Original Article

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Prospective evaluation of the clinical utility of whole-exome sequencing using buccal swabbing for undiagnosed rare diseases

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Purpose: Whole-exome sequencing (WES) has been a useful tool for novel gene discovery of various disease categories, further increasing the diagnostic yield. This study aimed to investigate the clinical utility of WES prospectively in undiagnosed genetic diseases.

Materials and Methods: WES tests were performed on 110 patients (age range, 0-28 years) with suspected rare genetic diseases. WES tests were performed at a single reference laboratory and the variants reported were reviewed by clinical geneticists, pediatricians, neurologists, and laboratory physicians.

Results: The patients' symptoms varied with abnormalities in the head or neck, including facial dysmorphism, being the most common, identified in 85.4% of patients, followed by abnormalities in the nervous system (83.6%). The average number of systems manifesting phenotypic abnormalities per patient was 3.9±1.7. The age at presentation was 2.1±2.7 years old (range, 0-15 years), and the age at WES testing was 6.7±5.3 years (range, 0-28 years). In total, WES test reported 100 pathogenic/likely pathogenic variants or variants of uncertain significance for 79 out of 110 probands (71.8%). Of the 79 patients with positive or inconclusive calls, 55 (50.0%) patients were determined to have good genotype-phenotype correlations after careful review. Further clinical reassessment and family member testing determined 45 (40.9%) patients to have been identified with a molecular diagnosis.

Conclusion: This study showed a 40.9% diagnostic yield for WES test for a heterogeneous patient cohort with suspected rare genetic diseases. WES could be the feasible genetic test modality to overcome the diversity and complexity of rare disease diagnostics.

Key words: Exome sequencing, Rare diseases, Gene discovery.

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Conflict of interest: The authors declare that they do not have any conflicts of interest.

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Introduction

To date, approximately 7,000 rare genetic diseases are known, and many other diseases fall into the undiagnosed area of disease [1]. Many of these diseases are extremely rare and have overlapping phenotype with other diseases, making it difficult to pinpoint a specific clinical diagnosis.

Therefore, even when the symptoms start presenting during childhood, it is not uncommon for a patient to remain undiagnosed until adulthood without appropriate genetic testing. Not having an accurate molecular diagnosis could adversely affect patient's quality of life, as they continuously undergo numerous unpleasant and costly unnecessary tests [2].

The diagnostic yield of rare genetic diseases could vary by the test methods, affected organ system, and patient population [3]. Whole-exome sequencing (WES) test that surveys the majority of the protein-coding exons of almost all genes, has revolutionized the diagnostic process for patients suspected of having rare monogenic disorders [4]. In addition, WES has been a useful tool for novel gene discovery of various disease categories, further increasing the diagnostic yield [5]. The diagnostic yield of WES test could vary from 25 to 52% by what the primary phenotype is [5-7].

Clinically, using innovative and minimally invasive diagnostic methods in children remains essential. A genetic test using a buccal swab is one of the ideal substitutes for blood and has high sensitivity and specificity and is a noninvasive approach [8]. The purpose of this study was to confirm the diagnosis rate by applying the WES using a buccal swab to undiagnosed patients suspected of monogenic disorders.

Materials and Methods

1. Patients

This prospective cohort study was conducted between July 2018 and July 2019. A series of 110 consecutive patients who presented at the Division of Pediatric Genetics and Metabolism, Rare Disease Center, Pusan National University Children's Hospital, Yangsan, South Korea with clinically suspected of having a rare genetic disease were recruited (age range, 0-28 years) from 110 nonconsanguineous families. Their detailed demographic and clinical characteristics, including age and diagnosis at presentation, sex, family history, laboratory findings, radiologic findings, and genetic testing results, were reviewed. Previous genetic tests before receiving WES test included chromosome analyses, chromosome microarray, single or targeted gene panel testing, or targeted gene panel testing by exome sequencing. According to a medicines regulatory point of view, in the USA, ultra-rare is defined as it affects 1 patient per 50,000 people [9].

Informed consent was obtained from all patients or their legal guardians after genetic counseling regarding the WES test. The study was approved by the Institutional Review Board of Pusan National University Yangsan Hospital (IRB no. L-2018-248-1).

2. Whole exome sequencing

A buccal swab sample was obtained from the patients for genomic DNA extraction. WES was performed as follows: coding exons of all known human genes (~22,000) were captured by the SureSelect kit (Version C2; Agilent Technologies, Inc., Santa Clara, CA, USA). The captured genomic regions were sequenced using the NovaSeq 6000 platform (Illumina, San Diego, CA, USA).

3. Bioinformatic analysis and variant interpretation

Sequencing reads were aligned to the human reference genome, UCSC assembly hg19, using BWA (v.0.7.12, MEM algorithm). The mean depth of coverage was ~100× (>×20=99.2%).

