Renal agenesis-related genes are associated with Herlyn-Werner-Wunderlich syndrome

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Objective: To explore the genetic causes of Herlyn-Werner-Wunderlich syndrome (HWWS) using whole-exome sequencing.
Design: Retrospective genetic study.
Setting: Academic medical center.
Patient(s): Twelve patients with HWWS.
Intervention(s): Whole-exome sequencing was performed for each patient. Sanger sequencing was used to confirm the potential causative genetic variants. In silico analysis and American College of Medical Genetics and Genomics guidelines were used to classify the pathogenicity of each variant.
Main Outcome Measure(s): Rare sequence variants associated with müllerian duct development and renal agenesis were identified and included in subsequent analyses.
Result(s): A total of 11 variants were identified in 10 of 12 patients (83.3%) and were considered to constitute a molecular genetic diagnosis of HWWS. These 11 variants were related to 9 genes: *CHD1L*, *TRIM32*, *TGFBR3*, *WNT4*, *RET*, *FRAS1*, *FAT1*, *FOXF1*, and *PCSK5*. All variants were heterozygous and confirmed by Sanger sequencing. The changes included one frameshift variant, one splice-site variant, and eight missense variants. All of the identified variants were absent or rare in Genome Aggregation Database East Asian populations. One of the 11 variants (9.1%) was classified as a pathogenic variant according to the American College of Medical Genetics and Genomics guidelines, and 8 of the 11 variants (72.7%) were classified as variants of uncertain significance.
Conclusion(s): To our knowledge, this is the first report of the genetic causes of HWWS. Renal agenesis-related genes, such as *CHD1L*, *TRIM32*, *RET*, and *WNT4*, may be associated with HWWS. Identification of these variants can not only help us understand the etiology of HWWS and the relationship between reproductive tract development and urinary system development, but additionally improve the level of genetic counseling for HWWS. (Fertil Steril® 2021;116:1360–9. ©2021 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Herlyn-Werner-Wunderlich syndrome, renal agenesis, whole-exome sequencing, CHD1L, TRIM32

Female genital tract anomalies are characterized as certain malformations in the uterus, cervix, fallopian tubes, and vagina, and have been reported to occur in 4.3% to 6.7% of women (1–3). Complex female genital malformations are generally defined as anomalies that include more than one organ or part of the female genital tract (4). Herlyn-Werner-Wunderlich syndrome (HWWS), also called obstructed hemivagina and ipsilateral renal anomaly syndrome (5), is a rare variant of the complex female genital malformations according to the

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classification of Acién and Acién (4). HWWS is typically characterized by concurrence of uterus didelphys, double cervix, and obstructed hemivagina, and is often accompanied by ipsilateral renal agenesis or other genitourinary malformations (6).

Herlyn-Werner-Wunderlich syndrome is caused by abnormal fusion of the müllerian ducts. During human embryonic development, the mesonephric ducts develop into the urinary system, and the müllerian ducts develop into the reproductive system. The development of the müllerian ducts depends on the mesonephric ducts; therefore, when a mesonephric duct is underdeveloped, the developmental process of the ipsilateral müllerian duct is affected, resulting in a series of malformations of the urinary and reproductive systems, such as the kidney, uterus, and vagina.

At present, the genetic factors that cause HWWS are unknown. As a rare type, HWWS may have similar pathogenic factors to other female genital tract anomalies. Previous studies have found that genetic alterations can lead to female genital tract anomalies. For example, *PAX8*, *TBX6*, *WNT4*, *WNT9B*, *BMP4*, *BMP7*, *HOXA10*, *LHX1*, and other genes are associated with Mayer-Rokitansky-Kuster-Hauser syndrome, septate uterus, or distal vaginal atresia (7–14). Whether genetic factors are associated with HWWS is a question still unexplored.

In this study, we aimed to explore the genetic causes of HWWS using whole-exome sequencing (WES) technology. We recruited 12 patients with HWWS and performed WES and family genetic analysis on these patients. We tried to find the genetic pathogenic factors related to HWWS.

MATERIALS AND METHODS

Patients

Twelve patients with HWWS were recruited at the Beijing Obstetrics and Gynecology Hospital between October 2018 and November 2020. The clinical conditions and manifestations of the patients are presented in Table 1. This study was approved by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital (2018-KY-027-01) and was conducted in accordance with the 1964 Helsinki Declaration and its later amendments. Written informed consent was first obtained from each patient and their corresponding relatives, and then 5-mL peripheral blood samples were collected for genetic analysis.

