의학박사 학위논문

분절 과증식 증후군 환자의 임상적 유전학적 특성과 치료 경과에 대한 고찰

Clinical and genetic analysis and treatment outcomes of patients with segmental overgrowth syndrome

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Abstract

Purpose: Segmental overgrowth syndrome refers to a group of proliferative disorders with diverse and overlapping symptoms. It is difficult to differentiate these diseases because their clinical aspects overlap with each other and the phenotype is very diverse within the same disease. Furthermore, effective treatments for these diseases have not yet been developed. This study investigated the clinical and genetic diagnosis and treatment outcomes of segmental overgrowth syndrome.

Methods: Fifteen patients with segmental overgrowth syndrome were enrolled. Clinical diagnosis with clinical characteristics and whole body magnetic resonance imaging (WB-MRI) findings were evaluated. Genetic diagnosis was made through targeted customized gene panel testing with affected tissues and peripheral white blood cells. Propranolol was administered and plasma cytokines and WB-MRI findings were examined. Clinical trial with the PIK3CA inhibitor or alpelisib was performed in two patients by managed access program.

Results: Clinically, 14 patients were suggested as having PIK3CA-related overgrowth spectrum (PROS) disorder; 12 patients as Klippel-Trenaunay syndrome and two patients as undetermined type of PROS. One patient was suspected to have Parkes-Weber syndrome. Seven patients (46.7%) were identified to have *PIK3CA* mutation. Two patients (13.3%) had *KRAS* mutation. Other identified mutations included *PTEN* (n=1, 6.7%), *MAP2K3* (n=1, 6.7%), *GNAQ* (n=1, 6.7%),

TBC1D4 (n=1, 6.7%) and *TEK* (n=1, 6.7%). Propranolol was administered in 12 patients and seven patients experienced improvement of symptoms. Alpelisib was administered in two patients and the WB-MRI after one year of treatment showed reduction of proliferated masses.

Conclusion: Targeted panel sequencing is useful in identifying the causative gene of segmental overgrowth syndrome. Propranolol could be used as an adjuvant therapy for reducing vascular symptoms of overgrowth syndrome. Targeted therapy considering genetic causes such as PIK3CA inhibitor would be the leading therapeutic strategy of overgrowth syndrome in the future.

Keywords: overgrowth syndrome, vascular malformation, PIK3CA-related segmental overgrowth syndrome, propranolol, alpelisib, targeted exome sequencing

차례

표 및 그림 목차

Introduction

Overgrowth syndrome refers to a group of the disorders with abnormally excessive tissue proliferation. Relatively well-known overgrowth syndromes include Beckwith-Wiedemann syndrome, Sotos syndrome, Weaver syndrome, Proteus syndrome and PIK3CA-related overgrowth spectrum (PROS).[1,2] These heterogeneous group of disorders can be divided into generalized or segmental overgrowth syndrome.[1,2] Segmental overgrowth syndrome has been known to be caused by somatic mutations of several genes and the affected patients show localized somatic and or visceral growth. Meanwhile, it is difficult to differentiate these diseases because their clinical aspects overlap with each other and the phenotype is very diverse within the same disease. Therefore, it is often difficult to identify the causative gene in patients with overgrowth syndrome.

Various signaling pathways in cells are known to influence proliferation of the cells. Especially, the PI3K/AKT/mTOR (Phosphoinositide-3-kinase/Protein Kinase B/mechanistic target of rapamycin) signaling pathway has the major role in cell growth, proliferation and differentiation.[3] The Ras/MAPK (Ras family of small GTPase proteins/mitogen-activated protein kinases) pathway also interacts with the PI3K/AKT/mTOR and regulates cell signaling.[3] There are also negative regulators of this pathway such as phosphatase and tensin homolog (PTEN) and tuberous sclerosis complex (TSC) 1 and 2.[3] The somatic mutations related in these pathways causes various

clinical symptoms of overgrowth; hemihypertrophy, megalencephaly, organomegaly, capillary malformation, and lymphatic malformation.[2,3] Phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) is a subunit of PI3Ks and its gain of function mutations could promote the PI3K/AKT/mTOR pathway and cause excessive growth in the affected tissue.[4-6] In addition, PIK3CA is related with oncogenesis.[2] The segmental overgrowth syndromes due to *PIK3CA* mutations, PROS, account for various subtypes of overgrowth syndromes; Klippel-Trenaunay syndrome, Megalencephaly-Capillary Malformation-Polymicrogyria syndrome (MCAP), fibroadipose hyperplasia and CLOVES syndrome.[3] In addition, there are other segmental overgrowth syndromes related with the activation PI3K-AKT-mTOR pathway such as Proteus syndrome, Cowden syndrome, and tuberous sclerosis.

There have been lots of efforts to develop inhibitors of PI3K, AKT and mTOR[3], but no practical treatment has yet been developed. The PIK3CA inhibitor named alpelisib (PIQRAY, Novartis Pharmaceuticals Corporation) has been reported to be efficient in the mouse models of PROS/CLOVES and is under investigation in clinical trials.[7,8] However, clinical trial on human is ongoing and verification of the effectiveness and stability is required. Propranolol has been used successfully for the treatment of hemangiomas, but the underlying mechanism of action is still uncertain. However, several previous studies explain the mechanism as propranolol induced reduction of angiotensin converting enzyme or inhibition of the AKT/mTOR pathway.[9-11] In this

regards, propranolol can be tried in overgrowth syndrome patients for inhibition of vascular proliferation. Indeed, propranolol has shown a partial effect in the regression of vascular masses of Klippel-Trenaunay syndrome patients.[12, 13]

Here we describe the clinical characteristics of segmental overgrowth syndrome patients and identify the causative genetic mutations. In addition, we report the efficacy and safety of long-term propranolol treatment to attenuate the phenotypes.

Materials and methods

1. Subjects and evaluation of clinical characteristics

This study was a single-center study performed at the Asan Medical Center, Seoul, Korea from February 2014 to May 2020. The study was approved by the Institutional Review Board of Asan Medical Center (no. 2020-1628). Fifteen patients with clinical features of segmental overgrowth were enrolled. The affected areas were evaluated by physical examination and whole body magnetic resonance imaging (WB-MRI). The findings of cutaneous capillary malformation such as port-wine stain, telangiectasia and angiokeratoma, varicosities and hypertrophy of soft tissues were analyzed. Medical photographs were taken yearly.

2. Genetic analysis

Genetic analysis was performed by targeted customized gene panel testing. Written informed consent was obtained for exome sequencing from all patients and the study was approved by the local ethics committee. Exome sequencing was performed using genomic DNA extracted from the affected tissue and peripheral blood leukocytes. The affected tissues were obtained by skin biopsy from the regions with hemangioma or cutaneous capillary malformation. Exomes were captured using the Agilent SureSelect Custom Panel (Agilent Inc., Santa Clara, CA, USA), which enriches a 372,068 bp region spanning 143 genes related with cell signaling pathway (Table 1).