Table 1. Delineation of demographic and clinical features of the 110 study participants

Items	Value (n=110)
Sex (male:female)	65:45 (59.1:40.9)
Age at first presentation (yr)	2.1±2.7 (0-15)
Age at time of WES (yr)	6.7±5.3 (0-28)
Head & neck abnormality, including facial dysmorphism	94 (85.4)
ID or DD including seizure	92 (83.6)
Abnormal brain MR findings	38 (34.5)
Visual system	40 (36.3)
Ear system	20 (18.2)
CV system	38 (34.5)
Respiratory system	14 (12.7)
GI system	18 (16.4)
Genitourinary system	30 (27.3)
Endocrine system	19 (17.2)
Blood and immune system	1 (0.9)
Skin involvement	16 (14.5)
Neoplasm	2 (1.8)
Connective tissue	11 (10.0)
Musculoskeletal system	81 (73.6)
Short stature	47 (42.7)
Behavior problem	9 (8.2)
Previous genetic analysis	88 (80.0)

Values are presented as number only, mean±standard deviation (range), or number (%).
WES, whole-exome sequencing; ID, intellectual disability; DD, developmental delay; MR, magnetic resonance; CV, cardiovascular; GI, gastrointestinal.

Variants were called using GATK (genomic analysis toolkit, https://gatk. broadinstitute.org/hc/en-us). EVIDENCE (https://3billion.io/), a streamlined variant interpretation system, was used for variant annotation, filtering, classification and sorting. EVIDENCE consists of three integrated modules: 1) variant annotation module using daily updated databases, 2) symptom similarity scoring module and 3) customized variant classification module based on the American College of Medi-

Table 2. Comparison of clinical features between confirmed cases and unconfirmed cases

Items	Confirmed cases (n=45)	Unconfirmed cases (n=65)	Total (n=110)	*P*-value	Odds ratio (95% confidence interval)
Sex, male	23 (51.1)	40 (61.5)	63 (57.3)	0.373	
Age at presentation[a]	2.0±2.7	2.2±2.7	2.1±2.7	0.632	
<1 yr	28 (45.2)	22 (48.9)	50 (46.7)		
1-2 yr	13 (21.0)	11 (24.4)	24 (22.4)		
2-5 yr	15 (24.2)	8 (17.8)	23 (21.5)		
>5 yr	6 (9.7)	4 (8.9)	10 (9.3)		
Age at time of WES[a]	5.6±5.4	6.9±5.4	6.4±5.4	0.239	
<1 yr	5 (7.7)	7 (15.6)	12 (10.9)		
1-2 yr	7 (10.8)	4 (8.9)	11 (10.0)		
2-5 yr	16 (24.6)	14 (31.1)	30 (27.3)		
5-10 yr	21 (32.3)	12 (26.7)	33 (30.0)		
>10 yr	16 (24.6)	8 (17.8)	24 (21.8)		
Dysmorphic face	43 (95.6)	47 (72.3)	90 (81.8)	0.004	8.23 (1.8-37.58), *P*=0.0065
Neurodevelopmental abnormalities[b]	41 (91.1)	55 (84.6)	96 (87.3)	0.475	
Abnormal finding in MR brain	19 (42.2)	21 (32.3)	40 (36.4)	0.389	
Organ involvement[a]	4.1±1.8	3.6±1.7	3.8±1.8	0.099	1.32 (1.02-1.69)
Visual system	23 (51.1)	18 (27.7)	41 (37.3)	0.022	2.73 (1.23 -6.06), *P*=0.0136
Ears, nose and throat system[c]	10 (22.2)	10 (15.4)	20 (18.2)	0.507	
Cardiovascular system	20 (44.4)	19 (29.2)	39 (35.5)	0.151	
Respiratory system	5 (11.1)	11 (16.9)	16 (14.5)	0.565	
Gastrointestinal system	4 (8.9)	14 (21.5)	18 (16.4)	0.133	
Genitourinary system	13 (28.9)	18 (27.7)	31 (28.2)	>0.999	
Endocrinology system	9 (20.0)	13 (20.0)	22 (20.0)	>0.999	
Blood and immune system[c]	1 (2.2)	0 (0.0)	1 (0.9)	0.409	
Integumentary system[c]	11 (24.4)	6 (9.2)	17 (15.5)	0.057	
Neoplastic disease[c]	0 (0.0)	3 (4.6)	3 (2.7)	0.268	
Connective tissue[c]	1 (2.2)	11 (16.9)	12 (10.9)	0.026	0.11 (0.01-0.90), *P*=0.0392
Musculoskeletal system	28 (62.2)	29 (44.6)	57 (51.8)	0.105	
Short stature	22 (48.9)	28 (43.1)	50 (45.5)	0.684	
Behavior problems[c]	2 (4.4)	9 (13.8)	11 (10.0)	0.194	
Previous genetic analysis	41 (91.1)	48 (73.8)	89 (80.9)	0.044	3.63 (1.13-11.65), *P*=0.0302
Karyotyping	39 (86.7)	48 (73.8)	87 (79.1)	0.165	
FISH[c]	0 (0.0)	2 (3.1)	2 (1.8)	0.512	
MLPA[c]	1 (2.2)	2 (3.1)	3 (2.7)	>0.999	
CMA	27 (60.0)	33 (50.8)	60 (54.5)	0.447	
Single gene analysis	13 (28.9)	17 (26.2)	30 (27.3)	0.921	
TES[c]	4 (8.9)	5 (7.7)	9 (8.2)	>0.999	