Whole-Genome Copy Number Variation Sequencing Analysis

Whole-genome copy number variation (CNV) sequencing was performed by MyGenostics, Inc. (Beijing, People's Republic of China) and Novogene Bioinformatics Technology Co. Ltd. (Tianjin, People's Republic of China). The bioinformatics analysis was performed as follows. First, Illumina sequencing adapters and low-quality reads (<80 bp) were filtered out with the use of Cutadapt (1.16) software (http://code.google.com/p/cutadapt/). After quality control, the clean reads were mapped to the UCSC hg19 human reference genome with the use of BWA (0.7.12) software (http://bio-bwa.sourceforge.net/). Only uniquely mapped reads were selected. The Genome Analysis Toolkit (GATK, 4.0.8.1) MarkDuplicates was used to remove duplicated reads. Mapped reads were classified into adjustable sliding windows, which were 50 kb in length with 5-kb increments. The coverage of each window was calculated on the basis of the read amount and underwent a two-step bias correction (GC correction and population-scale normalization). A binary segmentation algorithm was used to localize the segment breakpoints to identify the candidate CNV regions and determine the CNV genotype, and the U test and parallelism test were then used to estimate the genotype and significance of each segment.

Whole-Exome Sequencing Analysis

Libraries were generated with the use of the Agilent SureSelect Human All Exon V6 kit (Agilent Technologies, Santa Clara, CA, USA) after the manufacturer's recommendations. WES was performed by Annoroad Genomics (Beijing, People's Republic of China) and Novogene Bioinformatics Technology Co. Ltd. on Illumina NovaSeq6000 sequencers with a pair end of 150 bp (PE150) for each reaction. The raw sequencing reads were aligned to the reference genome GRCh37/hg19, and Genome Analysis Toolkit (GATK) software was used to call the variants, including single nucleotide polymorphisms and short insertions and deletions, which were further annotated with the use of the ANNOVAR software tool. The functional effects of variants (damaging or not) were predicted with the use of three algorithms (PolyPhen-2, SIFT, and MutationTaster). The criteria used for filtering the desired variants were missense, nonsense, frameshift, or splice-site variants and variants with minor allele frequency <1%. The minor allele frequency data were obtained by referring to the following databases: Genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/), the NHLBI Exome Sequencing Project (ESP6500), and the 1000 Genomes Project (1000G, http://browser.1000genomes.org/index.html).

Sanger Sequencing Analysis

Sanger sequencing was performed to validate the mutation of each identified gene and to determine if it was inherited from a parent. Primer pairs for each gene are listed in Supplemental Table 1 (available online). Forward or reverse primers were used to sequence polymerase chain reaction products. The sequencing reaction was performed on an ABI 3730 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

RESULTS

Whole-Exome Sequencing Analysis of the Patients

We recruited 12 affected female patients with HWWS in this study (Table 1). We first performed CNV sequencing analysis on each patient, and the results showed that there were no pathogenic CNVs found (data not shown).

We then performed WES on each affected patient. A total of 11 variants were identified in 83.3% (10 of 12) of the patients and were considered a molecular genetic diagnosis of HWWS. These 11 variants were related to 9 genes: *FRAS1*

TABLE 1

Clinical information of 12 patients with Herlyn-Werner-Wunderlich syndrome.

Patient no.	Age at onset of symptoms (y)	Diagnosis	Classification according to Acién and Acién (4)	European Society of Human Reproduction and Embryology and ESGE classification	Symptoms
Fc-H-1	24	Herlyn-Werner-Wunderlich syndrome (complete septate uterus, septate cervix, left oblique vaginal septum, left renal agenesis), endometriosis stage I	2A	U2bC1V2	Postmenstrual spotting and dysmenorrhea
Fc-H-2	24	Herlyn-Werner-Wunderlich syndrome (uterus didelphys, double cervix, left oblique vaginal septum with a communicating buttonhole, left renal agenesis)	2C	U3cC2V1	Vaginal pain during menstruation
Fc-H-3	11	Herlyn-Werner-Wunderlich syndrome (complete septate uterus, septate cervix, a small communication between the septate cervices, dysplasia of the right cervix, right oblique vaginal septum, right renal agenesis)	2B	U2bC1V2	Progressive worsening of dysmenorrhea
Fc-H-4	13	Herlyn-Werner-Wunderlich syndrome (uterus didelphys, double cervix, left oblique vaginal septum, left renal agenesis), endometriosis stage I	2A	U3cC2V2	Progressive worsening of dysmenorrhea
Fc-H-5	16	Herlyn-Werner-Wunderlich syndrome (uterus didelphys, double cervix, right oblique vaginal septum, right renal agenesis), endometriosis stage III, left ovarian endometrioma	2A	U3bC2V2	Abnormal vaginal discharge
Fc-H-6	12	Herlyn-Werner-Wunderlich syndrome (complete septate uterus, septate cervix, right oblique vaginal septum, right renal agenesis), endometriosis stage I, primary infertility	2A	U2bC1V2	Menstruation prolonged
Fc-H-7	17	Herlyn-Werner-Wunderlich syndrome (bicornuate communicating uterus, left cervical aplasia, left oblique vaginal septum, left renal agenesis), left adenomyosis, endometriosis stage II	2B	U3aC3V2	Postmenstrual spotting and abnormal vaginal discharge
Fc-H-8	12	Herlyn-Werner-Wunderlich syndrome (uterus didelphys, double cervix, right oblique vaginal septum, right renal agenesis)	2A	U3bC2V2	Progressive worsening of dysmenorrhea and abnormal vaginal discharge and pus
Fc-H-9[a]	25	Herlyn-Werner-Wunderlich syndrome (bicornuate communicating uterus, left cervical aplasia, left oblique vaginal septum, left renal agenesis)	2B	U3aC3V2	No obvious clinical symptoms
Fc-H-10[a]	12	Herlyn-Werner-Wunderlich syndrome (uterus didelphys, double cervix, blind right hemivagina with large hematocolpos, right renal agenesis)	2A	U3bC2V2	Right hysterectomy and right salpingectomy were performed in other hospitals due to dysmenorrhea when she was 12 years old
Fc-H-11[a]	19	Herlyn-Werner-Wunderlich syndrome (uterus didelphys, double cervix, right oblique vaginal septum, right renal agenesis), right uterus with adenomyosis, right hydrosalpinx, endometriosis stage II	2A	U3bC3V2	Progressive worsening of dysmenorrhea
Fc-H-12	12	Herlyn-Werner-Wunderlich syndrome (uterus didelphys, double cervix, left oblique vaginal septum, left renal agenesis)	2A	U3cC2V2	Dysmenorrhea and abnormal vaginal discharge