Table 1. The list of the targeted genes.

No.	Gene	Number of target region	Size of target region (bp)
1	*PIK3CD*	22	4,013
2	*MTOR*	58	9,865
3	*SDHB*	8	1,151
4	*PIK3R3*	11	1,877
5	*PRKAA2*	9	2,019
6	*JAK1*	24	4,425
7	*GLMN*	18	2,437
8	*NRAS*	4	730
9	*THEM4*	6	963
10	*SHC1*	12	2,235
11	*LMNA*	15	2,819
12	*GLUL*	6	1,362
13	*MAPKAPK2*	12	1,591
14	*PFKFB2*	15	2,184
15	*FH*	10	1,851
16	*AKT3*	14	2,044
17	*LPIN1*	24	3,975
18	*KCNK3*	2	1,248
19	*SOS1*	23	4,896
20	*ANTXR1*	21	2,304
21	*BCL2L11*	9	1,097
22	*ACVR1*	9	1,890
23	*PDK1*	13	1,769
24	*BMPR2*	13	3,637
25	*PIKFYVE*	41	7,921
26	*IRS1*	3	3,725
27	*EIF4E2*	10	1,198
28	*VHL*	5	951
29	*RAF1*	17	2,669
30	*MAPKAPK3*	10	1,549
31	*GSK3B*	12	1,773
32	*PIK3CB*	22	4,059
33	*PDCD10*	7	903
34	*PIK3CA*	20	3,979
35	*KDR*	30	5,258
36	*FAT4*	18	15,485

Table 1. continued

37	*GAB1*	11	2,615
38	*VEGFC*	8	1,512
39	*RICTOR*	39	6,733
40	*PRKAA1*	11	2,205
41	*PIK3R1*	17	2,977
42	*AGGF1*	15	2,670
43	*RASA1*	27	4,092
44	*PPP2CA*	7	1,210
45	*FLT4*	30	5,276
46	*ATXN1*	3	2,437
47	*MAPK14*	13	1,723
48	*CDKN1A*	3	708
49	*VEGFA*	10	1,538
50	*SGK1*	17	2,600
51	*CCM2*	11	1,868
52	*HSPB1*	3	738
53	*YWHAG*	2	824
54	*KRIT1*	16	2,851
55	*SND1*	24	3,693
56	*RHEB*	8	875
57	*PPP2CB*	7	1,210
58	*IKBKB*	22	3,244
59	*LYN*	12	2,019
60	*DEPTOR*	10	1,507
61	*PTK2*	36	4,900
62	*RPS6*	6	990
63	*TEK*	23	4,287
64	*GNAQ*	7	1,360
65	*SYK*	13	2,428
66	*MAPKAP1*	12	2,063
67	*ENG*	15	2,560
68	*PTPA*	11	1,424
69	*TSC1*	22	4,315
70	*MAP3K8*	7	1,677
71	*GDF2*	2	1,370
72	*DDIT4*	2	779
73	*PTEN*	11	1,999
74	*PIK3AP1*	17	3,098

Table 1. continued

75	*CHUK*	22	3,041
76	*PDCD4*	12	1,876
77	*HRAS*	5	852
78	*ILK*	12	1,839
79	*WEE1*	16	2,095
80	*RAG1*	1	3,172
81	*ATG13*	17	2,312
82	*VEGFB*	7	931
83	*BAD*	3	627
84	*CCND1*	6	1,048
85	*GAB2*	10	2,417
86	*SDHD*	4	640
87	*CDKN1B*	2	677
88	*KRAS*	5	887
89	*MDM2*	11	1,934
90	*ATXN2*	29	4,815
91	*NOS1*	29	5,556
92	*PXN*	12	2,400
93	*UBC*	1	2,098
94	*ULK1*	28	4,236
95	*SMAD9*	6	1,644
96	*FOXO1*	4	1,949
97	*TBC1D4*	22	4,704
98	*SOS2*	23	4,890
99	*HIF1A*	16	3,228
100	*PGF*	7	793
101	*AKT1*	13	1,963
102	*RASGRP1*	17	3,073
103	*MAP2K1*	11	1,622
104	*TSC2*	42	7,137
105	*MLST8*	8	1,319
106	*PDPK1*	14	2,231
107	*ABAT*	15	2,096
108	*EEF2K*	17	2,858
109	*PRKCB*	18	2,878
110	*MAPK3*	8	1,460
111	*CDH5*	12	2,760
112	*PLCG2*	32	5,034

Table 1. continued

113	*YWHAE*	6	982
114	*TP53*	11	1,672
115	*FLCN*	12	2,415
116	*MAP2K3*	12	1,524
117	*NF1*	59	10,808
118	*RPS6KB1*	16	2,234
119	*PRKCA*	17	2,699
120	*MAP2K6*	12	1,485
121	*RPTOR*	34	5,368
122	*SMAD4*	11	2,071
123	*CCBE1*	11	1,661
124	*PHLPP1*	23	5,332
125	*BCL2*	4	772
126	*STK11*	9	1,662
127	*MAP2K2*	11	1,630
128	*PIK3R2*	17	2,526
129	*AKT2*	13	1,966
130	*BAX*	6	959
131	*AKT1S1*	6	996
132	*PRKCG*	20	2,859
133	*PLCG1*	32	5,156
134	*YWHAB*	5	941
135	*ELMO2*	20	2,963
136	*SOX18*	4	1,073
137	*MYT1*	23	4,033
138	*MAPK1*	8	1,361
139	*YWHAH*	2	805
140	*PRR5*	9	1,599
141	*VEGFD*	7	1,326
142	*XIAP*	6	1,720
143	*GAB3*	10	2,151

The schematic diagram of the bioinformatics pipeline for exome sequencing is presented in figure 1. Sequencing was performed on the NextSeq platform (Illumina Inc.). The mean depth of coverage was 878 reads per base with a 30X coverage of 99.3% for the affected tissue extracted DNA sequencing (Table 2). The mean depth of coverage was 346 reads per base with a 30X coverage of 99.0% for the blood extracted DNA sequencing (Table 2). Sequence reads were aligned to the reference genome, hg19, using Burrow-Wheeler Aligner (version 0.7.12, MEM algorithm).[14] Duplicate reads were removed using Picard tools 1.96. The Genome Analysis Toolkit (GATK version 3.7) was used for local realignment and base quality recalibration. Variant calling was performed using GATK MuTect2 and HaplotypeCaller[15] for tissue and blood, respectively. Common variants with minor allele frequency ≥ 1% were filtered out using public databases such as Genome Aggregation Database (http://gnomad.broadinstitute.org/), Exome Variant Server (http://evs.gs.washington.edu/EVS/) and 1000 Genomes Browser (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). Population-specific common variants were further filtered out using the Korean Reference Genome Database (KRGDB) (http://coda.nih.go.kr/coda/KRGDB/index.jsp). Variants were annotated using Variant Effect Predictor 88 and Oncotator (version 1.9.2). Candidate variants were manually curated by using Integrated Genome Viewer.