Values are presented as number (%) or mean±standard deviation.
[a]Student *t*-test was performed for continuous variables. [b]Neurodevelopmental abnormalities is defined as intellectual disability, developmental delay or seizure. [c]Fisher's Exact test was performed for marked variable, whereas chi-squared test was performed for categorical variables not marked.
WES, whole-exome sequencing; MR, magnetic resonance FISH, fluorescence in situ hybridization; MLPA, multiplex ligation dependent probe amplification; CMA, chromosomal microarray; TES, targeted-exome sequencing.

cal Genetics and Genomics guidelines. Following EVIDENCE, a small subset of rare variants that fulfilled the criteria for being potentially disease-causing, were manually reviewed by medical geneticists to select pathogenic/likely pathogenic variants and variants of uncertain significance worthy of reporting. The selected variants were reported back to the referring physicians.

4. Validation by Sanger sequencing

Sanger sequencing was used to confirm all reportable variants. The variants were sequenced bi-directionally using ABI PRISM 3.1 Big Dye Terminator Kit (Applied Biosystems, Foster City, CA, USA) and ABI PRISM 3130XL sequencer (Applied Biosystems). The chromatograms were analyzed using Sequencer 4.9 (Gene Codes, Ann Arbor, MI, USA).

5. Statistics analysis

All statistical analyses were performed using the R studio software (version 3.5.1). Student *t*-test was performed for continuous variables. Chi-squared test or Fisher's exact test was performed for categorical variables according to their characteristics. The odds ratio and confidential interval were calculated for significant variables with *P*-value less than 0.05.

Results

1. Basic clinical manifestations

The demographic and clinical features of the 110 study participants are described in Table 1. The age at presentation was 2.1±2.7 years old (range, 0-15 years), and the age at WES testing was 6.7±5.3 years (range, 0-28 years). The average number of systems manifesting phenotypic abnormalities per patient was 3.9±1.7. Abnormalities in the head or neck, including facial dysmorphism, were the most common, identified in 85.4% of patients, followed by abnormalities of the nervous system (83.6%), musculoskeletal system (73.6%), eye system (36.3%), cardiovascular system (34.5%), ear system (18.2%), endocrine and metabolic system (17.2%), and gastrointestinal system (16.4%). Eighty-eight (80.0%) patients underwent one or more genetic tests prior to WES. The comparison of clinical features between confirmed cases and unconfirmed cases is depicted in Table 2. Nearly 50% of the patients had clinical presentation before the age of 1, and the age at which WES was administered was about 50% after the age of 5, which indicates that there was an average of 4 years of the time difference between the onset of clinical symptoms and the time of WES. The frequency of the dysmorphic face was statistically higher in the confirmed case compared to the unconfirmed case (95.6% vs. 72.3%, *P*=0.004; odds ratio [OR], 8.23). The frequency of the involvement of the visual system was statistically higher in the confirmed case compared to the unconfirmed case (51.1% vs. 27.7%, *P*=0.022; OR, 2.73). On the other hand, the frequency of connective tissue involvement was statistically lower in the confirmed case than in the unconfirmed case (2.2% vs. 16.9%, *P*=0.026; OR, 0.11). In the case of the frequency of the genetic evaluation before