Note: [a]The first operation on these patients was performed in another hospital, and we were unable to find photographs of the surgery and related imaging information. Therefore, the accurate clinical classification is unknown, and the current classification is on the basis of the medical records; ESGE = European Society of Gastrointestinal Endoscopy.

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TABLE 2

In silico analysis of sequence variants found in 12 patients with Herlyn-Werner-Wunderlich syndrome by whole-exome sequencing.

Patient no.	Zygosity	Gene	Reference mRNA no.	Mutation type	Variant	Amino acid change	GnomAD-East Asian (EAS)	PolyPhen-2/ SIFT/Mutation Taster/LRT/ FATHMM-MKL	ACMG
Fc-H-1	Hetero	*FRAS1*	NM_025074	Missense	c.3191G>T	p.R1064L	0.000172	B/T/N/N/N	LB: PM2+BP1+BP4
Fc-H-2	Hetero	*FAT1*	NM_005245	Missense	c.249C>G	p.F83L	0.0000556	D/D/D/D/D	VUS: PM2+PP3+BP1
Fc-H-3	Hetero	*FRAS1*	NM_025074	Missense	c.779G>C	p.R260T	0	B/T/N/N/N	LB: PM2+BP1+BP4
Fc-H-5	Hetero	*CHD1L*	NM_004284	Splice-site	c.348-1G>C		0.000388	NA/NA/D/NA/D	VUS: PP3+BS1
Fc-H-6	Hetero	*WNT4*	NM_030761	Missense	c.527G>C	p.S176T	0	B/T/D/D/D	VUS: PM2+PP2+BP4
Fc-H-8	Hetero	*CHD1L*	NM_004284	Splice-site	c.348-1G>C		0.000388	NA/NA/D/NA/D	VUS: PP3+BS1
Fc-H-9	Hetero	*FOXF1*	NM_001451	Missense	c.491A>G	p.N164S	0	B/T/D/D/D	VUS: PM2+PP2+BP4
Fc-H-10	Hetero	*TGFBR3*	NM_003243	Frameshift	c.1997_1998del	p.Val666Glufs*16	0	NA/NA/D/NA/NA	P: PVS1+PM2+PP3
Fc-H-10	Hetero	*TRIM32*	NM_012210	Missense	c.1012G>T	p.A338S	0	B/T/D/N/N	VUS: PM1+PM2+PP2+BP4
Fc-H-11	Hetero	*PCSK5*	NM_001190482	Missense	c.5041C>G	p.P1681A	0.000918	NA/D/D/D/D	VUS: BP1
Fc-H-12	Hetero	*RET*	NM_020630	Missense	c.1433G>A	p.C478Y	0	D/D/D/D/D	VUS: PM2+PP2+PP3

Note: The Genome Aggregation Database (gnomAD) is a resource developed by an international coalition of investigators, with the goal of aggregating and harmonizing both exome and genome sequencing data from a wide variety of large-scale sequencing projects. In this study, we referred to the allele frequencies in the East Asian (EAS) population. Pathogenicity items: PolyPhen-2: D, probably damaging; P, possibly damaging; B, benign. SIFT: D, damaging; T, tolerated. MutationTaster: D, disease-causing; N, polymorphism. LRT: D, deleterious; N, neutral. FATHMM-MKL: D, damaging; N, neutral; NA, not applicable. ACMG items: ACMG, American College of Medical Genetics and Genomics guidelines; VUS, variant of uncertain significance; P, pathogenic variant; LB, likely benign variant.

