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신경모세포종을 동반한 *EZH2* 유전자 변이가 확인된 위버증후군의 국내 첫 증례 보고

First Korean Case of Weaver Syndrome Along with Neuroblastoma and Genetic Confirmation of *EZH2* Variant

오인성 · 김보람 · 이지수 · 김만진 · 조성임 · 박성섭 · 성문우
Inseong Oh, M.D.[1], Boram Kim, M.D.[1,2], Jee-Soo Lee, M.D.[1], Man Jin Kim, M.D.[1], Sung Im Cho, M.T.[1], Sung Sup Park, M.D.[1,2], Moon-Woo Seong, M.D.[1,2]

서울대학교병원 진단검사의학과[1], 의생명연구원[2]
Department of Laboratory Medicine[1] and Biomedical Research Institute[2], Seoul National University Hospital, Seoul, Korea

Weaver syndrome (WS) is a rare congenital disorder characterized by overgrowth and accelerated osseous maturation. This syndrome is caused by a variant in the enhancer of zeste homolog 2 (*EZH2*) gene. No genetically confirmed cases of WS have been reported in Korea. In this case report, we discuss a case in which a variant in *EZH2* was detected and confirmed as WS in a patient showing overgrowth syndrome accompanied by neuroblastoma. A 7-month-old female presented to the out-patient pediatrics clinic of Seoul National University Hospital because of multiple palpable masses. Pathological examination confirmed that the mass was neuroblastoma. The patient's height, head circumference, and weight were ≥97% of those expected for her age. The c.2050C>T (p.Arg684Cys) variant of *EZH2* was confirmed through next-generation sequencing-based gene panel testing. Although overgrowth syndrome caused by variants in *EZH2* is rare, screening for this condition should be included in the gene panel to evaluate overgrowth syndrome. The possibility of WS should be considered in cases of overgrowth syndrome accompanied by neuroblastoma.

Key Words: Weaver syndrome, Neuroblastoma, Enhancer of zeste homolog 2, Congenital Disorder

INTRODUCTION

Weaver syndrome (WS; OMIM 277590), first reported in 1974, is a rare congenital disorder characterized by overgrowth, distinct facial features, and accelerated osseous maturation. According to a previous study, WS is caused by a variant in the enhancer of zeste homolog 2 (*EZH2*) gene [1]. *EZH2* variants were also identified in 48 patients with WS with the clinical phenotype described above [1]. Approximately 50% patients were *de novo* cases, whereas familial cases were inherited in an autosomal dominant pattern [2]. In Korea, although potential WS cases have been reported, an *EZH2* variant has not been confirmed [3]. In this case report, we discuss the first Korean case of WS along with genetic confirmation of an *EZH2* variant. The Institutional Review Board of Seoul National University Hospital (Seoul, Korea) approved the study (approval number: 2111-131-1275). Informed consent was obtained from the patient's legal guardians.

Corresponding author: Moon-Woo Seong, M.D., Ph.D.
https://orcid.org/0000-0003-2954-3677
Department of Laboratory Medicine, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul 03080, Korea
Tel: +82-2-2072-4180, Fax: +82-2-747-0359, E-mail: mwseong@snu.ac.kr

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CASE

A 7-month-old female was presented to the out-patient pediatrics clinic of Seoul National University Hospital with multiple palpable masses on her back, left side of the head, and extremities. The masses were approximately 1-3 cm in size and moved upon touch, but were reportedly not painful. The patient did not have

any perinatal problems and was delivered at full-term. However, her height, head circumference, and weight were ≥97% of those expected for her age. The patient was tall in stature and exhibited macrocephaly. Physical examinations revealed doughy skin and umbilical hernia; however, camptodactyly, hoarse cry, and abnormal tone were absent. Her characteristic facial features included frontal bossing, round face, retrognathia, wide-spaced eyes, low-set ears, and cleft chin (Table 1). In the magnetic resonance imaging examination, a 7 cm mass was found on the left adrenal gland. Pathological examination of the mass revealed neuroblastoma on the left adrenal gland, and bone marrow aspiration confirmed the presence of neuroblastoma cells in the bone marrow (Fig. 1A).