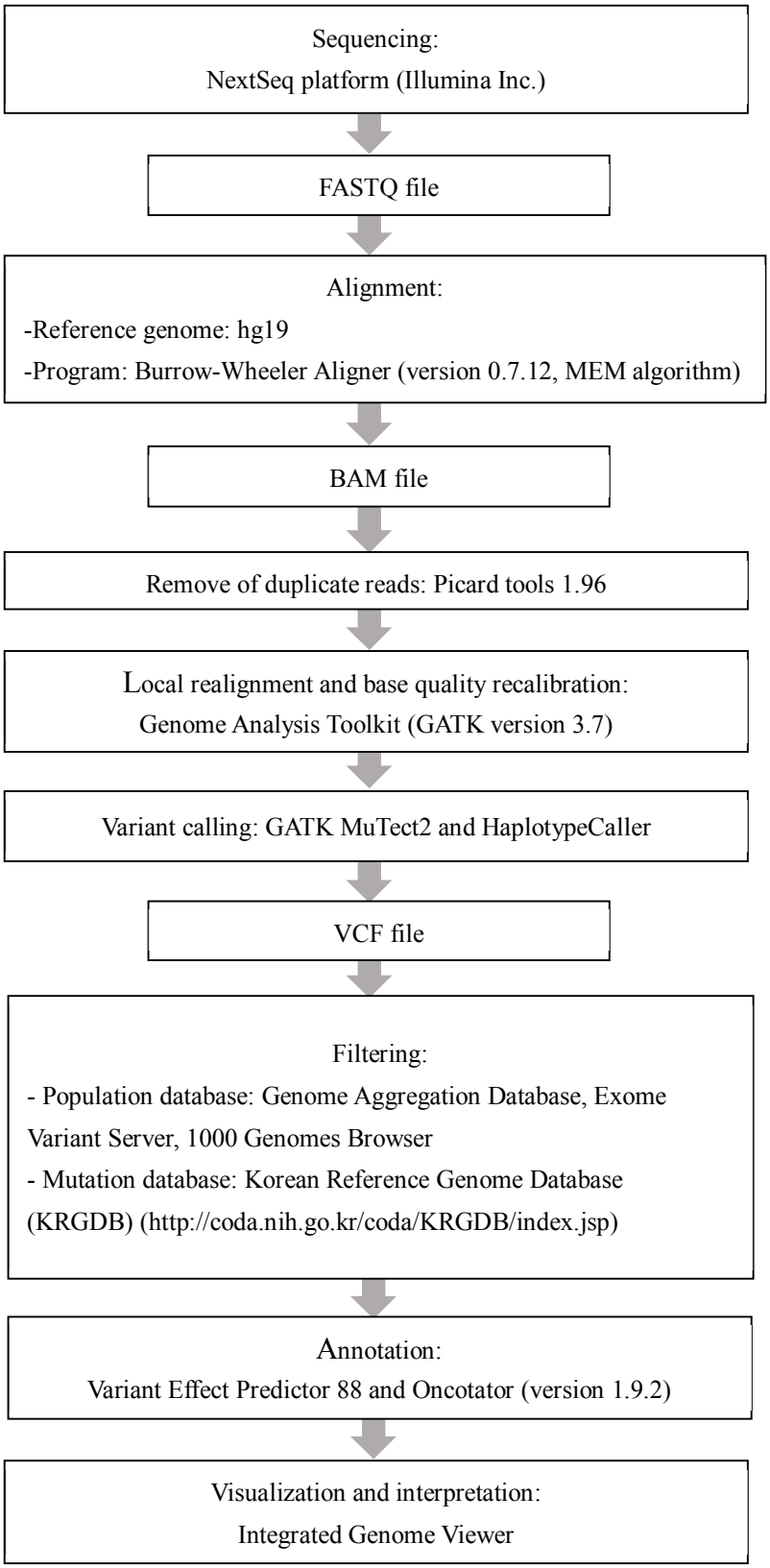

Fig. 1. The bioinformatics pipeline for exome sequencing

Table 2. The sequencing depth and coverage.

Patient No.	Tissue sample			Blood sample		
	Identified mutation	Mean Depth (X)	Coverage 30X (%)	Identified mutation	Mean Depth (X)	Coverage 30X (%)
1	+	513	99.63	-	594	99.63
2	+	765	98.8	-	326	98.7
3	+	721	99.0	-	323	98.0
4	+	633	98.3	-	352	98.5
5	+	1554	99.5	-	398	99.1
6	+	1149	99.3	-	314	98.5
7	+	890	99.6	+	239	99.3
8	+	1080	99.6	-	212	99.2
9	+	571	99.2	-	391	98.9
10	+	880	98.9	+	281	98.6
11	+	796	98.9	+	341	98.7
12	+	1038	99.6	-	248	99.4
13	+	952	99.6	-	273	99.4
14	+	544	99.63	-	567	99.63
15	-	1074	99.6	-	322	99.5
Mean		877	99.3		345	99.0
SD		278	0.4		108	0.49

3. Treatment strategies

Treatment with propranolol was attempted with a starting dose of 0.5 mg/kg/day to a maximum dose of 4mg/kg/day. Whole body MRI findings, erythrocyte sedimentation rate and vascular endothelial growth factor levels with other representative cytokines before and after propranolol trial were evaluated. The cytokine evaluation was performed by using the Millipore Human Cytokine/Chemokine Magnetic Bead Panel (Merck KGaA Inc., Darmstadt, Germany) with an interval of six months. SF-36 version 2 of short-form health survey questionnaire[16] was acquired every six months to evaluate the change in quality of life. SF-36 questions yield eight subscales and two summary of physical and mental component scores.

Alpelisib, an alpha-specific PI3K inhibitor which has been approved in the United States for medical use in breast cancer with *PIK3CA* mutation since May 2019, was administrated in two patients for clinical trial by managed access program approved by Novartis Pharmaceuticals Corporation (Novartis/CBYL719X2001I MAP ID 17746/17751). Alpelisib was provided as a 50 mg or 250 mg coated tablet and administered orally once daily. The volume change of the extremities was measured and compared by WB-MRI.

Results

1. Clinical presentation and diagnosis

Fifteen patients with symptoms of segmental overgrowth were enrolled in this study. There were 11 male and 4 female patients. The age of the patients was 14.8 ± 19.5 years (range, 0.25-53 years). None of the patients had any other family member affected by the similar phenotypes.

