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Prophylactic Mastectomy and Implant-Based Breast Reconstruction of BRCA1/2 Mutation-Positive Patients in Korea

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Purpose: Mastectomy is performed as a surgical treatment for patients with breast cancer who have the BRCA 1/2 mutation. In this study, we have reported the trends in Korea for both immediate breast reconstruction and prophylactic mastectomy. **Methods:** This retrospective study was conducted from 2019 to 2021. Both skin-sparing mastectomy and immediate implant-based breast reconstruction with prepectoral and/or subpectoral techniques were performed in five patients with BRCA 1/2 mutations. Data on age; body mass index; cancer stage; BRCA 1/2 mutation; estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 expression; diagnosis; and complications were collected. **Results:** The average (±standard deviation [SD]) age was 44.0±6.48 years old; BMI 24.5±2.25 kg/m^2; and breast volumes were 365.8±70.34 and 382.4±96.33 cc for right and left ones, respectively. The BRCA 1 and 2 were diagnosed in four and one patients, respectively. The estrogen and progesterone receptors and human epidermal growth factor receptor 2 were detected in one (20%), one (20%), and three (60%) patients, respectively. The applied implant-based breast reconstruction techniques for ten breasts were subpectoral technique (n=7, 70%) and prepectoral technique (n=3, 30%). For the cancer stage, those with I, II, and III stages were one (20%), two (40%), and one (20%), respectively. There were no major complications such as Infection, seroma. **Conclusion:** When mastectomy is performed as surgical treatment in BRCA 1/2 mutation positive breast cancer patients, it is possible to obtain a better outcome with both implant-based breast reconstruction and different circumstances between breast cancer and contralateral breast.

Key words: BRCA 1/2 mutation, Contralateral prophylactic mastectomy, Bilateral breast reconstruction

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INTRODUCTION

Recently, with the development of equipment and technology, the early diagnosis rate of breast cancer has improved. Concurrently, various genetic tests and treatment methods have also been developed to predict a good prognosis. Generally, once unilateral breast cancer is diagnosed, the amount of resection is determined according to the tumor size. During this process, a BRCA gene mutation test is also usually performed. Breast cancer is the most common cancer among women worldwide and in Korea, and approximately 7% cases are hereditary [1]. Hereditary breast cancer generally refers to a mutation in the BRCA1 or 2 genes, and a confirmed mutation can increase the risk of acquiring breast cancer by 60%–80% [1]. In Korea, the estimated cumulative risk of breast cancer by the age of 70 years is 72.1%/66.3% in the presence of BRCA 1/2 mutation, respectively [2]. Thus, a positive BRCA gene mutation can be considered to be associated with a high risk of breast cancer. In particular, BRCA2 mutation-positive breast cancer tends to show similar pathological features to those of sporadic breast cancer, whereas BRCA1 mutation-positive breast cancer shows features different from

those of sporadic breast cancer. Therefore, triple-negative breast cancer (TNBC) is considered in cases in which the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are all not expressed [3-5]. Other studies reported that 57% of BRCA1 mutations and 23% of BRCA2 mutations in TNBC [6]. It is reported that clinical outcomes are similar when comparing sporadic breast cancer and BRCA-related cancer [7]. Based on these studies, in a patient with a positive BRCA gene mutation diagnosed with breast cancer in one breast, the possibility of developing cancer in the contralateral breast cannot be eliminated. In this case, even if only one breast, a very important anatomical structure symbolizing femininity, is resected, there can be great impact and pain. Particularly, when bilateral mastectomy is decided because of a BRCA gene mutation, breast reconstruction is essentially considered. The oncological safety of unilateral breast reconstruction performed immediately together with mastectomy has already been confirmed, and such treatments have been increasing used recently. Previously, the concept of prophylactic mastectomy was viewed negatively due to oriental thinking. However, in recent years, owing to the improvement in socioeconomic and educational status of patients, the application of prophylactic mastectomy has been increasing. This has led to an increase in the rate of contralateral prophylactic mastectomy (CPM), especially in patients with early-stage, low-risk unilateral breast cancer [8,9]. Therefore, in this study, we have reported about bilateral implant-based breast reconstruction in BRCA 1/2 mutation-positive breast cancer.

METHODS

Study design

The Institutional Review Board of Kyungpook National University Chilgok Hospital (No. 2018-05-005-001) approved this prospective study, and all patients provided informed consent for collecting, analyzing, and publishing their data (including deidentified photographs) for research purposes.

From January 2019 to June 2021, immediate implant-based breast reconstruction was performed after nipple-sparing mastectomy on both sides in five patients with BRCA 1/2 mutation-positive breast cancer. Among them, one was not diagnosed with breast cancer. The following patient characteristics were evaluated: age; body mass index (BMI); preoperative breast volume; implant volume; cancer staging; smoking status (smoker/non-smoker); use of radiotherapy and chemotherapy; presence of BRCA 1/2 mutation; and ER, PR, and HER2 expression. Photometry was performed preoperatively and at 1, 3, 6, and 12 months postoperatively.

Patient selection

This prospective randomized study included patients (1) who were diagnosed with BRCA 1/2 mutation, (2) who were 30–60 years old at the time of surgery, and (3) who underwent immediate implant-based breast reconstruction after nipple-sparing mastectomy on both sides. This study excluded patients (1) who were diagnosed with advanced stage III or IV breast cancer, (2) who were unable to complete the self-reported questionnaire owing to a history of cognitive impairment, (3) who had a history of neurological and musculoskeletal disorders, and (4) who had a history of alcohol or drug abuse.

Surgical procedure

Selection of skin-sparing mastectomy incision

After discussing with an oncological breast surgeon and a plastic surgeon, (1) a lateral incision from the nipple to the axillar area, (2) an incision of approximately one-third of the lateral part of the inframammary fold using endoscopy, and (3) an approximately 4–5-cm incision in the mid-axillary line for robot-assisted mastectomy were preferred by the patients and were appropriately performed according to the breast size.

