.

1.1 | Ethical Compliance

This study was approved by the institutional review board of Columbia University Irving Medical Center. Informed consent was obtained from the proband's parents. Very few of the patient's demographics have been revealed, and the patient cannot be traced. All information in this report has been de-identified in accordance with HIPAA and institutional review board regulations.

2 | Clinical Presentation

The proband was a 3-year-old female who was a child of a non-consanguineous union between a father of Caribbean Black descent and a mother of Caribbean Black and American Indian descent. Her family history from the maternal side was positive for cleft lip (maternal grandfather's niece), adult-onset leukemia (maternal great-grandmother), and seizures (maternal uncle); her paternal side was positive for schizophrenia (paternal cousin). However, her family history was negative for intellectual disabilities/autism, neonatal or sudden death, young hearing loss, bleeding, or muscle disorders.

The proband's mother received prenatal care starting in the first trimester, and the prenatal history was significant for decreased fetal movements, polyhydramnios, and ultrasound findings of cleft lip. The proband was born vaginally at full-term gestation, weighing 5 lbs. to a then G3P2, 38-year-old female.

At 2 years of age, the proband was clinically diagnosed with gamma-delta T-cell acute lymphoblastic leukemia (T-ALL). She was sequentially treated with total marrow/lymphoid irradiation and haploidentical peripheral blood stem cell transplant from the mother. However, the disease relapsed, and she was deceased at the age of 3 years and 9 months.

Evaluation at the age of 3 years and 6 months showed she had global developmental delay, regression in social milestones, failure to thrive, microcephaly (< 1 percentile, $Z = -3.89$), left cleft lip and left partial gum cleft, eczema, primary adrenal insufficiency, diastolic dysfunction, alopecia, and seizure. Her x-ray results showed that her lower extremity was under mineralized and was consistent with osteopenia. No additional findings were observed for her eyes, ears, respiratory, endocrine, genitourinary, or musculoskeletal systems. She was non-dysmorphic and had normal fingers, fingernails, toes, and toenails bilaterally.

3 | Genetic Analysis

Chromosomal studies for the proband's bone marrow showed a highly complex karyotype with multiple subclones: 46,XX,t(8;9)(q22;p22),t(11;14)(p11.2;q32),del(18)(q21)[11]/46,idem,inv(6)(p22q21)[4]/46,idem,t(5;16)(q14;p13.2)[2]/46,idem,del(6)(q13q25)[2]/46,XX[25] (Figure 1A). Though such karyotypic aberrations predict poor clinical outcomes in all types of hematologic malignancies and are common in T-ALL, they are not specific to the diagnosis of T-ALL (Sun et al. 2019). FISH analysis using LSI 9p21/CEP9 probe showed heterozygous 9p21 (*CDKN2A*) deletion in 84% of cells, which was frequently seen in T-ALL (Figure 1B).

Chromosomal microarray analysis (CMA) using the proband's peripheral blood identified multiple copy number variants (CNVs) (arr[hg19] 8q11.23q12.1(55,081,007_56,692,301)x1, 9p22.1p21.3(19,807,298_21,920,647)x1, 9p21.3p21.3(21,928,299_22,235,519)x0[0.63], 9p21.3p21.2(22,241,295_26,851,119)x1, 18q12.2q12.3(35,749,370_37,759,778)x1). Specifically, a 7.044-Mb heterozygous (monoallelic) deletion including *MTAP*, *CDKN2A*, and *CDKN2B* was detected in all cells, while 63% of cells also had a smaller, 307 Kb deletion in the other allele, resulting in a homozygous (biallelic) loss of *CDKN2A* (Figure 1C). The CMA result was consistent with her karyotypic and FISH results and reflected somatic changes in blood cells of her T-ALL diagnosis. Repeated CMA with the proband's buccal swab specimen revealed a normal female chromosomal constitution. No CNVs except for those commonly seen in normal populations were detected.