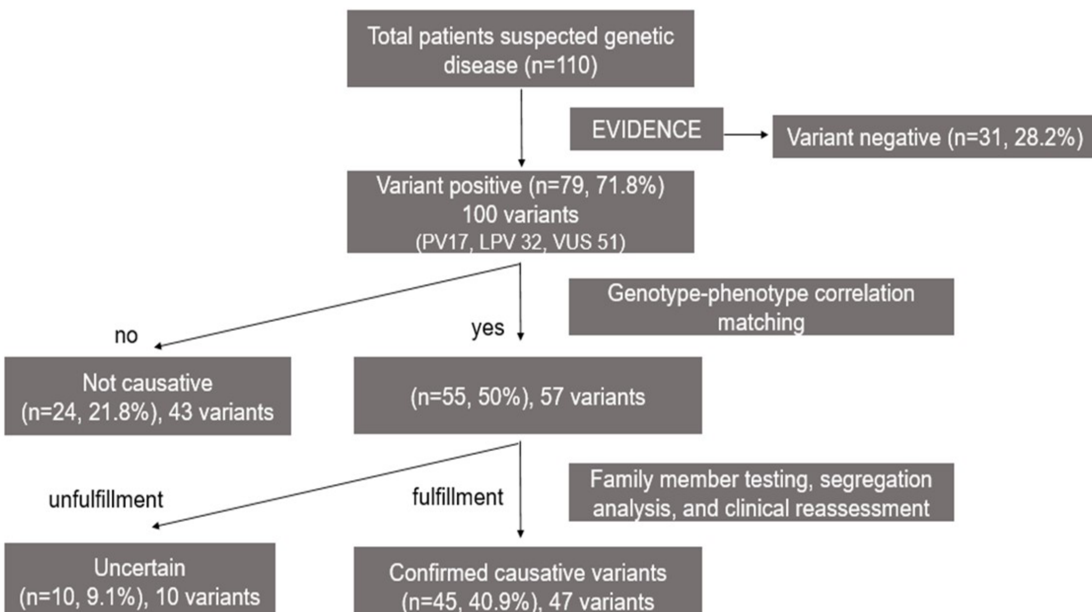

Fig. 1. Data analysis strategy. EVIDENCE, the streamlined variant prioritization software program, was applied in-house to prioritize variants based on American College of Medical Genetics and Genomics guidelines and the phenotype of each patient and to interpret these variants consistently.

WES, the confirmed case was statistically higher than the unconfirmed case (91.1% vs. 73.8%, P=0.044; Incidence rate ratio, 3.63).

2. Molecular genetic characteristics

The number of patients with variants and the identity of these variants have been summarized (Fig. 1, Supplementary Table 1). A total of 110 patients suspected to have the genetic disease were performed WES. False positives in WES from saliva (5.5%, 6/110), failure to meet the quality control (QC) criteria. False positives in next generation sequencing occurred at 5.5%. WES analysis using EVIDENCE identified 100 variants, including pathogenic variant (PV) 17, likely pathogenic variant (LPV) 32, and 121 variant of uncertain significance (VUS), in 79 (71.8%) of 110 probands. Among these, genotype-phenotype matching detected 57 variants in 55 (50.0%) patients. These patients were assessed by Sanger sequencing and family member testing and segregation analysis resulting in 47 variants, including 33 novel variants, in 45 (40.9%) patients confirmed as being responsible for 42 genetic disorders.

In the mode of inheritance for confirmed cases, autosomal dominant *de novo* was 64%, X-linked was 15%, autosomal dominant unknown inheritance was 11%, autosomal recessive was 9%, and autosomal dominant inherited was 2% (Fig. 2). Blended

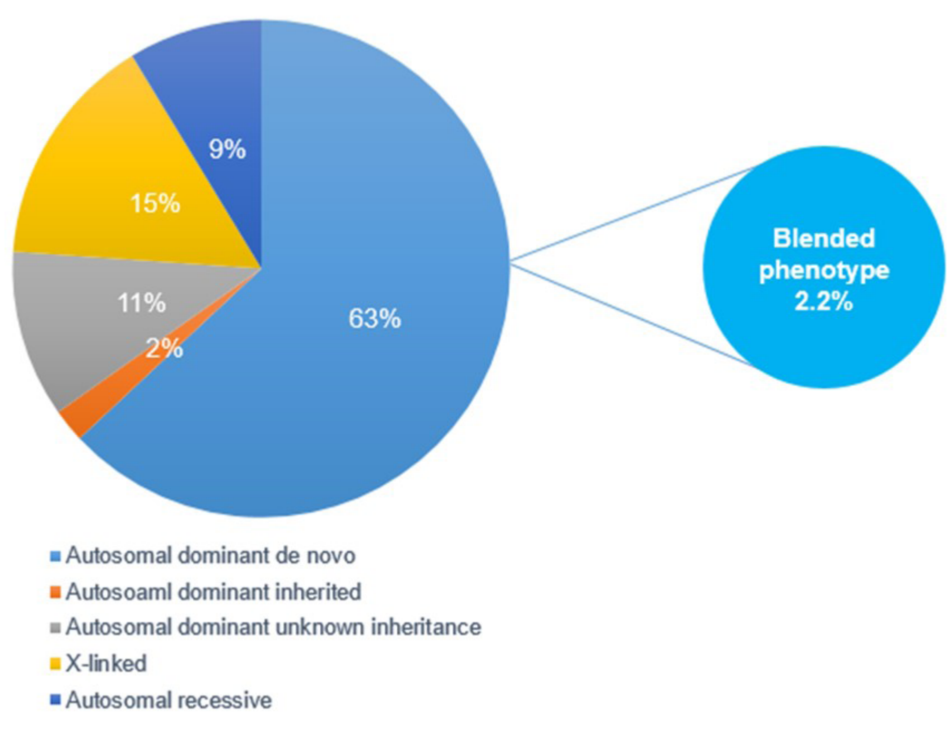

Fig. 2. Mode of Inheritance for confirmed cases.