Implant-based breast reconstruction: subpectoral and/or prepectoral technique

The appropriate technique was determined according to a skin sparing mastectomy and by checking the shape of the breast defect. If the breast height was longer than the breast width of the defect, the subpectoral plane was used to reduce the depression of the upper pole of the breast with a round implant and the upper part was covered with the pectoralis major muscle. The insufficient implant pocket of the inferolateral part was custom-made for patients with an acellular dermal matrix (ADM), and subsequently, the implant was inserted. However, if the width and height of the breast were similar or wider, a large ADM was used as a prepectoral plane according to the width to create a pocket with full coverage of the anterior part of the breast implant and then proceeded with sparing of the pectoralis major muscle (Fig. 1). In both techniques, to minimize foreign body reaction to the implant, after applying two lines of the negative suction drain, irrigation was performed using an antibiotic irrigation solution (povidone–iodine, 50 cc; isepamicin, 80 mg; cefazolin, 1 g in 500 mL of sterile saline). Then, after betadine redraping of the incision

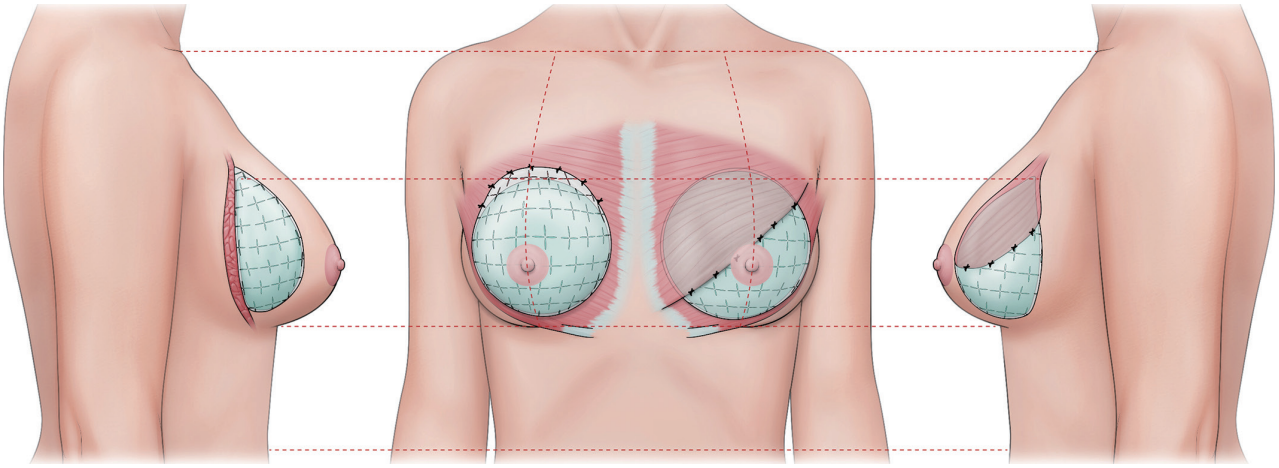


Fig. 1. Concept illustration of implant-based breast reconstruction in BRCA 1/2 positive patient. In the case of a BRCA 1/2 positive patient diagnosed with left breast cancer, the mastectomy area is wider for oncological safety in the affected left breast, and is smaller in the right breast based on the concept of contralateral prophylactic mastectomy, and the thickness of the breast skin flap is preserved. Because the finding on both sides is different, the size of the breast implant is different, and the technique is selected according to the patient's situation.

site and implant insertion, the ADM was checked in terms of the location of the drain; after positioning it at the desired location, the shape of the reconstructed breast, and symmetry of both sides was confirmed. However, before the final implant selection, the most appropriate profile for the patient was selected through an implant sizer, and the same implant of the same size was not always inserted into both breasts because of the difference in the mastectomy defects on the two sides.

RESULTS

For the five patients with BRCA 1/2 mutation positive, their average (±standard deviation [SD]) age was 44.0±6.48 years old; BMI 24.5±2.25 kg/m^2; and breast volumes were 365.8±70.34 and 382.4±96.33 cc for right and left ones, respectively. The BRCA 1 and 2 were diagnosed in four and one patients, respectively. The estrogen and progesterone receptors and human epidermal growth factor receptor 2 were detected in one (20%), one (20%), and three (60%) patients, respectively. For the axillary operation, lymph node biopsy (n=3, 60%) and axillary lymph node dissection (n=1, 20%) were reported. The applied implant-based breast reconstruction techniques for ten breasts were subpectoral technique (n=7, 70%) and prepectoral technique (n=3, 30%). Only one patient was found to receive radiotherapy as an adjuvant one and four patients were found to receive chemotherapy as a neo (n=2, 40%) and adjuvant (n=2, 40%). For the cancer stage, those with I, II, and III stages were one (20%), two (40%), and one (20%), respec-

Table 1. Patient's demographics. Average±Standard deviation

Category	n=5 (10 breasts)
Age	44.0±6.48
BMI	24.5±2.25
Breast volume (R/L)	365.8±70.34/382.4±96.33
BRCA 1/2	4 (80%)/1 (20%)
ER/PR/HER2	1 (20%)/1 (20%)/3 (60%)
TNBC/non-TNBC	1 (20%)/3 (60%)
Axillary OP	
- SLNB	3 (60%)
- ALND	1 (20%)
- None	1 (20%)
Subpectoral/prepectoral (breast)	7 (70%)/3 (30%)
Radiotherapy	
- Adjuvant	- 1 (20%)
- None	- 4 (80%)
Chemotherapy	
- Neoadjuvant	- 2 (40%)
- Adjuvant	- 2 (40%)
- None	- 2 (40%)
Cancer stage	
1/2/3	1 (20%)/2 (40%)/1 (20%)
Pathology	
- IDC	- 4 (80%)
- None	- 1
ADM (breast)	
- CGCryoderm	- 4 (40%)
- MegaDerm	- 3 (30%)
- BellaCell HD	- 3 (30%)

BMI, body mass index; BRCA, breast cancer gene; ER, estrogen receptor; PR, progesterone receptor; HER 2, human epidermal growth factor receptor 2; TNBC, triple negative breast cancer; SLNB, sentinel lymph node sampling; ALND, axillary lymph node dissection; OP, operation; IDC, invasive ductal carcinoma; ADM, acelluar dermal matrix.

tively. The invasive ductal carcinoma (n=4, 80%) accounted for most of the pathologic diagnosis. There were no major complications such as Infection, seroma, and implant rupture and both nipple necrosis was found in a patient (Table 1).

DISCUSSION

BRCA 1/2 is a gene that provides genetic information on breast cancer. Generally, it is diagnosed more often in younger patients than in older patients; this trend is similar worldwide and in Korea. This trend may be due to a selection bias as younger patients more readily agree to undergo genetic testing. In particular, patients with BRCA1 mutation tend to have higher histological and nuclear grades and a lower proportion of ductal carcinoma in situ (DCIS) than those without BRCA1 mutation. However, the T stage distribution was not significantly different in the BRCA2 group, and notably, the axillary lymph node involvement was found to be more frequent in the BRCA2 group. ER, PR, and HER2 statuses are very important factors for treatment and prognosis as they have crucial clinical implications. In particular, BRCA1 mutation tends to occur more frequently in triple-negative breast cancer [10-12]. In addition, it has been reported that BRCA-related breast cancer has a clinical outcome similar to that of sporadic breast cancer [6,7]. Research on the molecular basis for the phenotype specificity of BRCA1 is ongoing. A previous study has shown that the BRCA1 gene can induce ERα mRNA expression [13]. Therefore, ERα expression can be inhibited in the case of positive BRCA1 mutations [14]. Research at the molecular level and that on the relationship with PR or HER2 is ongoing. In the case of axillary lymph node involvement, the possibility of involvement is generally high when the tumor size is large, but the possibility is slightly lower in the case of positive BRCA 1 mutation [15]. Studies on the presence of BRCA 1/2 mutations; association with ER, PR, and HER2 receptors; and analysis of axillary lymph node involvement, prognosis, and treatment are ongoing. If the differences and similarities with patients with sporadic breast cancer are identified and the basics of the relationship at the molecular level are revealed, a positive outcome for breast cancer patients can be predicted in the future. Based on these analyses, surgical treatment should be administered and long-term analysis of cases in which immediate breast reconstruction was performed after mastectomy must be performed. Usually, breast cancer is diagnosed first, and genetic testing is performed later with patient consent while investigating the family history and specifics. If patients are positive for BRCA 1/2 mutation, they have a very high probability of being diagnosed with breast cancer even though they may not have it yet. Therefore, the option to remove the breasts simultaneously can be explained to patients. As life expectancy increases and the rate of early diagnosis increases, there will be a gradual change in the tendency to recommend contralateral prophylactic mastectomy (CPM) if there is a gene mutation. In addition, the cancer risk on the contralateral breast can be reduced by up to 95% in the case of CPM [8,9]. Surgical treatment is performed for these reasons, and the scope of mastectomy must be slightly different between patients diagnosed with breast cancer and those undergoing surgery due to a BRCA