Exome sequencing (ES) was performed on DNA derived from the buccal swab of the proband and her parental samples. Following the manufacturer's protocol, paired-end sequencing was performed on the Illumina HiSeq 2500 platform using the Agilent SureSelectXT (Human All Exon v.5 + UTRs)

capture kit. NextGENe (version 2.3; SoftGenetics LLC) software was used to align (hg19) and annotate the sequence data. A New York State–approved in-house-developed pipeline was used to filter and annotate variants. Variants were reviewed as part of the workflow for constitutional clinical exome sequencing as previously described (Kurtz et al. 2021). Overall, ES achieved average coverage of 98.95% for the proband and 97.5% for the father and mother for the coding region. Trio-based exome analysis identified a *de novo* heterozygous single base pair substitution in the clinically relevant gene, *EP300* (chr22:41572973G>A, GRCh37/hg19). This missense variant (NM_001429.4: c.5258G>A) resides in exon 31 (the last exon) which causes substitution of cysteine for tyrosine at position 1753 out of 2415 amino acids total (NP_001420.2: p.Cys1753Tyr) (Figure 2). This variant has not been reported so far in the literature or any human disease mutation databases.

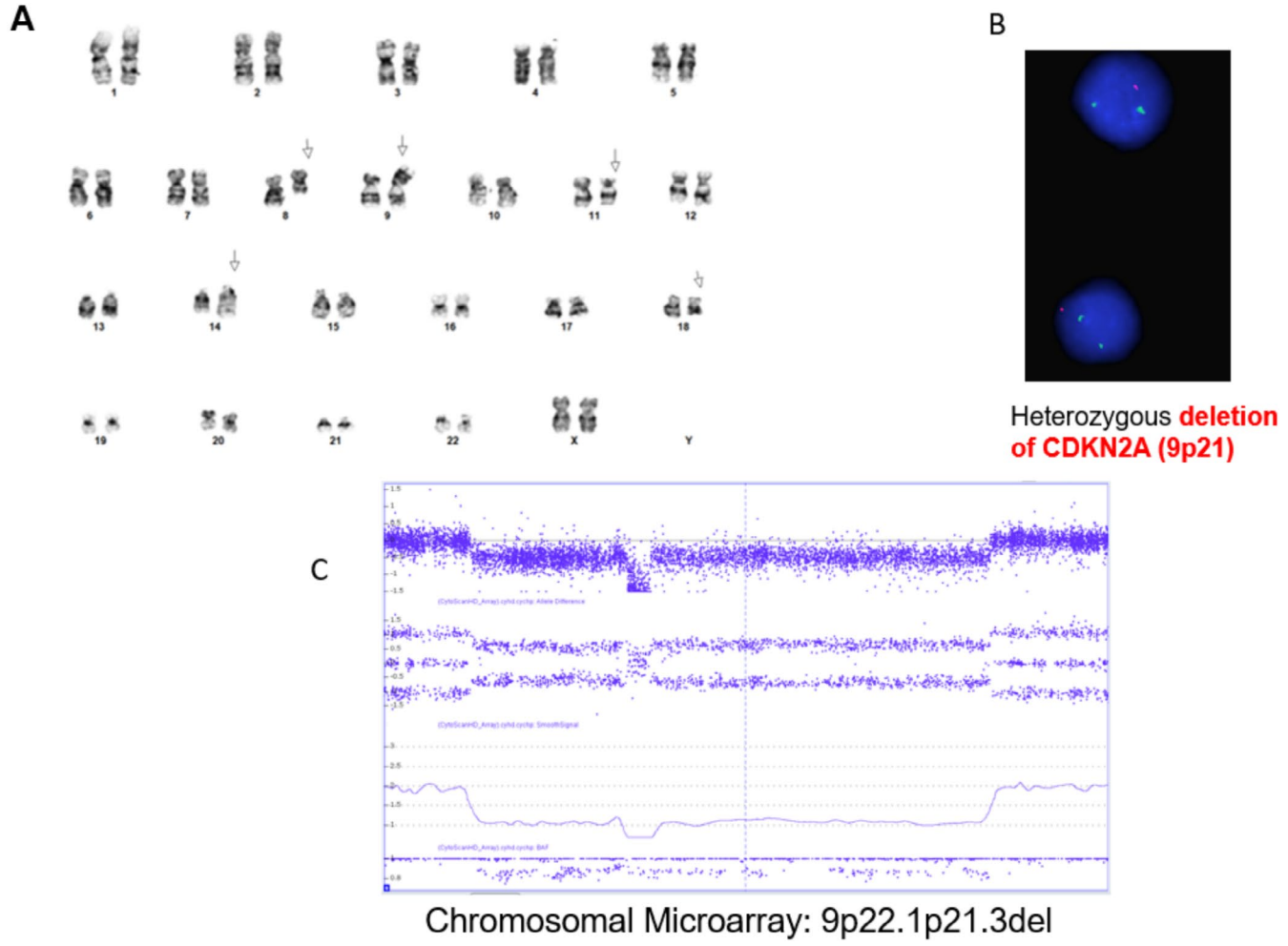

FIGURE 1 | (A) Chromosome analysis (bone marrow) showed a cell with complex karyotype: 46,XX,t(8;9)(q22; p22),t(11;14)(p11.2;q32),del(18)(q21). (B) FISH analysis using LSI 9p21(red)/CEP9(green) probe showed heterozygous 9p21 (*CDKN2A*) deletion in 84% of cells. (C) Chromosome microarray analysis (blood sample) revealed a large heterozygous loss in chromosomal location 9p22.1p21.2 and a smaller mosaic homozygous loss including *CDKN2A* in 9p21.3.

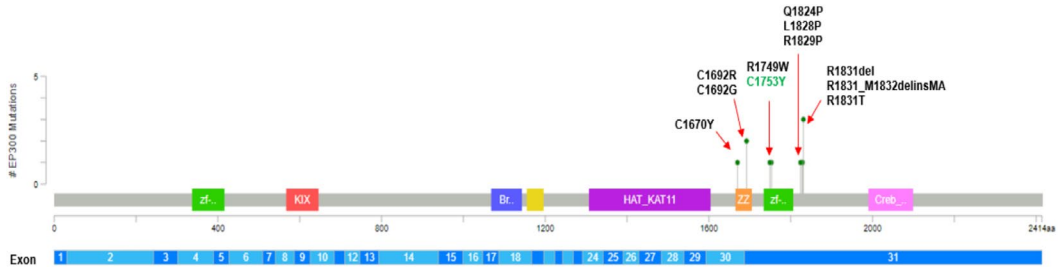

FIGURE 2 | Distribution of *EP300* variants associated with MKHK2. C1753Y variant (green) in the TAZ2 domain was detected in the current study. Other variants (black) were reported previously (Haghshenas et al. 2024; Menke et al. 2018). Domain: ZF/TAZ-type 1 (green, 331-417 aa), KIX (red, 566-645 aa), bromo (blue, 1067–1139 aa), CBP/p300-type HAT (purple, 1287-1663 aa), ZZ-type (Orange, 1665-1713 aa), TAZ-type 2 (Green, 1728-1809 aa).

It was also absent in the Genome Aggregation Database (gnomAD v4.1.0), indicating that it was not a common benign variant in the populations represented in these databases. This variant resides in the TAZ2 Zing finger domain of the EP300 protein, and it is predicted to be damaging/deleterious by most of the *in silico* prediction programs including Provean (score: −10.76), SIFT (score: 0), Revel (score: 0.94), and AlphaMissense (score: 1). Based on both clinical and genetic findings, this patient was diagnosed with Menke–Hennekam syndrome 2 (MKHK2).