As the patient was suspected of having overgrowth syndrome, she underwent next-generation sequencing-based gene panel testing, which revealed a heterozygous missense variant in NM_004456.4 (*EZH2*):c.2050C>T (p.Arg684Cys) (Fig. 1B). This variant is absent from large population databases such as gnomAD (https://gnomad.broadinstitute.org/) and the Korean Reference Genome Database (http://coda.nih.go.kr/coda/KRGDB/). A previous functional study demonstrated that, in the presence of the c.2050C>T

Table 1. Comparison of clinical phenotype of our patient with those of previous cases of Weaver syndrome

	Current study	Case 1 [14]	Case 2 [22]	Case 3 [23]	n/N* (%) [24]	n/N* (%) [2]
Facial features†	+	+	+	+	3/3 (100)	31/48 (65)
Hypertonia	-	-	+	-	1/3 (33)	11/39 (28)
Hypotonia	-	-	-	-	2/3 (67)	18/41 (44)
Camptodactyly	-	-	-	+	2/3 (67)	17/38 (45)
Umbilical hernia	+	-	+	-	3/3 (100)	17/40 (43)
Doughy skin	+	-	-	-	2/3 (67)	17/35 (49)
Hoarse cry	-	-	-	-	3/3 (100)	10/27 (37)
Malignancy	NB	AML	-	-	0/3 (0)	2/48 (4)‡
EZH2						
AA change	R684C	P132L	E745K	H240R		

*n/N refers to the proportion of patients exhibiting the corresponding phenotype among the total number of patients included in the reference reports describing the presence of a specific phenotype. †Facial features characterizing WS, namely ocular hypertelorism, frontal bossing, round face, and retrognathia, ‡Three types of cancer occurred in two patients with WS (lymphoma, NB and ALL).
Abbreviations: AA, amino acid; NB, neuroblastoma; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; WS, Weaver syndrome.

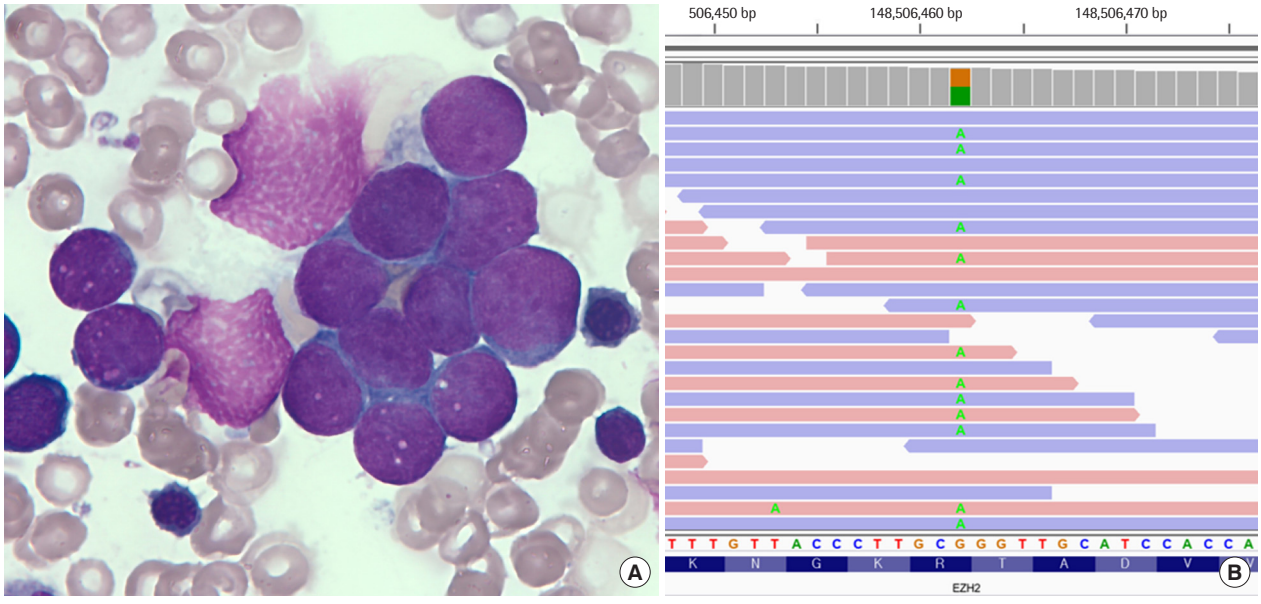

Fig. 1. Morphological characteristics of bone marrow aspirate and visualization of next-generation gene sequencing data of the patient. (A) Neuroblastoma cells cluster with fibrils (Wright-Giemsa stain, ×1,000). (B) Sequence visualization of enhancer of zeste homolog 2 (*EZH2*) gene showing a missense variant, c.2050C>T.

(p.Arg684Cys) variant of *EZH2*, the histone methyltransferase activity of the polycomb repressive complex-2 (PRC2) protein was significantly decreased compared to that of the wild-type enzyme [4]. In addition, a different missense change in the same codon (p.Arg684His) has been reported as a pathogenic variant [2]. *In silico* analyses using SIFT, Polyphen-2, and Mutation Taster supported the finding that this variant has deleterious effects on the protein function. This variant was previously reported as pathogenic [2, 4, 5]; thus, we subsequently classified it as pathogenic (PS3, PM2, PM5, PP3, and PP5) based on the 2015 American College of Medical Genetics and Genomics and Association for Molecular Pathology guidelines [6]. Other genes in the panel, including *AKT3, BRWD3, DNMT3, EED, GPC3, HIST1H1E, MTOR, NFIX, NSD1, PIK3CA, PPP2R5D,* and *PTEN*, did not contain variants. Moreover, the *EZH2* variant was detected in the adrenal gland biopsy tissue, and as a result, the patient was diagnosed with WS. She was started on chemotherapy with etoposide and doxorubicin, and her neuroblastoma showed a partial response after 10 cycles of treatment.