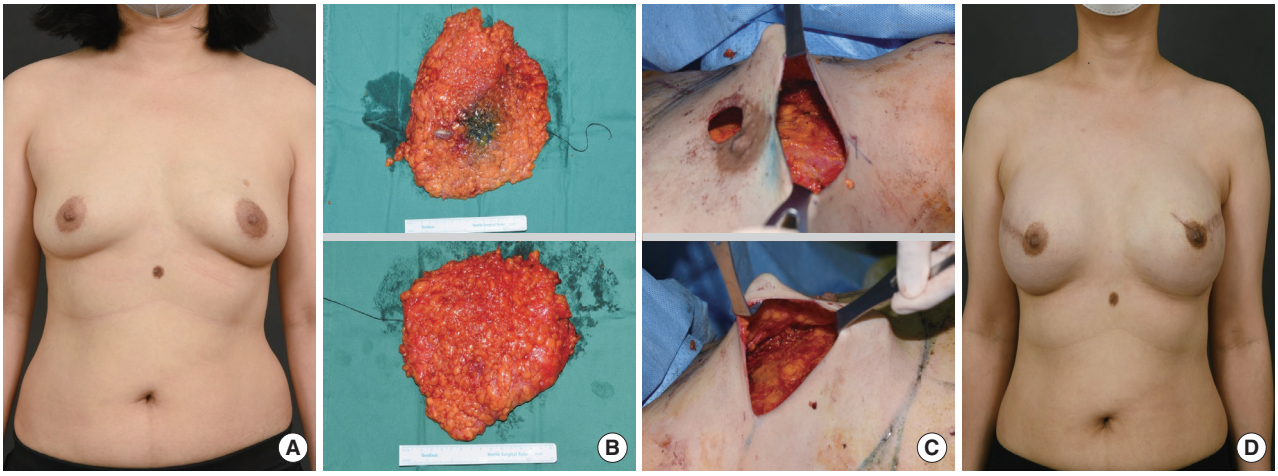


Fig. 2. Prophylactic mastectomy and immediate implant-based breast reconstruction. (A) Preoperative findings. Left breast cancer patient with BRCA 1 mutation positive patient. (B) Specimen. upper; Left breast, lower; Right breast. (C) Breast defect and skin flap after mastectomy. upper; Left breast, lower; Right breast. (D) Postoperative 1 year findings.

1/2 mutation. When cancer is diagnosed first, axillary lymph node sampling must be performed simultaneously and, if necessary, axillary lymph node dissection should be performed. Mastectomy also requires a safety margin; hence, rather than excising only the breast tissue, efforts are being made to reduce the recurrence rate by excising a sufficiently larger tissue than the range. However, in the case of CPM, as breast cancer is not diagnosed, axillary lymph node sampling is unnecessary, and only the mammary gland tissue is excised to preserve a thicker skin flap, which can be considered an advantage for breast reconstruction. As both sides are usually reconstructed, implant-based breast reconstruction rather than autologous tissue reconstruction is often chosen because the recovery time is lesser. This can be the reason why there is a possibility of using implants of different sizes in different ways for both breast reconstructions (Figs 1, 2). The thick skin flap reduces the appearance of the implant projected through it, resulting in a better aesthetic outcome. Complications such as sloughing and, in severe cases, partial necrosis, may be reduced, to some extent, to provide a better blood supply. It may cause, however, necrosis since the blood supply to the nipple-areolar complex (NAC) part is poor. This is considered the problem of difference in the vascular formation variation or preoperative chemotherapy or radiotherapy, rather than problem of the surgical technique. Recently, high-quality ADM (acellular dermal matrix) of various sizes can be used for implant-based breast reconstruction, which is very useful for creating implant pockets. Moreover, complications such as infection or seroma are gradually decreasing. As such, the possibility of occurrence, treatment, and prognosis of BRCA 1/2 mutation and breast cancer can be predicted, and surgical treatment is performed in parallel based on these factors. Recently, the quality of life of the patients, a very important factor, is increasingly. Because there is no correlation between cancer and immediate implant-based breast reconstruction, the trend is expected to change with the concept of prophylactic mastectomy and breast reconstruction. In the future, it can be expected that the cancer incidence rate will be predicted at the genetic level before cancer is diagnosed and that the concept of total care for a better life will flow into a safer, more practical, and aesthetic outcome for the rest of the lifetime.

In conclusion, despite the limitation of having a small sample size, we were able to investigate the association between BRCA 1/2 mutation and breast cancer incidence. Therefore, when mastectomy is performed as surgical treatment, it is possible to obtain a better outcome with both implant-based breast reconstruction by considering the symmetry of the breast and different circumstances between diagnosed and undiagnosed breast cancer.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest to disclose.

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Targeted Panel Exome Sequencing in Suspected Monogenic Diabetes: Single-Center Pilot Study

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Purpose:Maturity onset diabetes of the young (MODY) is the most common hereditary form of diabetes mellitus (DM), with similar clinical manifestations to type 1 or type 2 DM, leading to diagnostic ambiguity. Despite increased genetic research on monogenic DM, studies with Asian populations are limited. Therefore, we investigated mutation in possible monogenic DM and MODY in Korean children and aldolescents. Methods: Targeted panel exome sequencing including 32 targets genes was performed for 41 patients with suspected monogenic DM at Kyungpook National University Children's Hospital. Results: Variants were detected in 19 patients, including those in known MODY-associated genes (*HNF4A*, *GCK*, *HNF1A*, *CEL*, *PAX4*, *INS*, and *BLK*) and monogenic DM-associated genes (*WFS1*, *FRX6*, and *GLIS3*). Conclusion: MODY variants were detected more than expected. Targeted exon sequencing is helpful in diagnosing MODY or possible monogenic DM patients.