4 | Discussion

Compared with the well-known, relatively common RSTS, the newly discovered MKHK is much rarer, and MKHK2 caused by *EP300* mutations is even rarely reported. To date, there are only 11 *EP300* variants reported in MKHK2 patients, including 9 missense variants, one in-frame deletion, and one in-frame indel (Figure 2) (Haghshenas et al. 2024; Menke et al. 2018). All these patients are of European descent, and here we report the first MKHK individual in African Americans.

Recently Haghshenas et al. evaluated a cohort of 81 patients with variants within the MKHK region of *EP300* or *CREBBP* and found their common features included intellectual disability, autism spectrum disorder, and other behavioral problems, cerebral anomalies, strabismus, recurrent infections, feeding and gastrointestinal problems, vision and/or hearing impairments, low birth height and weight, and microcephaly (Haghshenas et al. 2024). In addition, by comparing their clinical and DNA methylation profiles, the authors proposed three domain-specific subtypes of MKHK: MKHK-ZZ, MKHK-TAZ2, and MKHK-ID4, rather than gene-specific subtypes (MKHK1 and MKHK2). Particularly, the MKHK-TAZ2 subtype included 27 individuals with variants located in the TAZ2 domain of CREBBP (26 individuals) or EP300 (one individual). They shared a domain-specific methylation profile and characteristics including hearing impairment, dental anomalies, cryptorchidism, muscle hypertrophy/hypertonia, contractures, anomalies of the extremities (mostly clubfeet), cleft palate, laryngeal anomaly, congenital heart anomaly, renal anomaly, epilepsy, hypothyroidism, kyphosis/scoliosis, hip dysplasia, hypermobility, and inguinal hernias (Haghshenas et al. 2024). Our patient's variant is in the TAZ2 domain and is the second reported *EP300* variant that belongs to this subtype. This cysteine residue in the TAZ2 domain is located at a zinc ion binding spot, and thus is important for stabilizing helical folding. We therefore hypothesize that the C1753Y variant detected in this study disturbs the zinc ion binding and affects the coordination of the two zinc finger domains, similar to many other MKHK variants in both *EP300* and *CREBBP* (Haghshenas et al. 2024). Most features of our patient have also been seen in other patients of this subtype. However, some phenotypes such as osteopenia and alopecia have not been reported previously, which may indicate a phenotypic expansion.