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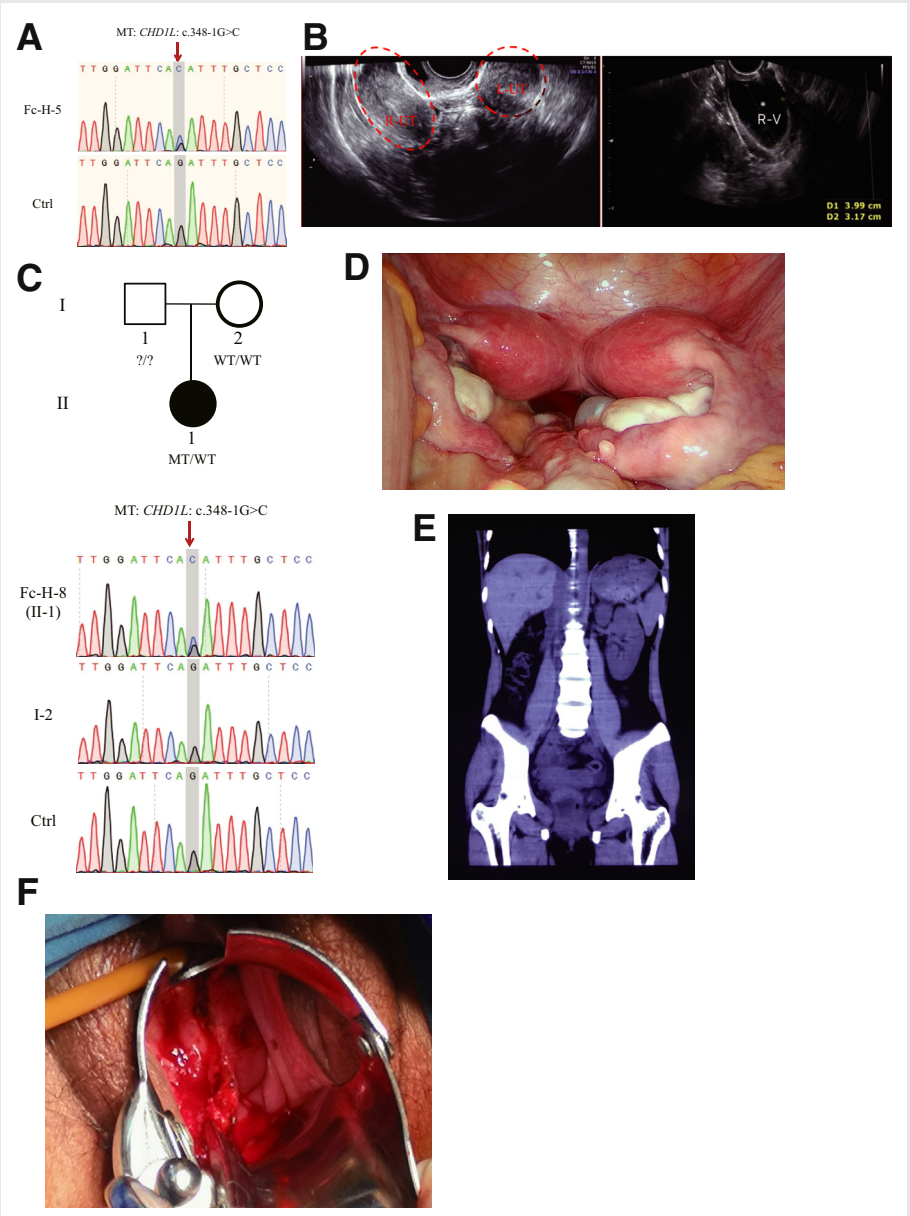
(Fc-H-1 and Fc-H-3), *FAT1* (Fc-H-2), *CHD1L* (Fc-H-5 and Fc-H-8), *WNT4* (Fc-H-6), *FOXF1* (Fc-H-9), *TGFBR3* (Fc-H-10), *TRIM32* (Fc-H-10), *PCSK5* (Fc-H-11), and *RET* (Fc-H-12) (Table 2). All variants were heterozygous. These changes included one frameshift variant, one splice-site variant, and eight missense variants (Table 2). One patient (Fc-H-10) harbored more than one variant in different genes (Table 2). All of the identified variants were absent or rare in the gnomAD East Asian populations (Table 2). One variant (1 of 11, 9.1%) was classified as a pathogenic variant according to the American College of Medical Genetics and Genomics (ACMG) guidelines, and most of the variants (8 of 11, 72.7%) were classified as variants of uncertain significance (VUS).