Key words: Diabetes mellitus, Genes, Koreans

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INTRODUCTION

Monogenic DM, which is associated with a single-gene disorder or hereditary diseases [1,2]. The most common form of monogenic diabetes is maturity-onset diabetes of the young (MODY), and this category also includes neonatal diabetes and maternally inherited diabetes and deafness [3]. MODY has similar charicteristics to those of type 1 and 2 DM, resulting in diagnostic ambiguity. In addition, disease treatment and progression differ between the two conditions, further emphasizing the importance of an accurate differential diagnosis [4,5]. Recently, there has been an explosion in research related to the genetic diagnosis of monogenic DM using targeted panel exome sequencing, leading to the identification of 14 MODY-specific genetic variants that change the function of beta cells to cause DM [6-8]. However, studies on the genotype of monogenic DM have mainly focused on Caucasian patients, with limited genetic screening performed for Asian patients to date. Therefore we performed targeted panel exome sequencing on pediatric patients suspected of having monogenic DM at Kyungpook National University Children's Hospital to determine how many Patients have MODY gene variation.

MATERIALS AND METHODS

Participants

Participants included pediatric patients from the Endocrinology Unit at Kyungpook National University Children's Hospital who were receiving insulin therapy by injection following a diagnosis of DM prior to the age of 25 years. A total of 41

patients who have a family history of two generation or more were selected for targeted panel exome sequencing. Patients who received a diagnosis of type 1 DM (C-peptide < 0.6 ng/mL, or anti-pancreas antibody-positive) were excluded from the study [9].

Clinical parameters and Targeted exome sequencing

Patient clinical characteristics were reviewd with respect to age, sex, family history, body mass index (BMI), and C-peptide levels. Targeted exome sequencing in the patient was performed using the TruSight One Sequencing Panel, 32 genes, including 14 identified MODYgenes (*HNF4A*, *GCK*, *HNF1A*, *PDX1*, *HNF1B*, *NEUROD1*, *KLF11*, *CEL*, *PAX4*, *INS*, *BLK*, *ABCC8*, *KCNJ11*, *APPL1*) and 18 monogenic DM-related genes (*EIF2AK3*, *FOXP3*, *GATA4*, *GATA6*, *GLIS3*, *IER3IP1*, *INSR*, *MNX1*, *NEUROG3*, *NKX2-2*, *PAX6*, *PTF1A*, *RFX6*, *SLC19A2*, *PTPRD*, *SYT9*, *WFS1* and *PAX1*).The pathogenicity of the geneticvariants was evaluated according to the American College of Medical Genetics and Genomics and Association for Molecular Pathology guidelines.

Statistical analysis

Data were using a t-test with PAWS Statistics 20 (SPSS Inc., Chicago, IL, USA) after confirming the normality of the data distribution. Significance was defined as $P < 0.05$

RESULTS

The characteristics of participants (Table 1)

Of the 41 patients, 23 were male (56.1%) and 18 were female (43.9%). At the time of diagnosis, the average age of all patients was 11.80 ± 3.7 years, the HbA1c level was 12.14 ± 2.42%, body weight was 59.95 ± 26.92 kg, BMI was 23.11 ± 4.72 kg/m^2, serum insulin level before and after meals was 6.40 ± 4.01 uU/mL and 10.72 ± 9.83 uU/mL, respectively, and the serum level of C-peptide before and after meals was 2.50 ± 1.61 ng/mL and 3.84 ± 3.38 ng/mL, respectively.

Table 1. The characteristics of participants

Variables	Patients
Number (M/F)	41 (23/18)
Age	11.8 ± 3.7
HbA1c	
At Diagnosis	12.1 ± 2.4
BMI (kg/m^2)	23.1 ± 4.7
Insulin (uU/mL)	
Pre-meal	6.4 ± 4.0
Post-meal	10.7 ± 9.8
C-peptide (ng/mL)	
Pre-meal	2.50 ± 1.61
Post-meal	3.84 ± 3.38

Identification of gene variants (Table 2)

Variants were identified in 19 of the 41patients (46.3%), including variants in seven MODY-identified genes (*HNF4A*, *GCK*, *HNF1A*, *CEL*, *PAX4*, *INS*, and *BLK*) and three monogenic DM-related genes (*WFS1*, *RFX6*, *GLIS3*).

DISCUSSION

In this study, targeted panel exome sequencing was performed on pediatric patients suspected of having monogenic DM. In a review of the literature, Kim et al. [10] confirmed that the MODY3 and MODY2 genotypes account for 20% and < 5% of all MODY cases in Korea and China, respectively. In this study, monogenic DM variants were identified in 19 of 41 (46.3%) patients. Actually, the frequency of PV or LPV variations excluding the actual VUS is 9.75% (4 of 41), and the frequency of PV or LPV variations excluding the VUS variations is more reliable as the frequency of the actual Monogenic DM [11]. However, compared to studies where the previous VUS and PV/LPV ratios were 6% and 2.1%, respectively, we can see a high positive rate [12]. Instead, Yang et al. [13] reviewed studies of Korean patients with monogenic DM, highlighting that the prevalence of MODY, which has been studied mainly in Western populations to date, may be different for Asians. Similarly, we found a high prevalence of the MODY8 genotype (4/17, 21.0%), which has been reported at a low frequency in previous studies [14].

Based on a review of the state of monogenic DM in Korea, MODY exhibits various pathophysiological mechanisms and clinical manifestations [13]. Unlike type 1 DM, which is caused by the destruction of pancreatic beta cells, some MODY subtypes do not require insulin therapy and can be improved with oral antidiabetic medications, diet management, and treatment with sulfonylurea. However, we did not find any difference in disease progression according to the presence or absence of variants, with respect to a similar significant in HbA1c and BMI.

The limitation of this study were many patients with gene variants showed VUS. Further more, there are not many patient numbers. In order to secure these limitations in the future, it is necessary to conduct a family examination on patients with confirmed genetic variation, and increased targeted study numbers.