Hypertrophy of soft tissues were seen in 13 patients (13/15, 86.7%). Eight of these patients showed lower extremities hypertrophy; six for right, one for left, and one for both. Five patients (5/15, 33.3%) showed both lower and upper extremities hypertrophy; four for left and one for right. Eight patients showed limb length discrepancy. Cutaneous capillary malformation presented as port-wine stain was seen in 12 patients (12/15, 80%). One patient had a pigmentary skin macule presented as an epidermal nevus on the left trunk and extremities (Figure 2). Venous engorgement of extremities were identified in 6 patients (6/15, 40%) by WB–MRI. Other clinical features included splenomegaly with lymphangioma and hemangioma (n=2, 13.3%), facial hemangioma (n=1, 6.7%), tongue hemangioma (n=1, 6.7%), leg or spinal arteriovenous malformation (n=2, 13.3%), facial asymmetry with hemifacial bone prominency (n=1, 6.7%), hemimegalencephaly (n=1, 6.7%), seizure (n=1, 6.7%), and one-sided blindness (n=1, 6.7%) (Table 3). Clinically, 14 patients (93.3%) were diagnosed with PROS; 12 patients (80%) as Klippel-Trenaunay syndrome and 2 patients (13.3%) as undetermined type of PROS. One patient (6.7%) was suspected to have Parkes-Weber syndrome (Table 3).

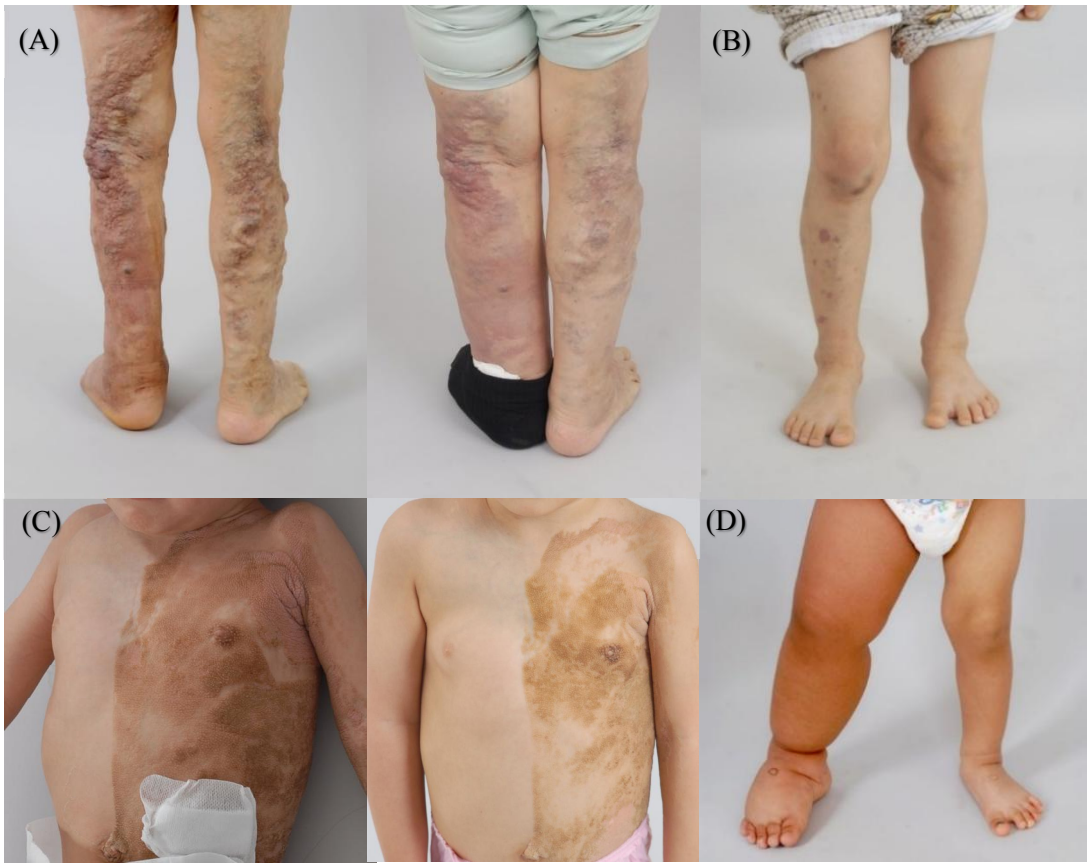

Fig. 2. Clinical manifestations of patients. (A) Patient 5 with a *PIK3CA* mutation with prominent superficial venous engorgement. The superficial venous engorgement showed improvement after three years of propranolol administration. (B) Patient 1 with a *PIK3CA* mutation showing port-wine stain of skin and hypertrophy of the right leg. (C) Patient 10 with a *PTEN* mutation presented with epidermal nevus. The color of the epidermal nevus faded after six months of propranolol administration. (D) Patient 9 with a *KRAS* mutation showing hypertrophy of the right leg.

Table 3. Clinical characteristics of the patients.

Patient (Gender/age)	Clinical features				Clinical diagnosis
	Vascular malformation	Soft tissue hypertrophy	Bone hypertrophy	Others	
1 (M/7y)	+	+	-	-	PROS (KTS)
2 (M/8y)	+	+	-	Seizure, ipsilateral long 2nd toe	PROS (KTS)
3 (F/39y)	+	+	-	-	PROS (KTS)
4 (M/10m)	+	-	Hemimegalencephaly	-	PROS (undetermined)
5 (F/53y)	+	+	-	Pulmonary thromboembolism	PROS (KTS)
6 (F/4m)	+	+	-	-	PROS (KTS)
7 (M/4m)	+	+	-	-	PROS (KTS)
8 (M/7y)	-	+	-	-	PROS (KTS)
9 (M/2y)	Lumbosacral AVM	+	-	Lymphangioma	Parkes-Weber syndrome
10 (F/2y)	Chest wall AVM	+	-	Lymphangioma	PROS (undetermined)
11 (M/3y)	-	+	Tibia, Lt.	-	PROS (KTS)
12 (M/6y)	+	+	-	ipsilateral eye blindness mental retardation	PROS (KTS)
13 (M/47y)	+	+	-	-	PROS (KTS)
14 (M/5y)	+	-	-	-	PROS (KTS)
15 (M/43y)	+	+	Hemifacial prominency	-	PROS (KTS)

PROS, PIK3CA-related overgrowth spectrum; KTS, Klippel-Trenaunay syndrome; AVM, arteriovenous malformation