Novel HWWS Candidate Genes

CHD1L. We identified the *CHD1L* variant in two unrelated patients. Both Fc-H-5 and Fc-H-8 harbored the same *CHD1L* splice-site variant, c.348-1G>C, which was confirmed by Sanger sequencing (Fig.1A). Fc-H-5 was diagnosed as HWWS (European Society of Human Reproduction and Endocrinology [ESHRE] classification: U3bC2V2) with uterus didelphys, double cervix, right oblique vaginal septum, and right renal agenesis (Table 1 and Fig. 1B). In addition, Fc-H-8 was diagnosed as HWWS (ESHRE classification: U3bC2V2) with uterus didelphys, double cervix, right oblique vaginal septum, and right renal agenesis (Table 1 and Fig. 1D–F). Blood samples from the parents of patient Fc-H-5 could not be obtained; however, blood samples from the mother of patient Fc-H-8 were available. The results of Sanger sequencing suggested that Fc-H-8's mother did not carry the mutant allele of *CHD1L* (Fig. 1C), so the *CHD1L* variant was segregated with HWWS. The allele frequency of the c.348-1G>C variant in the gnomAD East Asian population was 0.000388, and the variant was predicted to be a damaging variant by the MutationTaster and FATHMM-MKL algorithms (Table 2). This variant was classified as VUS (PP3+BS1) according to the ACMG guidelines. This variant may affect the acceptor splice-site (AG) on intron 3. In silico analysis suggested two potential mechanisms caused by the c.348-1G>C variant. One mechanism involves skipping exon 4, leading to the synthesis of the truncated CHD1L p.P119* protein. The other mechanism induces the retention of intron 3, predicted to generate the truncated CHD1L p.F117Yfs*23 protein.

***TRIM32* and *TGFBR3*.** We identified a *TRIM32* variant in patient Fc-H-10. Fc-H-10 was diagnosed as HWWS (ESHRE classification: U3bC2V2) with primary infertility (Table 1). Fc-H-10 harbored the *TRIM32* missense variant c.1012G>T; p.A338S (Table 2). This variant was confirmed by Sanger sequencing (Supplemental Fig. 1A, available online) and was classified as VUS (PM1+PM2+PP2+BP4) according to the ACMG guidelines (Table 2). In addition, Fc-H-10 harbored a frameshift variant in *TGFBR3*, c.1997_1998del;p.V666Efs*16 (Supplemental Fig. 1A), which was classified as a pathogenic variant (PVS1+PM2+PP3). Therefore, potential digenic inheritance might be associated with HWWS in patient Fc-H-10.

FIGURE 1



CHD1L was mutated in patients with Herlyn-Werner-Wunderlich syndrome (HWWS). **(A)** Sanger sequencing validated the *CHD1L* variant in patient Fc-H-5. The *red arrow* indicates the variant site (c.348-1G>C). **(B)** Images of patient Fc-H-5 with a diagnosis of HWWS. In the transvaginal ultrasound, two uterine contours (*left*) and the hydrocolpos (*right; asterisk*; the liquid dark area) of the right vagina can be seen in the pelvic cavity. (**C**) Sanger sequencing confirmed the heterozygous *CHD1L* variant in patient Fc-H-8. The patient's mother harbored two wild-type alleles. A sample from the patient's father was unavailable. The *red arrow* indicates the variant site (c.348-1G>C). **(D)** Images of the uterus of patient Fc-H-8 under laparoscopy indicated European Society of Human Reproduction and Endocrinology and European Society of Gastrointestinal Endoscopy classification category U3b, and an external fundal indentation completely divided the uterine corpus up to the level of the cervix. **(E)** In the coronal computed tomographic image of Fc-H-8, the absence of the right kidney (on the same side of the vaginal septum) is evident. **(F)** Cervical morphology of patient Fc-H-8 after excision of the obstructed vaginal septum. The image shows the residual traces of the obstructed hemivagina after resection, and the right side is the cervix behind the obstructed hemivagina cavity. L-UT = left uterus; MT = mutated allele; R-UT = uterus; R-V = right vagina; WT = wild-type allele.