Table 2. Clinical and molecular characteristics of patients with MODY gene variant

Pt	Gen	Age (yr)	BMI	HbA1c	c pep	Gene	DNA	AA Change	Zyg	Disease	Class	Clin	Novelity	In silico analysis score
1	F	9.9	1.0	9.0	5.19	*HNF4A*	c.778G>A	p.(Asp260Asn)	Het	MODY1	VUS	none		S/P/M Deleterous
2	M	3.2	-0.43	6.7	0.42	*GCK*	del (chr.44184714-44186245)	exon 9-11	Het	MODY2	PV	none		/
3	F	3.2	1.29	6.7	1.99	*GCK*	c.775G>A	p. (Ala259Thr)	Het	MODY2	PV	none		S/P/M Deleterous
4	M	6.9	1.83	6.4	1.70	*GCK*	c.579+1G>T	p.(?)	Het	MODY2	PV	none		M Tolerate
5	F	12.2	0.67	9.0	2.1	*HNF1A*	c.773T>C	p.(leu258pro)	Het	MODY3	VUS	none	Y	S/P/M Deleterous
6	M	15.5	1.47	6.1	2.6	*CEL*	c.1421C>T	p.(Thr474Met)	Het	MODY8	VUS	none		S Deleterous P/M Tolerate
7	F	13.3	1.35	13.2	1.61	*CEL*	c.2106_2171del	p.(Thr7004_Val725del)	Het	MODY8	VUS	none		/
8	M	10.1	1.75	14.7	1.47	*CEL*	c.1154C>T	P.(Thr385Met)	Het	MODY8	VUS	none		S/P Deleterous M Tolerate
9	M	14.1	1.49	14.3	1.97	*CEL*	c.1627C>T	p.(Arg543Cys)	Het	MODY8	VUS	none		S/P Deleterous M Tolerate
10	M	14.1	2.12	12.3	3.8	*PAX4*	p.(Arg121Trp)	p.(Arg 121 Trp)	Het	MODY9	VUS	none		S/P/M Deleterous
11	M	14.2	1.54	12.8	0.2	*PAX4*	c.374_412+1del	p.(Arg192His)	Het	MODY9	LPV	none	Y	S/P Deleterous M polymorphism automatic
12	M	10.2	1.36	17.3	1.0	*INS*	c.67G>A	P.(Ala23Thr)	Het	MODY10	VUS	deafness		S/P/M Tolerate
13	M	14.2	1.55	12.4	4.0	*BLK*	c.337del	p.(val113TrpfsTer52)	Het	MODY11	VUS	none		/
14	F	11.2	1.71	13.0	3.3	*WFS1*	c.1846G>T	p.(Ala616Sat)	Het	WFS1	VUS	none		S/P/M Tolerate
15	F	14.2	0.35	11.6	1.1	*WFS1*	c.76C>T	P.(Arg26Ter)	Het	WFS1	LPV	none		M Deleterous
16	M	16.2	0.23	12.4	0.8	*WFS1*	c.2417C>G	p.(Ala806Gly)	Het	WFS1	VUS	none	Y	S Tolerate P/M Deleterous
17	F	16.2	1.74	12.6	2.0	*WFS1*	c.1264G>T	p.(Ala422Ser)	Het	WFS1	VUS	none		S/M Deleterous P Tolerate
18	F	17.2	-0.51	10.3	1.2	*RFX6*	c.280G>C	p.(Asp94His)	Het	MTCHRS	VUS	none		S/M Deleterous P Tolerate
19	M	17.2	-0.01	11.8	1.3	*GLIS3*	c.838G>C	p.(Glu280Gln)	Het	Diabetes Mellitus	VUS	none	Y	S/P/M Tolerate

Pt, patient; Gen, Gender; Age, Age when DM diagnosis/exon sequencing did; BMI, Body mass index, Z score; C.pep, C-peptide; Zyg, zygomatic; Ds, disease; C.pep, C.peptide; Path, pathophysology; Het, heterozygosity; Clin, Clinical Pathology; VUS,variants of unknown significance; PV, pathogenic variants; LPV, likely pathogenic variants; WFS, wolfram-like sydrome; Y, novel variation; Zyg, zygonite; AA, aminoacid; in silico analysis S, SIFT; P, Polyphen2; M, MutationTaster.

In conclusion, using targeted panel sequencing, we were able to make molecular genetic diagnoses for 17/39 patients (44%) with MODY. MODY variants were detected more than expected in Korean children and aldolescents with suspected monogenic DM.

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None

DISCLOSURE

All authors have no potential conflicts of interest.

ETHICAL STATEMENT

This study was approved by the Institutional Review Board (approval number: 2017-05-006) of Kyungpook National University Children's Hospital. This was a retrospective study; hence, it was exempted from the requirement of obtaining informed consent from patients.

AUTHOR CONTRIBUTIONS

Conceptualization: Moon JE. Data curation: Lee JM, and Lee SW. Formal analysis: Lee JM, Lee R, Lee MS, Lee SW and Moon JE. Methodology: Moon JE. Writing - original draft: Lee SW, Lee JM and Moon JE. Writing - review & editing: Lee JM and Moon JE.

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A Heterogeneous Genetic Disorder: Primary Ciliary Dyskinesia

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Primary ciliary dyskinesia (PCD) is a genetic disorder that affects approximately 1 in 15,000–30,000 people, with the majority of patients inheriting the disorder via autosomal recessive inheritance. PCD is characterized by abnormal ciliary ultrastructure and/or function, which results in impaired mucociliary clearance and recurrent respiratory infections. Despite the presence of symptoms from birth, many patients with PCD remain undiagnosed until adulthood. Many advances in the diagnosis of PCD have occurred in recent years, including nasal nitric oxide assays, ciliary motility tests, and genetic sequencing. Early diagnosis and symptom management may reduce morbidity and mortality from PCD improving the patient's quality of life.

Key words: Primary ciliary dyskinesia, Genetic disorder, Diagnosis

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INTRODUCTION

Primary ciliary dyskinesia (PCD, MIM 244400) is a rare heterogeneous genetic disorder characterized by abnormal ciliary function or ultrastructure [1]. PCD Inheritance is mostly autosomal recessive, with an estimated prevalence of 1:15,000–30,000 [2]. PCD can be caused by mutations in genes that code for the axonemal structure or functional components of motile cilia [3]. PCD is a respiratory ciliopathy characterized by decreased mucociliary clearance of the airway [3]. There is no single gold standard diagnostic test for PCD [4]. Early PCD diagnosis is important for proper clinical management and better prognosis of patients with PCD.

CLINICAL FEATURES

The clinical manifestations of PCD typically begin in childhood. They may differ by the sites of dysfunctional cilia [5]. Mucociliary clearance is affected by ciliary dysfunction, resulting in chronic airway infections and inflammation. The primary manifestations are a situs inversus and unexplained neonatal respiratory distress in the neonatal period (82%), chronic otitis media (92%), pansinusitis (100%), and/or bronchiectasis in the infancy to preschool period, and infertility in the adult period [5]. Laterality defect is present in approximately half of PCD cases and is caused by the dysfunction of embryonic nodal cilia [6]. The prevalence of infertility and subfertility in PCD is still unknown. According to a systematic review, 58% of women and 100% of men are infertile [5].

PATHOGENESIS

Cilia are classified into three types: primary cilia, nodal cilia, and motile cilia.

Motile cilia are long, thin protrusions that extend up to 20 μm from the cell surface and move fluids along surfaces of respiratory epithelium such as the nasal and sinus cavities, eustachian tubes in-ear, lungs, brain ependyma, and fallopian tubes [7]. Sperm tails have an ultrastructure that is similar to cilia. The axoneme of motile cilia is made up of nine peripheral doublet microtubules with attached inner and outer dynein arms and radial spokes that surround a central complex made up of two central microtubules surrounded by a central sheath (9+2 axonems) [8]. Cilia play an important role in mucociliary clearance. Therefore, the ultrastructure of cilia is critical for improving the clearance of the lower respiratory tracts.