2. Genetic diagnosis

Significant somatic mutation was selected if the variant was observed in the affected tissue with variant allele frequency (VAF) > 0.01 and its VAF was also compared in non-affected tissue (blood leukocytes) in each patient. Significant mutations were observed from the affected tissue in 14 patients. The identical mutations were also identified from the blood in patient 7, 10 and 11. The variant allele frequency of the *PTEN* mutation found in the tissue and blood of patient 10 were 0.712 and 0.481, respectively. A causative mutation was not found in patient 15. Seven patients (46.7%) were identified to have *PIK3CA* mutation. Two patients (13.3%) had *KRAS* mutation. Other identified mutations included *PTEN* (n=1, 6.7%), *MAP2K3* (n=1, 6.7%), *GNAQ* (n=1, 6.7%), *TBC1D4* (n=1, 6.7%) and *TEK* (n=1, 6.7%) (Table 1). All of the identified mutations were known somatic mutations. All somatic mutations except for *TEK* were found in the Catalogue Of Somatic Mutations In Cancer (COSMIC) database. The somatic mutation of *TEK* was previously reported from a patient with sporadic venous malformation.[17] The c.755A>T* germline mutation of *PTEN* has been reported previously.[18,19] The type of mutations, variant allele frequency, and in silico results are described in table 4 and 5.

Table 4. The characteristics of identified mutations.

Patient	Mutated gene	DNA sequence	Amino acid change	VAF (tissue)	VAF (blood)	Depth (X) (tissue)	Depth (X) (blood)	COSMIC ID
1	*PIK3CA*	c.1636C>A	p.Gln546Lys	0.048	0	513	594	COSM766
2	*PIK3CA*	c.2740G>A	p.Gly914Arg	0.182	0	765	326	COSM3205660
3	*PIK3CA*	c.1345C>A	p.Pro449Thr	0.131	0	721	323	COSM18601
4	*PIK3CA*	c.1633G>A	p.Glu545Lys	0.023	0	633	352	COSM763
5	*PIK3CA*	c.1357G>A	p.Glu453Lys	0.127	0	1554	398	COSM12584
6	*PIK3CA*	c.3073A>G	p.Thr1025Ala	0.081	0	1149	314	COSM771
7	*PIK3CA*	c.2908G>A	p.Glu970Lys	0.023	0.036	890	239	COSM94980
8	*KRAS*	c.35G>A	p.Gly12Asp	0.041	0	1080	212	COSM521
9	*KRAS*	c.35G>A	p.Gly12Asp	0.058	0	571	391	COSM521
10	*PTEN*	c.755A>T	p.Asp252Val	0.712	0.481	880	281	COSM3368151
11	*MAP2K3*	c.696+1G>A	p.(?)	0.042	0.088	796	341	COSM560209
12	*GNAQ*	c.548G>A	p.Arg183Gln	0.039	0	1038	248	COSM52975
13	*TBC1D4*	c.667G>A	p.Asp223Asn	0.026	0	952	273	COSM6797461
14	*TEK*	c.3324_3334del	p.Glu1109Leufs Ter5	0.046	0	544	567	-
15	Not found		-	-	-	1074	322	-

VAF, variant allele frequency; COSMIC, Catalogue Of Somatic Mutations In Cancer

Table 5. In silico results of identified mutations.

Patient	Mutated gene	DNA sequence change	SIFT	Mutation taster	LRT	PROVEAN	CADD phred scrore
1	*PIK3CA*	c.1636C>A	Tolerated	Disease causing	Deleterious	Neutral	25.2
2	*PIK3CA*	c.2740G>A	Deleterious	Disease causing	Deleterious	Deleterious	31
3	*PIK3CA*	c.1345C>A	Deleterious	Disease causing	Deleterious	Deleterious	28.4
4	*PIK3CA*	c.1633G>A	Deleterious	Disease causing	Deleterious	Deleterious	33
5	*PIK3CA*	c.1357G>A	Tolerated	Disease causing	Deleterious	Neutral	32
6	*PIK3CA*	c.3073A>G	Tolerated	Disease causing	Deleterious	Deleterious	23.2
7	*PIK3CA*	c.2908G>A	Tolerated	Disease causing	Neutral	Neutral	22.8
8	*KRAS*	c.35G>A	Deleterious	Disease causing	Deleterious	Deleterious	25.3
9	*KRAS*	c.35G>A	Deleterious	Disease causing	Deleterious	Deleterious	25.3
10	*PTEN*	c.755A>T	Deleterious	Disease causing	Deleterious	Deleterious	27.3
11	*MAP2K3*	c.696+1G>A	-	Disease causing	-	-	26.8
12	*GNAQ*	c.548G>A	Deleterious	Disease causing	Deleterious	Deleterious	35
13	*TBC1D4*	c.667G>A	Deleterious	Disease causing	Deleterious	Deleterious	34
14	*TEK*	c.3324_3334del	-		-	-	-

SIFT, Sorting Intolerant For Tolerant; LRT, Likelihood Ratio Test; PROVEAN, Protein Variation Effect Analzyzer; CADD, Combined Annotation Dependent Depletion

3. Treatment outcomes

Propranolol was administrated to twelve patients except for patient 4, 9 and 14. The total duration of treatment was 26.7 ± 14.9 months (range, 12-50 months). The initial starting dose of propranolol was 0.76 ± 0.29 mg/kg/day (range, 0.4-1.3 mg/kg/day) and the escalated maximum dose decided by the patients' tolerance was 3.6 ± 1.6 mg/kg/day (range, 0.5-6.4 mg/kg/day).

Seven patients experienced mild improvement of symptoms; relief of pain, extended range of motion, mild decrease of cutaneous swelling and capillary lesion (Figure 2). The SF-36 version 2 of short-form health survey questionnaire data was available in seven patients. The mean physical component score of SF-36 increased from 50.8 ± 31.9 to 65.1 ± 23.3 after treatment. The mental component score also showed an increase from 58.4 ± 25.1 to 65.6 ± 18.2. Three patients showed a prominent improvement of SF-36 scores. However, there was no obvious improvement in the size of the lesions on regular follow-up whole body MRI findings. Patient 5 and patient 11 showed aggravation of symptoms after discontinuance of propranolol, but they regained the treatment effect again after re-introducing propranolol administration. Patient 5 showed improvement of cutaneous vascular symptoms with a three year administration of propranolol (figure 2). Three patients experienced transient side effects with dizziness or bradycardia, but they continued treatment by sustaining the minimal dose or performing gradual dose escalation.