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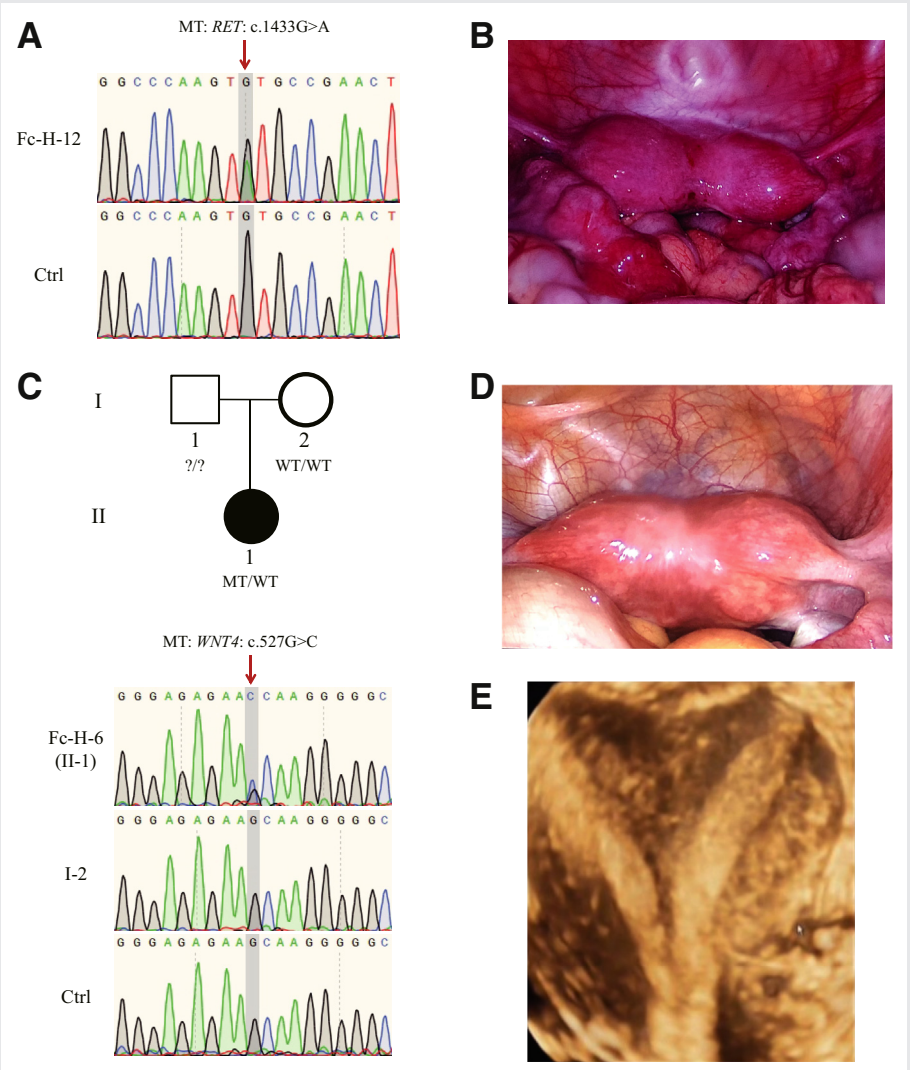
Other genes. In addition, we identified variants in the *RET*, *WNT4*, *PCSK5*, *FOXF1*, *FAT1*, and *FRAS1* genes. Variants other than the two *FRAS1* variants were classified as VUS according to the ACMG guidelines (Table 2). A heterozygous missense variant in *RET*, c.1433G>A; p.C478Y, was found in patient Fc-H-12 (Table 2 and Fig. 2A), who was diagnosed

FIGURE 2



RET and *WNT4* variants were associated with Herlyn-Werner-Wunderlich syndrome (HWWS). **(A)** Sanger sequencing validated the *RET* variant in patient Fc-H-12. The *red arrow* indicates the variant site c.1433G>A (*RET*). **(B)** Image of the pelvic cavity of patient Fc-H-12 under laparoscopy, showing that an external fundal indentation partly divided the uterine corpus at the level above the cervix. On the basis of a combination with three-dimensional ultrasound and hysteroscopy images, the patient was considered as European Society of Human Reproduction and Endocrinology and European Society of Gastrointestinal Endoscopy classification category U3c. **(C)** Sanger sequencing confirmed the heterozygous *WNT4* variant in patient Fc-H-6. The patient's mother harbored two wild-type alleles. A sample from the patient's father was unavailable. The *red arrow* indicates the variant site c.527G>C (*WNT4*). **(D)** The shape of the uterus in patient Fc-H-6 under laparoscopy was normal, and the bottom of the uterus was slightly depressed. **(E)** In the three-dimensional ultrasound image of patient Fc-H-6, the shape of the fundus of the uterus is still flat, and a septal echo can be seen from the fundus of the uterus to the cervix. The two cervical canals can be seen, showing the shape of a complete septate uterus. In addition, combined hysteroscopy and laparoscopy confirmed a complete septate uterus and septate cervix. MT = mutated allele; WT = wild-type allele.