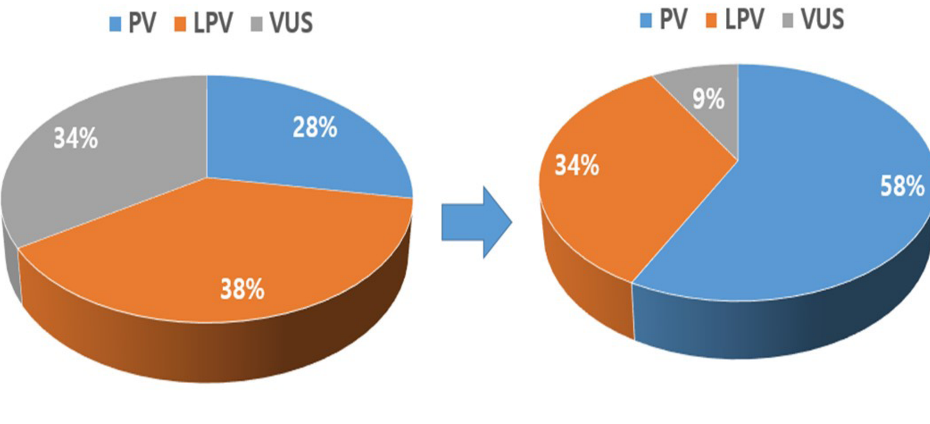

Fig. 3. Distribution of the causative variants. PV, pathogenic variant; LPV, likely pathogenic variant; VUS, variant of uncertain significance.

phenotype was seen in 2.2% of patients. Looking at the distribution of causative variants, PV accounted for 28%, LPV 38%, and VUS for 34% after family member testing and segregation (Fig. 3). However, after re-classification by clinical reassessment, PV accounted for 58%, LPV 34%, and VUS 9% (Fig. 3). In the mutant type of confirmed causative variants, missense was the most common with a frequency of 62%, followed by nonsense 16%, frameshift 11%, splice site 6%, and in the frame in/del 5% (Fig. 4). The ultra-rare disease identified in this study was 66.6%, 28 out of 42 genetic diseases (Fig. 5).

3. Identification of ultra-rare genetic diseases and its impact on clinical management

The confirmation of an ultra-rare genetic disease by WES affected patient management (Fig. 6).

Disease monitoring was initiated in 46.6% of patients. In addition, the systemic involvement of specific genetic diseases was investigated in 22.2%, the change of estimated inheritance pattern of genetic disease was 11.1%, and the prognosis was changed in 15.5%. Medical initiation occurred in 4.4% and reproductive planning was initiated in 2.2%. For example, a patient presented with an intellectual disability at the age of 11 years. He experienced recurrent epistaxis at age 10. Family history revealed father and grad father had recurrent epistaxis and delayed bleeding time after the surgical procedure. He showed dysmorphic facial features such as strabismus, thick eyebrows, smooth philtrum, a long face with a prominent chin, a prominent forehead, and protruded teeth. T2/FLAIR image revealed hyper-intense white matter signals in the periventricular region. His CBC profile revealed 6200-6.9-350K and elevated ALP 798 (86-315) IU/L. PLT functional test (Epi-PFA, ADP-PFA) (Blood) was normal. Ristocetin cofactor assay (Blood) was slightly decreased to 45% (range, 56-187). VWF Ag (Blood) was low at 38% (range, 47-197). WES was done in the patient, resulting in the SATB2 gene (NM_001172509) variant in the c.1136A>C (p.Gln379Pro) on exon8 which was *de novo* VUS, and the *VWF* gene (NM_00055212) variant in the c.4585G>C (p.Asp1529His) on exon 28 which was derived from his father (Fig. 7). These two variants were conserved among the different species. Finally, he had *SATB2*-associated