DISCUSSION

EZH2 encodes a histone-lysine N-methyltransferase that is a component of PRC2, which is associated with epigenetic silencing in cells. PRC2 exhibits histone methyltransferase activity and methylates histone H3 on lysine 27; the *EZH2* variant causes a partial loss of this methyltransferase function [7]. Variants in other PRC2 elements or other histone methyltransferase-related genes are genetically distinct from WS; however, some cases exhibit an overgrowth phenotype [8]. Particularly, these mechanisms are similar to the impaired histone methyltransferase activity of the variant of nuclear receptor binding SET domain protein 1 (*NSD1*), which causes Sotos syndrome, a type of overgrowth syndrome [9]. Sotos syndrome shows a high level of phenotypic overlap with WS [10]; therefore, overgrowth is expected to occur in cases of impaired methylation regulation [11]. However, analysis of EZH2 protein in patients with WS did not reveal a clear correlation between the severity of clinical symptoms and greatly decreased histone methyltransferase activity [4]. Therefore, phenotypic differences among patients with WS may be related to additional factors.

Both somatic gain-of-function and loss-of-function mutations of *EZH2* are associated with solid tumors, such as breast and prostate cancer, or hematologic cancers [12]. In contrast, evidence for the increased risk of malignancy in patients with *EZH2* germline variants is not sufficient [13]. Previous studies reported that cancers such as neuroblastoma, acute myeloid leukemia, acute lymphoblastic leukemia, teratoma, ovarian tumor, and lymphoma occur at an early age in patients with WS [1, 2, 14]. Three cases of neuroblastoma have been reported in 3-, 8-, and 9-month-old patients with WS, whereas only one case has been reported for each of the other cancer types [2]. Our patient was 6 months old at the time of neuroblastoma onset (7 months old when she presented to the clinic), which is consistent with similar cases. This case supports the finding that germline variants in *EZH2* may be associated with cancer, specifically neuroblastoma [15].

Diagnosing overgrowth syndrome is important for providing genetic counseling for cancer surveillance and prognosis [16]. Particularly, patients with Beckwith-Wiedemann syndrome show an increased risk of Wilms' tumor [17]. The incidence of cancer is reportedly increased in patients with other overgrowth syndromes, and variants in genes such as DNA (cytosine-5)-methyltransferase 3A (*DMNT3A*) are associated with solid or hematologic cancer [18-20]. The regular cancer screening plan has been proposed for patients with theses syndromes [21]. As neuroblastoma occurs increasingly in WS, we recommend that patients with WS should also undergo cancer surveillance.

This is the first Korean case of genetically confirmed WS, accompanied by the rare occurrence of early onset neuroblastoma. Although *EZH2* variant-induced overgrowth syndrome is rare, it should be included in the gene panel when evaluating overgrowth syndrome. Moreover, if overgrowth is accompanied by neuroblastoma, the probability of WS is high, and genetic testing for germline variants can help to confirm this diagnosis.

요 약

위버증후군은 과성장과 가속화된 골성숙을 특징으로 하는 드문 선천성 질환이다. 이 증후군은 *EZH2* 유전자의 변이에 의해 발병되는 것으로 알려져 있으며, 국내에서는 *EZH2* 유전자변이를 확인한 위버증후군은 보고된 증례가 없다. 본 증례에서는 신경모세포종이 동반된 과성장증후군 환자에서 *EZH2* 유전자의 변이가 검출되어 위버증후군으로 확인된 사례를 보고하고자 한다. 출생 후 7개월 된 환아가 신체에서 촉지되는 다발성의 종괴를 주소로 내원하였다. 종괴는 조직검사상 신경모세포종으로 확인되었으며, 환아

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는 키, 머리둘레, 몸무게 모두 소아발육표준치에 비해 상위 97% 이상인 특징을 보였다. 과성장증후군과 관련된 차세대염기서열분석 유전자 패널 검사에 의해 *EZH2* 유전자의 c.2050C>T (p.Arg684Cys) 변이를 확인하였다. 이는 *EZH2* 유전자의 변이에 의한 과성장증후군이 드물지만 과성장증후군을 평가하기 위한 유전자 패널에 포함되어야 하며, 신경모세포종이 동반되는 과성장증후군의 경우 위버증후군의 가능성을 고려해야 함을 시사하였다.

Conflicts of Interest

None declared.

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