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with HWWS (ESHRE classification: U3cC2V2) with uterus didelphys, double cervix, left oblique vaginal septum, and left renal agenesis (Table 1 and Fig. 2B). In addition, we identified a *WNT4* heterozygous missense variant, c.527G>C;p.S176T, in Fc-H-6 (Table 2 and Fig. 2C), who was diagnosed with HWWS (ESHRE classification: U2bC1V2) with complete septate uterus, septate cervix, right oblique vaginal septum, and right renal agenesis (Table 1 and Fig. 2D and E). The other variants in the *PCSK5*, *FOXF1*, *FAT*, and *FRAS1* genes were in addition confirmed by Sanger sequencing (Supplemental Fig. 2A–E). The clinical images of patients Fc-H-11 and Fc-H-9 are shown in Supplemental Fig. 3.

DISCUSSION

In this study, WES was used to study the genetic pathogenic factors of HWWS. We identified several genes and variants that may be related to the pathogenesis of HWWS. On the

basis of previous studies, these genes are thought to be related to renal agenesis. During the development of the human embryo, the kidneys develop from the mesonephric ducts and the reproductive tract develops from the müllerian ducts. The development of the ipsilateral müllerian duct is dependent on the mesonephric duct. In addition, the present study found that the genes and variants leading to renal agenesis may cause abnormalities in the reproductive tract and further expand the genetic variants and disease spectrum. Below, we discuss these genes individually.

CHD1L. *CHD1L* encodes the chromodomain helicase DNA-binding protein 1-like protein, a chromatin-remodeling enzyme that catalyzes nucleosome sliding. *CHD1L* is strongly expressed in human fetal kidneys and is localized in the early ureteric bud, the critical stage of kidney development (15), suggesting that *CHD1L* expression is important in the developing kidney. Heterozygous sequence variants in *CHD1L* have been found to be associated with congenital anomalies of the kidneys and urinary tract (CAKUT) (15–18). Thus, a previous study suggested that *CHD1L* variants might lead to renal dysplasia because of impaired chromatin remodeling (15). Variants in *CHD1L* were found in two unrelated patients with HWWS in our study. One splice-site variant, c.348-1G>C, was found in two patients. In silico analysis suggested two potential mechanisms. Regardless of the mechanism, it is predicted that the c.348-1G>C variant produces a CHD1L truncated protein. Further minigene assays are needed to validate the effect of the c.348-1G>C variant.

TRIM32. The *TRIM32* gene has been reported to be associated with Bardet-Biedl syndrome (19, 20), which is defined by obesity, mental retardation, retinal degeneration, polydactyly, renal dysfunction, and müllerian fusion anomalies in females. A heterozygous microdeletion containing the *TRIM32* gene was identified in two unrelated patients with uterus didelphys and a complete septate uterus (21). In addition, a missense *TRIM32* variant, c.1012G>T;pA338S, was found in patient Fc-H-10, who harbored a frameshift variant in *TGFBR3*, c.1997_1998del;p.Val666Glufs*16, a gene also related to CAKUT (16), suggesting a digenic mode of inheritance.

RET. In murine models, kidney formation is controlled by several proteins, including *RET* (22). In humans, *RET* mutations have been found to cause renal agenesis or CAKUT (17, 18, 23–27), and studies have additionally suggested that heterozygous mutations in *RET* may be the cause of abnormal kidney development (23, 26). In this study, in addition, we found a heterozygous missense variant in *RET*, c.1433G>A;p.C478. This variant was predicted to be a damaging variant by all of the in silico algorithms used (Table 2). Therefore, we hypothesized that abnormal kidney development caused by this *RET* variant might further affect the formation of the müllerian ducts, leading to HWWS.

WNT4. Female *Wnt4*-knockout mice fail to develop müllerian ducts (28). In addition, human *WNT4* variants were found to be associated with müllerian duct abnormalities. Heterozygous mutations E226G, A233T, L12P, and R83C in *WNT4* were found to be associated with atypical Mayer-Rokitansky-Kuster-Hauser syndrome (29–32). In addition, *Wnt4*-knockout mice showed kidney hypodysplasia (33). In addition, genetic studies have found some mutations associated with bilateral or unilateral renal agenesis (29, 34, 35) or renal hypodysplasia (36). On the basis of previous findings, *WNT4* mutations were associated with both müllerian duct anomalies and renal agenesis. In our study, we found the *WNT4* heterozygous c.527G>C;p.S176T variant in a patient with complete septate uterus, septate cervix, right oblique vaginal septum, and right renal agenesis. Therefore, our study supports the association of the *WNT4* variant with HWWS.