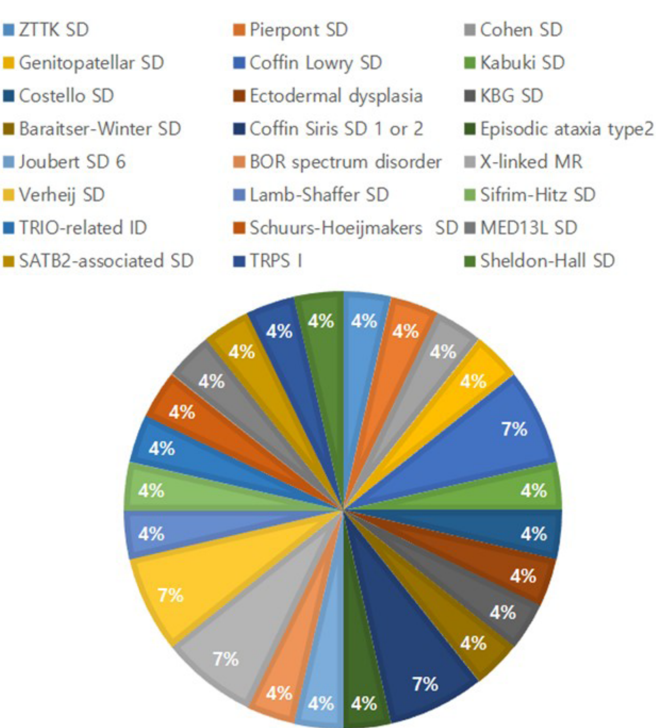

Fig. 5. Ultra-rare diseases identified in the study. SD, syndrome; ID, intellectual disability; MR, mental retardation.

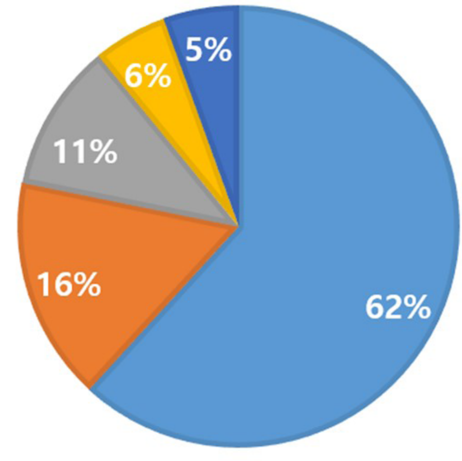

Fig. 4. Mutation type of confirmed causative variants.

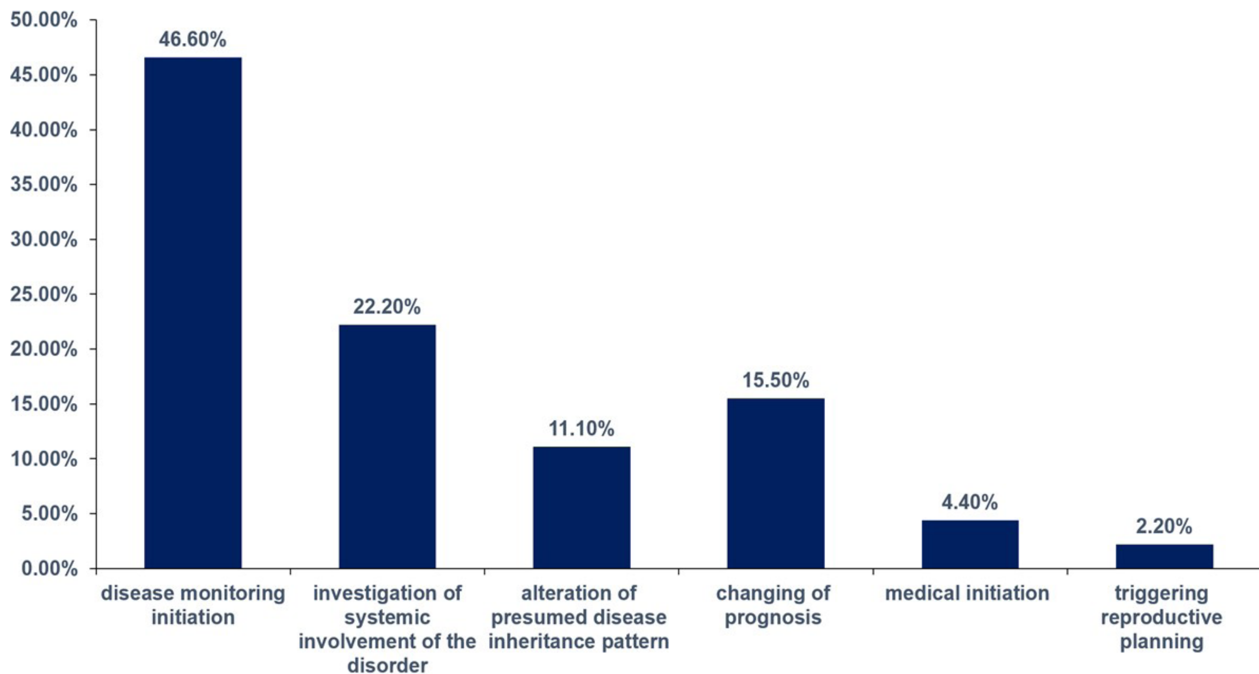

Fig. 6. The results of whole-exome sequencing affect management for confirmed patients with the genetic disease.