The nodal cilia are important in establishing left-right body orientation during embryogenesis. The nodal cilia are found in embryonic nodal plate cells and have a "9+0" microtubule arrangement [8]. Abnormalities in the nodal cilia can result in laterality defects, which may be associated with congenital heart defects [9].

PCD has various ciliary ultrastructural defects. In patients with PCD, cilia are stiff, ineffective, or absent, resulting in a build-up of mucus in the nose, sinuses, ears, and lungs, as well as a fertility problem.

DIAGNOSIS

There is no gold standard test for PCD. Several tests for PCD diagnosis have been developed, including cilia structural and functional assessments, a nasal nitric oxide (nNO) assay, and genetic testing [10]. The European Respiratory Society (ERS) guidelines, recently published, provide evidence-based recommendations on the diagnosis of PCD and offer an algorithm for diagnostic testing, which should be based on clinical symptoms, nNO assessment, high-speed video microscopy analysis (HSVMA), transmission electron microscopy (TEM) and genetic testing [10]. A definitive positive diagnosis of ERS comes with a non-ambiguous bi-allelic mutation or hallmark ciliary ultrastructural defect, according to PCD classification guidelines [10]. A highly likely diagnosis is given with compatible history, very low nNO, and either highly abnormal ciliary beat pattern on HSVMA or highly abnormal ciliary beat pattern on HSVMA following cell culture [10]. Modest or non-suggestive history and normal or high nNO renders the diagnosis of PCD extremely unlikely [10].

Traditional diagnostic tests of PCD have incorporated TEM to identify ciliary ultrastructural defects and HSVMA from airway epithelium biopsies to assess the ciliary beat frequency and motility [11]. However, chronic respiratory infection and inflammation often result in additional secondary ciliary damage, which modifies the primary ciliary abnormality. Diagnostic tests for cilia ultrastructure or function are not widely available or standardized. Furthermore, approximately 30% of patients with PCD have normal ciliary ultrastructure and/or subtle functional deficits [12,13]. Therefore, readily available diagnostic tests for PCD, such as the nNO assay and genetic testing, have benefited diagnosis.

The nNO assay has proven to be a useful PCD test. nNO is measured using a chemiluminescent nitric oxide analyzer accordance with international guidelines [14]. nNO levels are low in patients with PCD (10%–20% of normal values). However, low nNO levels have been found in some patients with cystic fibrosis, and on acute viral infections, acute bronchiolitis, and acute sinusitis [15]. Furthermore, a recent meta-analysis found high levels of heterogeneity among studies, and there is no agreement on appropriate thresholds, particularly for children under the age of 6 [16].

A genetic test should be considered as one of the diagnostic tests. PCD is a genetically heterogeneous autosomal recessive disorder. Previous studies reported over 40 pathogenic genes causing PCD [17]. Based on homozygosity and candidate gene testing, PCD-causing mutations were identified in 11 genes from 1999 to 2010 [18,19]. Exome sequencing has revealed PCD-causing mutations in 10 additional genes since 2011 [3]. There are several other newly discovered novel genes with PCD mutations [3].

Most of these genes code proteins in the axonemal structure or functional components of motile cilia. Mutations in PCD-causing genes affect the outer dynein arm (ODA), inner dynein arm (IDA), cytoplasmic proteins, microtubular disorganization, nexin link, central pair microtubules, and radial spoke proteins (Table 1) [10,20]. The majority of PCD mutations are loss-of-function variants, such as nonsense, frameshift, or splice mutations, with missense mutations occurring in a minority of cases [3].

Genetic testing is useful for diagnosing PCD in patients who have limitations with the nNO assay and electron microscopy assessment. Several studies have identified the causative genes up to 65% of cases [21,22]. Candidate gene analysis remains the primary tool for gene identification, though next-generation sequencing technologies may offer a greater advance. To establish the genetic diagnosis, non-ambiguous bi-allelic mutations in autosomal recessive and hemizygous mutations in X-linked PCD should be identified. Furthermore, to confirm

Table 1. Overview of primary ciliary dyskinesia causing genes

Sites of Defect	Gene	Locus
ODA	*DNAI1*	9p21-p13
	DNAI2	17q25.1
	DNAH5	5p15
	DNAL1	14q24.3
	CCDC103	17q12
	NME8/TXNDC3	7p14.1
	CCDC114	19q13.33
	CCDC151	19p13.2
	ARMC4	10p21
	TTC25	17q21.2
	DNAH8	6p21.2
ODA+IDA	*LRRC50/DNAAF1*	16q24
	KTU/DNAAF2	14q21.3
	C19orf51/DNAAF3	19q13
	LRRC6	8q24
	HEATR2/DNAAF5	7p22.3
	DYX1C1/DNAAF4	15q21
	ZMYND10	3p21.3
	SPAG1	8q22
	C21orf59	21q22.1
	PIH1D3	Xq22.3
MT disorganization+IDA	*CCDC39*	3q26
	CCDC40	17q25
Normal/subtle: N-DRC links missing with occasional MT disorganization	*CCDC164/DRC1*	2p23
	CCDC65/DRC2	12q13.12
Normal/subtle: increased frequency of transposition defects	*HYDIN*	16q22
Normal/Central pair defects	*RSPH9*	6p21
	RSPH4A	6q22
	RSPH1	21q22.3
	DNAH6	2p11.2
	DNAJB13	11q13.4
	STK36	2q35
	RSPH3	6q25.3
Normal/subtly abnormal: increased frequency of MT misalignment	*GAS8/DRC4*	16q24.3
Reduction of cilia number	*CCNO*	5q11.2
	MCIDAS	5q11.2
Variable	*RPGR*	Xp21.1
Unknown	*OFD1*	Xp22
Normal	*DNAH11*	7p15-21

ODA, outer dynein arm; IDA, inner dynein arm; MT, microtubular; N-DRC, nexin-dynein regulatory complex.

the genetic cause of PCD, the genetic variants should be correlated with the ultrastructural defects.

MANAGEMENT

The management of patients with PCD is not standardized and the evidence-based treatment for patients with PCD is lacking. Therefore, management strategy is based on expert consensus and the clinician's experience [4].

According to European guidelines, patients should be checked every three months for respiratory assessment [4,23]. Because patients with PCD have impaired mucociliary clearance, routine airway clearance management, elimination of inflammatory triggers, and use of antibiotics when patients with PCD have infectious diseases are required. High-resolution computed tomography can detect bronchiectasis in its early stages, before changes in lung function or plain chest radiography [24].