ESR was 15.1 ± 13.8 mm/hr before treatment and decreased to 6.4 ± 7.7 mm/hr at 12 months of treatment. The cytokine levels were examined in nine patients and the mean levels after treatment

were compared with the initial level. The changes of the cytokine levels did not show a consistent pattern among the whole patients. However, similar patterns of changes were observed in four patients (Figure 3). The growth factors such as epidermal growth factor (EGF), fibroblast growth factor 2 (FGF-2), transforming growth factor alpha (TGF-α) and vascular endothelial growth factor (VEGF) decreased (Table 6). There were also changes in the factors related with regulation of angiogenesis such as fractalkine, soluble cluster of differentiation 40 ligand (sCD40L) and interferon gamma induced protein 10 (IP-10) (Table 6).

Alpelisib was administrated for a year in patient 2 and 3 with a *PIK3CA* mutation who showed hypertrophy of extremities. Patient 2 had a left hemihypertrophy of the left leg and was 10 years old at the start of clinical trial (50 mg qd). In patient 2, the volume of the left and right leg before treatment were 8351.6 cm^3 and 7758.6 cm^3, respectively. After one year of treatment with alpelisib the volume of the left and right leg increased to 9013.2 cm^3 and 8542.2 cm^3, respectively. The volume increase rate of the left and right leg were 7.9% and 10.1% respectively. There was a 7.6 % difference between the volumes of both legs before treatment, but it decreased to 5.5 % after one year of treatment. Patient 3 had hypertrophy of the both lower legs and the clinical trial (250 mg qd) was started at age of 42 years. In patient 3 the volume of the left and right leg before treatment were 19867.3 cm^3 and 18239.1 cm^3, respectively. After one year of treatment with alpelisib the volume of the left and right leg decreased to 18000.9 cm^3 and 16570.3 cm^3, respectively. The volume decrease rate of the left and right leg was 9.4% and 9.1%, respectively (Figure 4).

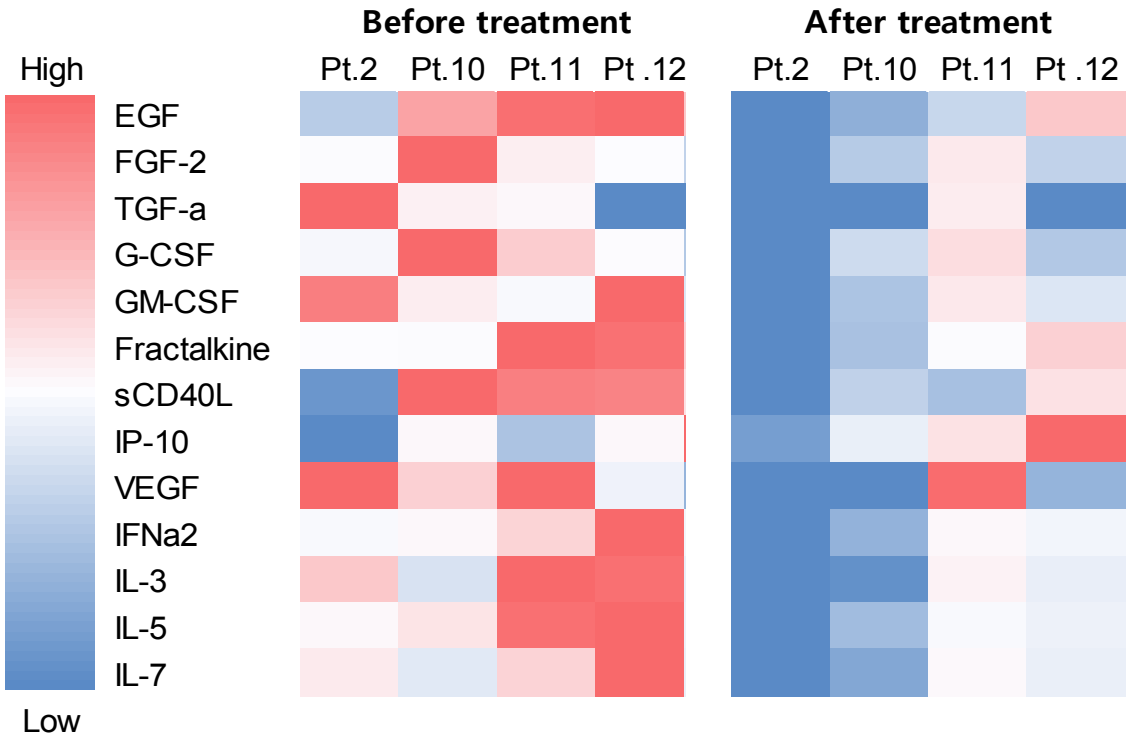

Fig. 3. Heatmap presenting the changes of cytokine levels before and after administration of propranolol in four patients who showed similar patterns of change.

Table 6. The cytokine levels of four patients (2, 10, 11, 12) that revealed changes after propranolol administration.

Cytokine (pg/ml)	Before treatment	After treatment
EGF	31 ± 12.6	12.8 ± 11.6
FGF-2	97.9 ± 30.8	52.5 ± 24.7
TGF-α	0.96 ± 0.68	0.58 ± 0.10
VEGF	90.7 ± 13	52.6 ± 29
G-CSF	159.5 ± 100.7	69.8 ± 47.9
GM-CSF	10.5 ± 1.1	5.9 ± 3.9
Fractalkine	136.1 ± 45.9	72.4 ± 45.9
sCD40L	342.3 ± 197.7	123.7 ± 84.5
IP-10	389.1 ± 78	531.9 ± 244
INFα2	49.9 ± 25.6	25.6 ± 20.3
IL-3	1.93 ± 0.77	0.73 ± 0.49
IL-5	1.52 ± 0.29	0.79 ± 0.46
IL-7	5.5 ± 3.0	1.7 ± 0.9

EGF, epidermal growth factor; FGF-2, fibroblast growth factor 2; TGF-α, transforming growth factor alpha; VEGF, vascular endothelial growth factor; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; sCD40L, soluble cluster of differentiation 40 ligand; IP-10, interferon gamma induced protein 10; INFα2, interferon alpha 2; IL, interleukin.