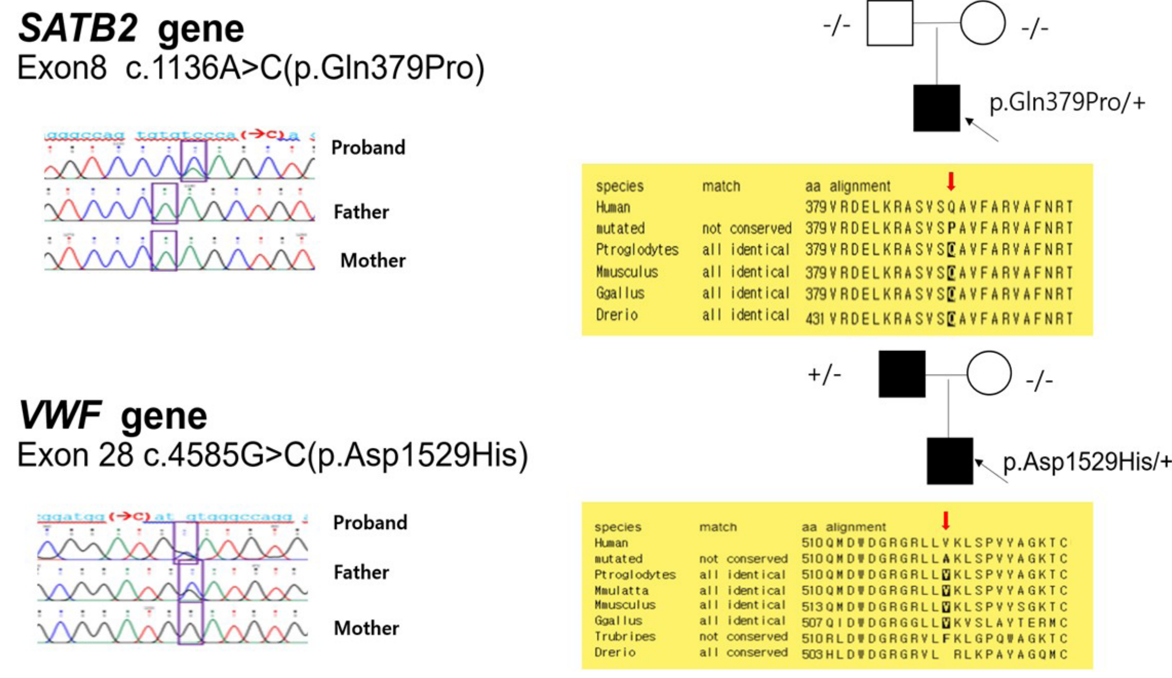

Fig. 7. Whole-exome sequencing revealed the *SATB2* gene variant (NM_001172509) in the c.1136A>C (p.Gln379Pro) on exon8 which was *de novo* variant of uncertain significance, and the *VWF* gene variant (NM_00055212) in the c.4585G>C (p.Asp1529His) on exon 28 which was derived from his father. These two variants were conserved among the different species.

syndrome and type 2 Von Willebrand disease simultaneously and displayed blended phenotypes. The results of WES affect his management regarding discontinuation of iron medication and investigation of systemic involvement such as heart, kidney, bone density, and spine deformity as well as regular monitoring of bleeding tendency.

Discussion

Implementation of WES in clinical practice is at a turning point because approximately 80% of rare diseases are estimated to have a genetic origin. The application of WES in diagnostics has transformed the clinic and helped systematic management of the disease when the causative variants are revealed [10]. This study shows a 40.9% diagnostic yield in 110 patients in whom initial diagnostic tests have negative results. To find causative genes related to rare genetic diseases, WES using buccal swabs as non-invasive specimens was applied in the patients suspected of having rare genetic diseases. The Sanger sequencing and WES agreement was 94.5% (104/110), and the QC criteria were met at 97.3% (107/110) which was relevant. Said et al. [11] reported that the DNA yield from buccal epithelial cells, per set of experiments, was significantly higher than whole blood.

In our study, in the comparison between the confirmed case and the unconfirmed case, it was identified that the frequency of the dysmorphic face was higher in the confirmed case. In the case of a genetic syndrome, if the dysmorphic face is seen as a phenotype, it is more likely that there is a genetic cause. Actually, when seeing patients, descriptions using accurate Human Phenotype Ontology terms for facial appearance will be important in clinical practice. Huang et al. [12] also reported that patients with syndromic short stature having facial dysmorphism had a significantly higher diagnostic rate than those without the corresponding phenotype, which suggested this phenotype might be applied as a predictor for the etiology of rare genetic disease. In addition, there may be differences in the likelihood of a genetic cause depending on which part of the body is involved in the actual patients.

In this study, the frequency of involvement of the visual system in confirmed cases was much higher (51.1%) than that in unconfirmed cases. Retterer et al. [5] also reported a high diagnostic yield of 47% in individuals with problems with the visual system when examining the diagnostic yield for a definitive diagnosis based on the primary phenotype using WES. According to a recent paper, Zamani et al. [13] reported that WES revealed disease-causing variants in 82% of the enrolled cases with vision impairments, suggesting that WES has a great influence on the efficiency of diagnosis depending on which cohort group it is tested.