The treatment of otitis media aims to improve hearing levels while also preventing delays in speech and language development [25]. PCD patients frequently have chronic otitis media and/or effusion, which causes conductive hearing loss. Therefore, episodes of otitis media should be treated using standard methods, but data on surgical intervention are lacking. Hearing needs to be evaluated on a regular basis.

Sinusitis is a major issue for PCD patients. Nasal irrigation, nasal steroids, and intermittent courses of systemic antibiotics are used to treat the condition [26]. Clearing the nose allows patients to breathe more easily, improves their senses of smell and taste, and improves their sleep quality. Sinus irrigation is a safe, simple, inexpensive, and generally well-tolerated treatment option, but more studies are needed to determine its effectiveness in PCD [4].

An in vitro study with aminoglycosides demonstrated the efficacy of premature termination codon read-through stimulation in PCD-causing mutations. Because this precision medicine for PCD was a preclinical cell-line study, more study is required to validate its efficacy [27].

In the future, therapies that can potentially correct the basic disease defect or the resulting defective proteins will be required in genetic disorders such as PCD [28].

CONCLUSION

Despite the fact that symptoms are present from birth, PCD remains underdiagnosed. Significant advances in PCD diagnosis have been made in the last decade. Early diagnosis and treatment may reduce morbidity and mortality, delay irreversible lung damage, and improve quality of life.

CONFLICTS OF INTERESTS

No potential conflict of interest relevant to this article was reported.

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The Role of Genetic Diagnosis in Hemophilia A

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Hemophilia A is a rare X-linked congenital deficiency of clotting factor VIII (FVIII) that is traditionally diagnosed by measuring FVIII activity. Various mutations of the *FVIII* gene have been reported and they influence on the FVIII protein structure. A deficiency of or reduction in FVIII protein manifests as spontaneous or induced bleeding depending on the disease severity. Mutations of the *FVIII* gene provide important information on the severity of disease and inhibitor development. *FVIII* mutations also affect the discrepant activities found using different FVIII assays. *FVIII* activity is affected differently depending on the mutation site. Long-range PCR is commonly used to detect intron 22 inversion, the most common mutation in severe hemophilia. However, point mutations are also common in patients with hemophilia, and direct Sanger sequencing and copy number variant analysis are being used to screen for full mutations in the *FVIII* gene. Advances in molecular genetic methods, such as next-generation sequencing, may enable accurate analysis of mutations in the factor VIII gene, which may be useful in the diagnosis of mild to moderate hemophilia. Genetic analysis is also useful in diagnosing carriers and managing bleeding control. This review discusses the current knowledge about mutations in hemophilia and focuses on the clinical aspects associated with these mutations and the importance of genetic analysis.

Key words: Hemophilia A, Genetic testing, Factor 8, Mutation

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INTRODUCTION

Hemophilia A is the most common inherited bleeding disorder and is caused by an abnormal or decreased activity of factor VIII (FVIII) protein. The *FVIII* gene is located on the X chromosome, and hemophilia A is an X-linked recessive disorder that predominantly affects males [1]. Its prevalence is 1 in 5,000–10,000 male births. Females are commonly heterozygote carriers; however, although rare, they can develop hemophilia as a result of X-chromosome inactivation, such as in Turner's syndrome, or if both parents carry abnormal genes [2].

Hemophilia A is diagnosed via factor assays to identify FVIII deficiency. The plasma concentrations of FVIII correlate with the degree of bleeding, which is classified as mild, moderate, or severe, and is related to the factor levels (mild, 6%–40% of normal; moderate, 1%–5% of normal; severe, < 1% of normal) [3].

According to the 2019 annual report of the Korea Hemophilia Foundation (KHF), there were was 1,746 patients with hemophilia A in Korea in that year [4]. Given the size of the Korean population, this is much fewer than expected. Many hemophilia patients do not register for the KHF because KHF registration is not obligatory. Therefore, a national registration system is needed to properly measure the incidence of hemophilia in the Korean population.

Moreover, a high proportion of these enrolled patients had severe hemophilia, with only 11% having mild disease. Generally, mild hemophilia is diagnosed only when an injury or medical intervention results in prolonged bleeding. A patient who is unaware that he has hemophilia may ignore the symptoms until they become severe or until complications develop.

The development of inhibitors is the most serious complication of FVIII replacement therapy; however, fewer hemophilia patients in Korea have inhibitors than in other countries [5]. Therefore, a more accurate diagnostic method, such as genetic analysis, is needed for the proper diagnosis and treatment.

This review discusses the current knowledge about mutations in hemophilia A, and focuses on the clinical aspect associated with these mutations, and the importance of genetic analysis.

STRUCTURE OF THE *FVIII* GENE

Hemophilia is caused by mutation of the *FVIII* gene, which spans 187 kb on the long arm of the X chromosome (Xq28) and contains 26 exons. FVIII protein has three distinct domain arranged as (NH_2) A_1-A_2-B-A_3-C_1-C_2 (COOH) [6]. The protein folds into a heterodimer in which the heavy (A_1-A_2-B domains) and light (A_3-C_1-C_2 domains) chains are linked by metal ion-dependent bonds [7]. This heterodimer circulates in a complex with von Willebrand factor (VWF) [8]. A_3-C_1-C_2 effectively competes with activated FVIII (FVIIIa) for binding to activated factor FIX (FIXa). The C_2 domain also contributes to VWF binding, which is essential for stabilizing FVIII in the circulation [9].

Various mutations of the *FVIII* gene can influence on the protein structure, reduce the synthesis or secretion of FVIII, and impair FVIII activity. A deficiency of or reduction in FVIII protein results in loss of normal hemostasis and manifests as spontaneous or induced bleeding depending on the disease severity. Mutations of the *FVIII* gene seem to be correlated with clinical findings and provide important information on the severity of disease and inhibitor development.

MUTATIONS OF THE *FVIII* GENE

More than 2,000 unique variants of the *FVIII* gene have been reported in the FVIII variant database [10]. The majority (66.5%) of hemophilia A cases are associated with single nucleotide variants (SNVs). Of these, missense mutations are the most common type in hemophilia A. Nonsense and splice site variants are more frequently associated with severe disease [11]. Severe hemophilia A is also associated with a higher proportion of deletions than mild or moderate hemophilia A.

Although mutations causing hemophilia A are scattered throughout the *FVIII* gene, there are two hotspots for recurrent genetic changes, inversion of introns 22 and 1 [12]. The size of the introns in the *FVIII* gene is variable and intron 22 is the largest. Intron 22 is the hotspot for the most common genetic defects leading to severe hemophilia A. Intron 1 is another hotspot, and mutations there cause severe hemophilia A in 1-6% of patients [13].

Long-range PCR is generally used to detect these two inversions. If they are not present, full mutation screening of the *FVIII* gene was done using direct Sanger sequencing, covering all exons, intron and the promoter region. Copy number variant (CNV) analysis, such as array comparative genomic hybridization and multiplex ligation-dependent probe amplification, has recently been used to detect large deletions or duplications.