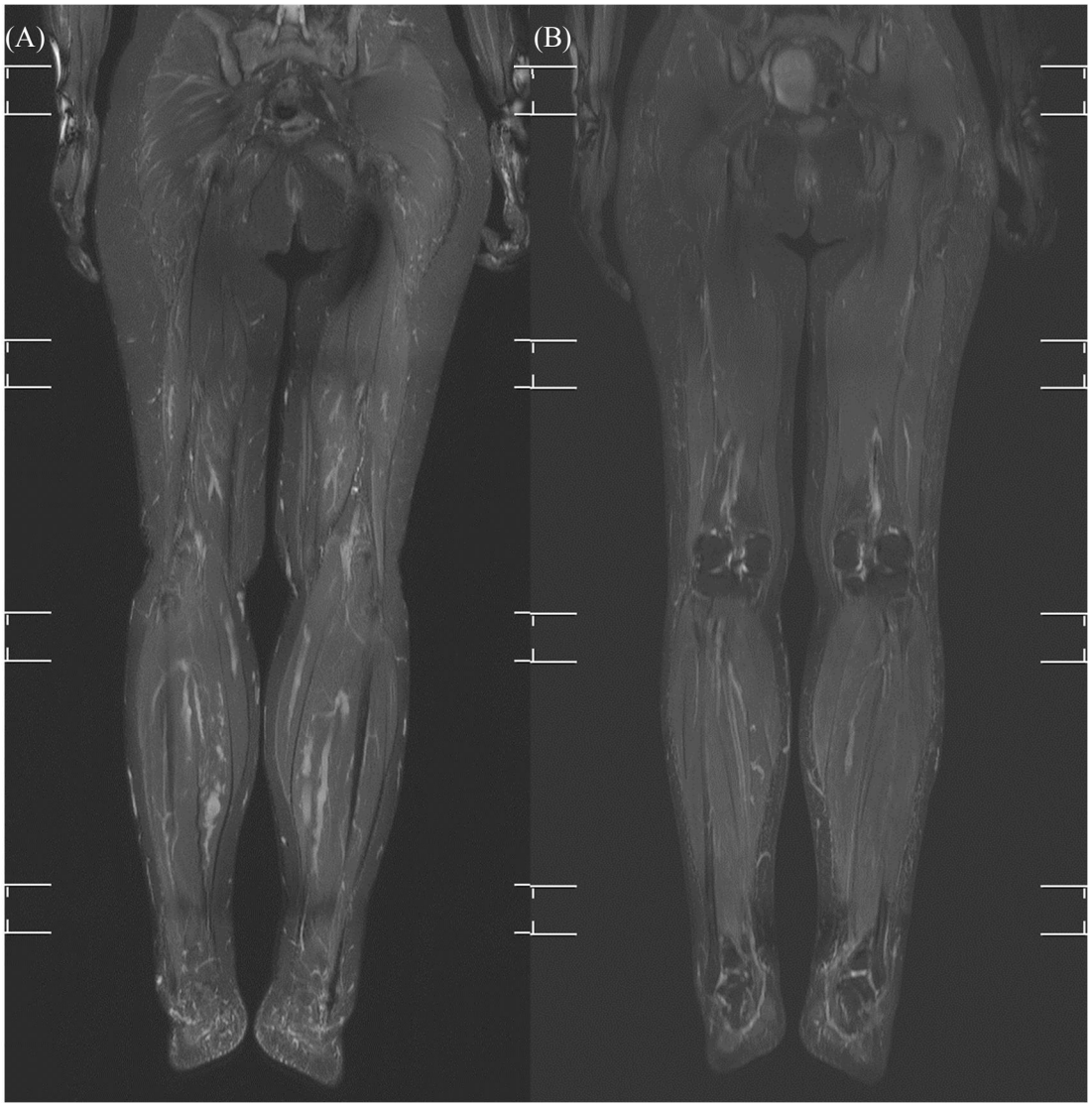

Fig. 4. The change of MRI findings in patient 3 after administration of alpelisib. (A) The MRI image of both lower legs before treatment (B) The MRI image of both lower legs after treatment. The extent of fine stranding of subcutaneous layer of bilateral distal lower legs decreased. Minimal improvement of tortuous and dilated deep and superficial venous structure in lower extremities was observed.

Discussion

Most of the patients were clinically diagnosed with PROS, but genetic testing revealed variable genetic causes. Careful physical examination of dysmorphic features can help identify a recognizable pattern in some cases, but there still remains a limitation in accurate clinical diagnosis. Differential diagnosis through genetic testing is important because segmental overgrowth syndromes share similar symptoms. Currently, the increased usage of exome sequencing has expanded the knowledge of genetic causes in overgrowth syndrome. The affected vascular malformation tissue was obtained and genetically tested in this study and it was possible to identify the causative gene in most cases. The mutation was also identified through genetic testing using peripheral white blood cells in three patients. Two of these mutations which showed a low mutant burden were considered to be derived from somatic mutation. The *PTEN* mutation identified in patient 10 with a high mutant burden from both the tissue and blood was considered as a germline mutation. This patient was an adopted child, and the genetic examination of her biological parents could not be performed. It has been reported that the levels of mosaicism is low as below 5% from tissue biopsies in some patients with PROS.[20] The overall molecular diagnostic yield of somatic overgrowth conditions was up to 45% in a single center study, but the detection rate was highly influenced by the submitted tissue.[21] It is important to acquire the ideal tissue from an affected area to increase the detection rate. Additional genetic testing with a blood sample may be helpful for diagnosis because the causative gene may not be identified through

only a tissue sample. Besides, germline mutation in a PROS patient has been reported and this highlights the need for germline filtering.[22]

Genetic variants related with RAS/MAPK pathyway such as *KRAS* and *MAP2K3* were identified in three patients. Mosaic Ras/MAPK variants have been reported to be related with vascular malformation[23] and the three patients in our study presented vascular malformation as skin pigmentation with localized hypertrophy of an ipsilateral leg. One patient with epidermal nevus and mild vascular malformation of the buttock and thigh was identified to have a *PTEN* mutation. One patient was diagnosed with Sturge-Weber syndrome proved by *GNAQ* mutation which has been reported to be identified in patients with capillary malformation involving overgrowth.[24-26] *TEK* mutation was identified in one patient with a port-wine stain and venous malformation of the buttock and leg. The *TEK* mutation has been reported to cause multiple sporadic venous malformation.[17,27,28] Although the association with the disease is not clear, the *TBC1D4* mutation was found in one patient. The same somatic mutation has been reported to be found from an large intestinal adenocarcinoma tissue.[29] TBC1D4 is a GTPase-activating protein that functions downstream from AKT, and seems to regulate proliferation of multiple cell types.[30-32] However, there are still no reports suggesting an association of *TBC1D4* mutation with overgrowth syndrome. As such, genetic mutations that are known to cause vascular malformations or tumors are often identified in patients with overgrowth syndrome. Increasing opportunities for genetic testing will increase our knowledge of many genetic causes that we did not know.