In the current study, the genetic diagnosis helped us understand the systemic constellation of symptoms and mutations related to each disease and to look out for concomitant organ phenotypes in the affected patients; neurological investigations including brain imaging and EEG, ophthalmic evaluation including strabismus and cataract, endocrinal assessment and skeletal survey as well as planning for the reproductive issue such as infertility.

This study has some limitations. Firstly, false positives in WES from saliva was 5.5% (6/110) which was relevant. The reasons for false positives are as follows. Among them, three cases showed low variant allelic frequency (around 20-25) suggesting low levels of mosaicism while the rest were considered technical errors such as PCR error. Secondly, since this study conducted only the proband-only WES test, a trio-WES or trio-WGS test will be required later for the remaining 59.1% who have not been diagnosed. Lastly, for 9% of VUS, it seems necessary to identify pathogenicity through additional functional studies.

In conclusion, this study shows a 40.9% diagnostic yield for the undiagnosed genetic disease by WES. Patients with dysmorphic facial features and visual system involvement are more likely to have an underlying genetic etiology. WES facilitates genetic diagnosis, eventually enabling us to better understand the ultra-rare disease and serve as a guide for establishing appropriate genetic counseling, surveillance, and management strategies.

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Author's Contributions

Conception and design: CKC. Acquisition of data: CKC, YBS, SYK. Analysis and interpretation of data: CKC, GHS, HL, CK, SHO. Drafting the article: CKC. Critical revision of the article: CKC, SHO, GHS, HL. Final approval of the version to be published: CKC.

References

1. Haendel M, Vasilevsky N, Unni D, Bologa C, Harris N, Rehm H, et al. How many rare diseases are there? Nat Rev Drug Discov 2020;19:77-8.
2. Moon D, Park HW, Surl D, Won D, Lee ST, Shin S, et al. Precision medicine through next-generation sequencing in inherited eye diseases in a Korean cohort. Genes (Basel) 2021;13:27.
3. Ghaoui R, Cooper ST, Lek M, Jones K, Corbett A, Reddel SW, et al. Use of whole-exome sequencing for diagnosis of limb-girdle muscular dystrophy: outcomes and lessons learned. JAMA Neurol 2015;72:1424-32.
4. Tan TY, Dillon OJ, Stark Z, Schofield D, Alam K, Shrestha R, et al. Di-

agnostic impact and cost-effectiveness of whole-exome sequencing for ambulant children with suspected monogenic conditions. JAMA Pediatr 2017;171:855-62.

5. Retterer K, Juusola J, Cho MT, Vitazka P, Millan F, Gibellini F, et al. Clinical application of whole-exome sequencing across clinical indications. Genet Med 2016;18:696-704.
6. Nolan D, Carlson M. Whole exome sequencing in pediatric neurology patients: clinical implications and estimated cost analysis. J Child Neurol 2016;31:887-94.
7. Srivastava S, Love-Nichols JA, Dies KA, Ledbetter DH, Martin CL, Chung WK, et al.; NDD Exome Scoping Review Work Group. Meta-analysis and multidisciplinary consensus statement: exome sequencing is a first-tier clinical diagnostic test for individuals with neurodevelopmental disorders. Genet Med 2019;21:2413-21. Erratum in: Genet Med 2020;22:1731-2.
8. Wang F, Zhu L, Liu B, Zhu X, Wang N, Deng T, et al. Noninvasive and accurate detection of hereditary hearing loss mutations with buccal swab based on droplet digital PCR. Anal Chem 2018;90:8919-26.
9. Harari S. Why we should care about ultra-rare disease. Eur Respir Rev 2016;25:101-3.
10. Monroe GR, Frederix GW, Savelberg SM, de Vries TI, Duran KJ, van der Smagt JJ, et al. Effectiveness of whole-exome sequencing and costs of the traditional diagnostic trajectory in children with intellectual disability. Genet Med 2016;18:949-56.
11. Said M, Cappiello C, Devaney JM, Podini D, Beres AL, Vukmanovic S, et al. Genomics in premature infants: a non-invasive strategy to obtain high-quality DNA. Sci Rep 2014;4:4286.
12. Huang Z, Sun Y, Fan Y, Wang L, Liu H, Gong Z, et al. Genetic evaluation of 114 Chinese short stature children in the next generation era: a single center study. Cell Physiol Biochem 2018;49:295-305.
13. Retterer K, Juusola J, Cho MT, Vitazka P, Millan F, Gibellini F, et al. Clinical application of whole-exome sequencing across clinical indications. Genet Med 2016;18:696-704.
14. Zamani M, Sedighzadeh S, Seifi T, Negahdari S, Zeighami J, Sedaghat A, et al. Whole-exome sequencing deciphers the genetic profile of visual impairments in patients from Southwest Iran. Mol Genet Genomics 2022;297:1289-300.