Other genes. In addition, we found several genes, including *PCSK5*, *FOXF1*, *FAT1*, and *FRAS1*, that may be associated with HWWS. Heterozygous mutations in conserved residues in *PCSK5* have been shown to be associated with vertebral anomalies, anal atresia, cardiac defects, tracheoesophageal fistula and/or esophageal atresia, renal and radial anomalies, and limb defects (VACTERL) (37, 38). In addition, an ethylnitrosourea-induced recessive mouse mutation, C470R, was identified and the mouse phenotype also resembled the VACTERL association phenotype in the human (37). FOXF1 is a transcription factor that has an important role in epithelium–mesenchyme signaling. A *FOXF1* de novo mutation (p.Gly220Cys) was identified in a patient with a VACTERL association-like phenotype, including left renal agenesis (39). In situ hybridization analyses in mouse embryos suggested that Foxf1 was expressed in genital tubercle, esophageal, tracheal, vertebral, and anal tissues (39). In addition, other studies found that mutations or microdeletions of *FOXF1* were associated with VACTERL or renal malformations (40–43). The *FAT1*-encoded protein is a member of the cadherin superfamily and is expressed at high levels in a number of fetal epithelial tissues, including renal glomerular epithelial cells. Sequence variants in *FAT1* have been identified in patients with CAKUT (16). Recessive mutations in *FRAS1* have been identified as the main cause of Fraser syndrome (a syndromic CAKUT) and renal agenesis (44–46). Although *FRAS1* heterozygous missense mutations have been reported to be a new cause of nonsyndromic CAKUT or renal agenesis in humans (25, 47, 48), our study suggests that these four genes may also be candidate genes associated with müllerian duct development, especially HWWS disease.

In addition, we should discuss the limitations of this study. HWWS is a rare disease with an incidence of 0.0032% to 0.3% (49). In this study, we recruited 12 patients in our hospital in approximately 2 years, and we only performed genetic analysis on these 12 patients. Thus, a limitation of this study is the failure to recruit more patients for genetic analysis. A study including more HWWS patients might enable us to find more pathogenic genes or more important major pathogenic genes associated with HWWS. Therefore, in the future, we need to continue to recruit patients with HWWS and perform multicenter studies in cooperation with other hospitals and centers to further explore the genetic pathogenic factors of HWWS. In addition, more functional experiments to elucidate whether the variants are

pathogenic are needed. Most of the variants found in this study were VUS, and whether these variants cause adverse changes in protein function is still unknown.

In summary, to our knowledge, this study is the first to demonstrate that renal agenesis-related genes may be associated with HWWS. Our study provides insights into the potential molecular mechanisms underlying HWWS.

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Genes relacionados con la agenesia renal se asocian con el síndrome de Herlyn-Werner-Wunderlich.

Objetivo: Explorar las causas genéticas del síndrome de Herlyn-Werner-Wunderlich (HWWS) mediante la secuenciación del exoma completo.

Diseño: Estudio genético retrospectivo.

Entorno: Centro médico académico.

Paciente (s): Doce pacientes con HWWS.

Intervención (es): Se realizó la secuenciación del exoma completo para cada paciente. Se utilizó la secuenciación de Sanger para confirmar la posible causa de las variantes genéticas. Se utilizaron análisis in silico y las guías del American College of Medical Genetics and Genomics para clasificar la patogenicidad de cada variante.

Principales medidas de resultado: Se identificaron variantes de secuencias raras asociadas con el desarrollo de los conductos müllerianos y con la agenesia renal y se incluyeron en análisis subsecuentes.

Resultado (s): Un total de 11 variantes fueron identificadas en 10 de 12 pacientes (83.3%) y se consideró que constituían un diagnóstico genético molecular de HWWS. Estas 11 variantes estaban relacionadas con 9 genes: *CHD1L, TRIM32, TGFBR3, WNT4, RET, FRAS1, FAT1, FOXF1 y PCSK5*. Todas las variantes fueron heterocigóticas y confirmadas con la secuenciación Sanger. Los cambios incluyeron una variante de desplazamiento del marco de lectura, una variante de sitio de empalme y ocho variantes de sentido erróneo. Todas las variantes identificadas estaban ausentes o eran raras en la base de datos de Agregación del Genoma de las Poblaciones de Asia Oriental. Una de las 11 variantes (9,1%) fue clasificada como variante patogénica de acuerdo a las guías del American College de Genética Médica y Genómica, y 8 de las 11 variantes (72,7%) se clasificaron como variantes de significado incierto.

Conclusión (es): Según nuestro conocimiento, este es el primer reporte de las causas genéticas de HWWS. Los genes relacionados con la agenesia renal, como *CHD1L*, *TRIM32, RET y WNT4* podrían asociarse con HWWS. La identificación de estas variantes no solo podría ayudarnos a comprender la etiología de HWWS y su relación entre el desarrollo del tracto reproductivo y el desarrollo del sistema urinario, sino que adicionalmente, a mejorar el nivel de asesoramiento genético para HWWS.