There was no case with obvious improvement of the affected area on the whole body MRI findings after propranolol administration. However, some patients showed improvement of pain, range of motion, and cutaneous symptoms. We also observed a certain degree of improvement of quality of life scale through questionnaires. In particular, rapid worsening of symptoms were observed in some of the patients after discontinuation of propranolol. Interestingly, several cytokine levels tended to change with similar patterns in four patients after propranolol administration. Growth factors such as vascular endothelial growth factor, epidermal growth factor, fibroblast growth factor and transforming growth factor alpha decreased. The sCD40L which is known to produce angiogenesis-associated factors[33-35] also decreased. The cytokine IP-10, which is secreted by several cell types in response to interferon gamma, showed a tendency of increase. IP-10 is known to block vascular endothelial growth factor induced endothelial motility and angiogenesis.[36,37] IP-10 has been also reported to enhance the anticancer activity of agents targeting tumor vasculature in soft tissue sarcomas.[38] Despite the effective use of propranolol for hemangiomas, the mechanism of action has not been clearly identified. Recent researches identified the possibility of propranolol inhibiting the activity of PI3K and AKT pathway.[9,10] When considering these findings together, propranolol seems to block the cytokines related with angiogenesis and subtly reduce the burdens caused by vascular malformations. Propranolol is a drug with uncommon side effects, so it could be used as an adjunct to relieve vascular symptoms in patients with segmental overgrowth syndromes.

There have been efforts to develop inhibitors of PI3K, AKT and mTOR pathways considering cancer and overgrowth diseases.[3] The mTOR inhibitor sirolimus has been clinically tried with a low dose and showed modest reduce in overgrowth. However, 72 % of participants showed at least one adverse event and risk-benefit evaluations must be carefully considered.[39] The PIK3CA inhibitor, alpelisib has been on clinical trial for several PIK3CA-dependent tumors and PROS.[7, 40,41] The use of alpelisib has been approved for certain breast cancer [42], but only a few reports exists regarding its application to PROS. The first evidence reported to use alpelisib in patients with PROS had supported promising efficacy and no substantial side effects.[7] There was an improvement in vascular tumor size, congestive heart failure, hemihypertrophy and scoliosis.[7] An organized clinical trial with alpelisib for patients with PROS is in progress since September, 2019.[43] Two PROS patients included in our study also participated in the alpelisib clinical trial, and it was confirmed that the degree of hypertrophy became lesser after administration of one year. There were no side effects in these patients with a one year trial of alpelisib. Some common side effects of alpelisib include, hyperglycemia, diarrhea, nausea, fatigue, stomatitis and pneumonitis.[42] PI3K/AKT/mTOR inhibitors not only inhibits activity of abnormal cells, but also would affect the metabolism of healthy cells such as dysregulation of glucose metabolism.[3] Besides, the need for life-long therapy with these inhibitor raises concerns with unknown side effects. Further research results on PROS patients with alpelisib will be needed to secure stability against these possible side effects.

This study includes several limitations. The sample size was small to detect enough patients with new causative genetic variations. The identified mutations have also been found in other reports, but more evidence is needed to prove the association with the disease. The age and severity of patients who took propranolol were different and it was unable to identify the exact mechanism of action or efficacy of propranolol in much more patients. In order to predict the change of cytokine levels after propranolol administration, a more precise and repetitive study performed in a larger number of patients is needed. Alpelisib was administrated with a fixed dose of 250 mg in an adult patient, but the pediatric dose has not been established. In order to prove the effectiveness of alpelisib in children, a differentiated dosage should be determined and administrated according to body weight.

Conclusion

In conclusion, exome sequencing using customized gene panel could enhance the genetic diagnosis rate of segmental overgrowth syndrome patients. Propranolol could be used as an adjuvant therapy for decreasing vascular symptoms in segmental overgrowth patients. Targeted therapy considering genetic causes would be the leading therapeutic strategy of overgrowth syndrome in the future.

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국문요약

목적: 분절 과증식 증후군은 체내 여러 조직의 증식을 동반하는 여러 질환들을 포함하며 다양한 증상을 보인다. 서로 다른 질환간에도 임상 증상이 겹치고, 원인이 되는 유전자가 전부 밝혀지지는 않아 감별 진단이 어려운 실정이다. 또한, 조직의 증식을 억제하고 합병증의 발생을 막는 효과적인 치료법이 아직 개발되지 않아 근본적인 치료가 어렵다. 이 연구는 분절 과증식 증후군 환자들의 유전적 원인을 발견하고 치료법을 모색하기 위해 진행되었다.

방법: 분절 과증식 증후군으로 진단된 열 다섯 명의 환자들이 연구에 참여하였다. 임상증상을 바탕으로 한 임상 진단과 함께 환자들의 전신자기공명영상을 분석하였다. 변이 유전자의 분석은 상용화된 유전자 패널을 이용하여 환자의 이환 된 조직 세포와 혈액의 백혈구 세포로부터 각각 DNA 를 분리하여 엑솜 시퀀싱을 진행함으로써 이루어졌다. 프로프라놀롤을 이용하여 치료를 하였으며, 치료 전후의 혈중 사이토카인 수치와 전신자기공명영상 결과의 변화를 살펴보았다. 두 명의 환자에게 PIK3CA 억제제인 알페리십을 일년간 사용하였으며, 치료 전후의 임상양상과 전신자기공명영상 결과를 비교하였다.

결과: 14 명의 환자들은 PIK3CA 연관 과증식 증후군(PROS)을 가지고 있는 것으로 임상 진단이 이루어졌다; 12 명은 임상적으로 Klippel-Trenaunay syndrome 으로 진단되었고, 두 명은 PROS 에 해당되는 질환 중 하나 일 것으로 짐작되었다. 한 명의 환자는 임상적으로 Parkes-Weber syndrome 으로 진단되었다. 유전자 검사상 일곱 명

(46.7%)의 환자에게서 *PIK3CA* 유전자 변이가 발견되었다. 두 명(13.3%)에게서는 *KRAS* 유전자 변이가 발견되었다. 다른 변이들로 *PTEN* (n=1, 6.7%), *MAP2K3* (n=1, 6.7%), *GNAQ* (n=1, 6.7%), *TBC1D4* (n=1, 6.7%), 그리고 *TEK* (n=1, 6.7%) 유전자 변이가 발견되었다. 프로프라놀롤은 12 명의 환자에게 투약되었으며, 이 중 일곱 명의 환자가 통증이나 운동 제한 증상의 완화를 보였다. 하지만 전신자기공명영상에서 현저한 호전 소견은 확인되지 않았다. 알페리십은 두 명의 환자에게 일 년간 투약되었으며, 치료 전후 전신자기공명영상에서 이환 부위의 경미한 호전을 확인할 수 있었다.

결론: 유전자 패널을 통한 엑솜 시퀀싱 검사는 분절 과증식 증후군 환자의 감별진단에 유용하다. 프로프라놀롤은 혈관 증식으로 인한 증상을 줄이는데 보조적인 치료법으로 이용될 수 있다. 추후에는 PIK3CA 억제제와 같이 특정 유전자의 작용을 억제하는 표적 요법이 과증식 증후군 치료법의 주류를 이룰 것으로 예상된다.

중심단어: 과증식 증후군, 혈관 기형, PIK3CA 연관 과증식 증후군, 프로프라놀롤, 알페리십, 표적 엑솜 시퀀싱