The routine order of genetic analysis for hemophilia A is based on the disease severity: severe cases first typically undergo intron 22 and 1 inversion analysis with long-range PCR before CNV analysis or direct sequencing. These routine molecular methods can identify pathogenic variants in approximately 95% of hemophilia A cases [14]. Next-generation sequencing such as whole genome sequencing, provides an enhanced opportunity to characterize molecular defects in patients, particularly in those whose molecular defect has not yet been determined.

In South Korea, it has been reported that intron 22 inversion and point mutations account for about 90% of all hemophilia mutation [5,15]. Therefore, in case of severe hemophilia A, long-range PCR analysis of the intron 22 inversion mutation should be performed at first. If intron 22 inversion is negative, direct sequencing analysis can be performed. MLPA can be useful for detecting large dosage mutations. In most cases, it is difficult to routinely perform genetic testing to diagnose hemophilia A except for research purposes.

The variant types except for the inversion of introns 22 and 1 span a broad spectrum. The American College of Medical Genetics and Genomics and the Association for Molecular Pathology have published guidelines for the clinical interpretation of novel genetic variants [16]. Therefore, there is a need to re-evaluate and update previous genetic reports in the hemophilia databases.

FVIII INHIBITOR DEVELOPMENT

Inhibitor formation is the most severe treatment complication and occurs in 20%–45% of previously untreated patients with severe hemophilia A. The risk factors for inhibitor development are divided into treatment-related (number of exposure days, intensity of exposure, replacement product type, and age

at first exposure) and patient-related (family history of inhibitors, severity of hemophilia, and FVIII mutation) factors [17].

Large deletions and nonsense mutations are associated with an increase in inhibitor development. Inhibitors are more common in patients with mutations within domains A_3, C_1, and C_2. In South Korea, high inhibitor risk is associated with large deletions and point mutations in domains A_3 and C_2 [5].

In severe hemophilia A, the patient's immune system drives inhibitor development, whereas in mild cases, other mechanisms may underlie inhibitor development. In particular, missense mutation in domains C_1 or C_2 may alter the immunogenicity of FVIII protein, leading to inhibitor formation [18]. Therefore, it is important to perform mutation screening in all novel hemophilia cases to predict and manage the risk of developing inhibitors. There may also be a role for genotyping in predicting the outcome of immune tolerance induction and for future gene-based hemophilia therapies [19].

FVIII ACTIVITY DISCREPANCY

FVIII mutations also help to explain the discrepant activities found using different FVIII assays. The one-stage clotting assay (OSA) measures the ability of reference plasma to shorten the activated partial thromboplastin time (APTT) of hemophilic plasma. This method is simple, rapid, and widely used for clinical monitoring. The chromogenic substrate assay (CSA) is an indirect method of measuring FVIII activity and is a two-stage clotting assay similar to the OSA. First activated factor X (FXa) is generated, then it is reacted with a chromogenic substrate and subjected to photometric measurement. This method does not require FVIII-deficient plasma and has lower inter-laboratory variability than the OSA; however, it is technically complex and more expensive than the OSA.

In most cases of severe hemophilia A, the FVIII activity measured with both assays is low enough that the correct diagnosis will be made. However, some patients with mild hemophilia A show FVIII activity within the normal range when it is measured with one assay but lower FVIII activity when it is measured with the other [20]. In these patients, the diagnosis may be missed if only one assay is used. This can lead to failure to diagnose hemophilia A or the severity of the disease being classified incorrectly.

Depending on the mutation site, FVIII activity is affected differently, influencing the OSA or CSA results. Dissociation of the A_2 subunit correlates with inactivation of FVIIIa and this mutation is related to plasma FVIII activity discrepancies between OSA and CSA [21]. Mutations located in the three A-domains are associated with lower levels in the CSA, whereas mutations affecting thrombin cleavage sites or FIX binding sites, such as the C-domains, have lower values in the OSA.

Replacement therapy with plasma-derived FVIII (pdFVIII) or recombinant FVIII (rFVIII) concentrates requires accurate potency determination to ensure optimal safety and efficacy. The OSA and CSA are used to assign potency and the FVIII activities indicated by the two assays are often comparable. However, discrepancies have been reported in some patients with specific mild hemophilia A phenotypes [20] and in patients being treated with pdFVIII and rFVIII products that modify the B-domain in FVIII [22]. The CSA is reported to be less affected by these modifications than OSA. Product manufacturers should provide appropriate guidance on recommended monitoring methods of factor activity and the laboratory has to prepare these methods. When the genetic analysis is routinely performed in new patient of hemophilia A, the mutation screening could confirm whether the mutation is known to cause discrepancies in OSA or CSA.

MUTATIONS IN HEMOPHILIA A CARRIERS

Although FVIII levels are decreased in carriers, factor levels and coagulation tests such as APTT are not good predictors of bleeding [23]. FVIII activity shows considerable variability and normal FVIII activity does not rule out carrier status. Labarque et al. [24] proposed that a cut-off value of 0.9 for the ratio of FVIII activity to the vWF antigen level may predict carrier status. The ratio is somewhat useful, but with few applications. It is also difficult to differentiate between hemophilia A and von Willebrand disease based on factor levels and coagulation test results alone. A molecular diagnosis is important for distinguishing between bleeding disorder with similar clinical presentations but different underlying genetic causes.

Identification of causative *FVIII* gene mutations can provide useful information for carriers and family members with a history of hemophilia A [12]. Hemophilia A carriers are predominantly female, and postpartum hemorrhage is the most serious of the reported abnormal bleeding patterns. Awareness among pregnant women and healthcare providers could lead to safer childbirth and appropriate management of postpartum bleeding. Therefore, genetic analysis is important for diagnosing carriers and for proper bleeding management [25].

CONCLUSIONS

It is important to incorporate the knowledge of molecular and genetic aspects of hemophilia into routine clinical care. Genetic analysis of the *FVIII* gene is useful for diagnosis of hemophilia A as well as for the selection of therapeutic regimens, such as FVIII concentrates or recombinant substitutes, by confirming susceptibility to inhibitor development. Advances in molecular methods for detecting mutations, such as next-generation sequencing, may enable active genetic analysis in the treatment of hemophilia patients. Furthermore, genetic screening of hemophilia carriers may predict disease and help prevent bleeding.

CONFLICTS OF INTEREST

There are no potential conflicts of interest relevant to this article.

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The total manuscript length should not exceed 1,200 words, excluding references and abstract. Brief reports can include a maximum of 20 references and two figures or tables.

6. Image

Images that may help make clinical decisions while being interesting and educational in terms of the treatment of endocrinology and metabolism should be prepared with a manuscript. The manuscript should not be more than one page (A4), with the number of references limited to five.

7. Letters to the editor

A letter should contain constructive criticisms or comments on a specific paper published by the journal within the previous 6 months. The manuscript should be no more than one page (A4), with the number of references limited to five

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