Due to the extensive roles of *EP300* and *CREBBP* in tumorigenesis, it is important to assess the cancer risk for individuals carrying germline variants of these two genes. Early studies of RSTS patients suggested that they have an increased incidence of cancer, with 5% diagnosed with childhood tumors of neural crest origin (Miller and Rubinstein 1995). However, a later study with molecular confirmation did not support evidence for an increased risk for malignant tumors in Dutch individuals with RSTS, though the incidence of specific benign tumors including meningiomas and pilomatricomas was significantly elevated in this population (Boot et al. 2018). While the overall cancer risk is not well-defined due to the rarity of the RSTS syndrome, there have been several reports showing that RSTS patients diagnosed with cancer (Boot et al. 2018; Johannesen et al. 2015; Sy et al. 2018). In fact, loss-of-function (LOF) mutations of p300/CBP occur frequently in various types of human cancers (Mullighan et al. 2011; Pasqualucci et al. 2011), though their roles in hereditary cancer syndromes are unclear. A recent international consensus statement for RSTS diagnosis and management recommends "oncologic surveillance of individuals with RSTS should follow national healthcare standards without need for additional surveillance" (R44) due to no evidence for an increased cancer risk in individuals below 40 years of age and uncertain value of additional surveillance at an older age (Lacombe et al. 2024). As for MKHK, to the best of our knowledge, no incidence of cancer development has been reported previously in these patients. Our patient therefore raises the possibility of an increased cancer risk in MKHK patients. As all the reported variants in MKHK patients are missense and in-frame indel variants, unlike the LOF variants in RSTS, it has been hypothesized that the disease mechanism for MKHK is gain-of-function (GOF). This hypothesis was supported by three-dimensional facial images of MKHK patients, which resemble those with a duplication of 16p13.3 including *CREBBP*, while are opposite to those with RSTS (Menke et al. 2018). Interestingly, several recent studies indicated that the TAZ2 domain of EP300, where the variant in this presented child is located, has an autoinhibitory role for p300, and mutations found in cancer that interfere with autoinhibition by TAZ2 allosterically activate p300 (Ibrahim et al. 2022; Xu et al. 2023; Yu et al. 2023). We also found that a total of 29 missense variants and one in-frame insertion variant in the TAZ2 domain were detected in diverse cancer types in the COSMIC database. Among them, two missense variants (p.K1783E and p.A1787S; COSM7349764, COSM4774848) and one in-frame insertion variant (p.F1805_C1806insGP, COSM7350949) were detected in patient samples from acute lymphoblastic T-cell leukemia (Neumann et al. 2015; Richter-Pechanska et al. 2017). Therefore, MKHK patients could be prone to cancer due to a GOF disease mechanism.

In conclusion, we report the first MKHK patient of African American descent and the second individual with an *EP300* variant in the TAZ2 domain. The clinical features of our patient may help to expand the phenotypic spectrum of this disorder and raise the question of whether germline MKHK variants are predisposed to an increased cancer risk, as somatic variants of *EP300* and *CREBBP* have been reported extensively in different types of cancers. However, further studies are necessary to establish a definitive link between any specific germline variants and cancer susceptibility. Clinicians should exercise caution when interpreting these findings, as the rarity of the disorder and limited available data necessitate additional research to confirm potential associations. Until more is known, careful

monitoring and long-term follow-up of affected individuals may be considered to assess potential oncogenic risks.

Author Contributions

S.B. and J.L. performed molecular genetic studies and wrote the manuscript. V.V.M. performed cancer cytogenetic studies. A.I. collected clinical data and made diagnosis. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

All data generated or analyzed during the present study are included in this published article.

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