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Construction of Copy Number Variation Map Identifies Small Regions of Overlap and Candidate Genes for Atypical Female Genitalia Development

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Abstract: Copy number variations (CNVs) have been implicated in various conditions of differences of sexual development (DSD). Generally, larger genomic aberrations are more often considered disease-causing or clinically relevant, but over time, smaller CNVs have been associated with various forms of DSD. The main objective of this study is to identify small CNVs and the smallest regions of overlap (SROs) in patients with atypical female genitalia (AFG) and build a CNV map of AFG. We queried the DECIPHER database for recurrent duplications and/or deletions detected across the genome of AFG individuals. From these data, we constructed a chromosome map consisting of SROs and investigated such regions for genes that may be associated with the development of atypical female genitalia. Our study identified 180 unique SROs (7.95 kb to 45.34 Mb) distributed among 22 chromosomes. The most SROs were found in chromosomes X, 17, 11, and 22. None were found in chromosome 3. From these SROs, we identified 22 genes as potential candidates. Although none of these genes are currently associated with AFG, a literature review indicated that almost half were potentially involved in the development and/or function of the reproductive system, and only one gene was associated with a disorder that reported an individual patient with ambiguous genitalia. Our data regarding novel SROs requires further functional investigation to determine the role of the identified candidate genes in the development of atypical female genitalia, and this paper should serve as a catalyst for downstream molecular studies that may eventually affect the genetic counseling, diagnosis, and management of these DSD patients.

Keywords: atypical female genitalia; differences of sexual development; genitourinary development; copy number variation; genomics

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1. Introduction

The development of female genital structures is controlled by a set of intricate biological processes. Disruptions at the genetic, endocrine, structural, and/or environmental level can lead to atypical or differences of sexual development (DSD), conditions associated with malformation and/or dysfunction of the reproductive system.

At the genetic level, development of the internal female reproductive tract depends on three finely-regulated processes: development of the Müllerian ducts, regression of the Wolffian ducts, and differentiation of the Müllerian ducts [1–3]. The reproductive tract is initially undifferentiated in the form of the Wolffian duct, which is derived from the mesonephros of the urinary tract and present in all embryos [4]. Expression of genes such as *WNT4* induce formation of the Müllerian duct, which uses the preexisting Wolffian duct as a scaffold [2]. The presence of a Y chromosome, specifically the *SRY* gene, determines whether the primitive gonads differentiate into ovaries or testes. Without *SRY*, no anti-Müllerian hormone (AMH) or testosterone is produced, resulting in the maintenance of the Müllerian ducts and regression of the Wolffian ducts [2–5]. Maturation of the Müllerian

ducts further depends on additional genetic pathways which result in development of the uterus, oviducts, cervix, and upper third of the vagina. Improper fusion of the Müllerian ducts or a failure of primitive tissue regression can lead to atypical structures such as bicornuate uterus or uterine or vaginal septum [1,4]. Development of the external female genitalia is less understood; however, *TP63* has been identified as a major genetic player in the differentiation of urogenital mucosa and septation of the cloaca [6].

Despite tight genetic regulation, environmental factors can throw female reproductive tract development into disarray. Endocrine disrupting chemicals, which mimic or antagonize estrogens and androgens, are found throughout the environment, from nature and wildlife to commerce and laboratories. These xenoestrogens can affect fetal development in utero and even during pre-conception. For example, in the mid-1900s, physicians prescribed diethylstilbestrol (DES) to prevent miscarriage—it was later discovered that females exposed to DES in utero developed "T-shaped" uteruses which in turn predisposed them to worse pregnancy outcomes [2,7]. Furthermore, changes at the epigenetic level (e.g., DNA methylation, histone acetylation, etc.) can affect gene expression pre- and postnatally, leading to atypical reproductive structures and function [8].

Typically, DSD is classified into three major groups: 46, XY DSD, 46, XX DSD, and sex chromosome DSD [9,10]. Additionally, DSD can result from a standalone genetic change (i.e., isolated) or as a clinical feature of a greater disorder with congenital abnormalities unrelated to the reproductive system (i.e., syndromic) [3,11]. Some specific conditions include Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, congenital adrenal hyperplasia (CAH), ovotesticular DSD, and nonsyndromic testicular DSD, among others [1,10]. Overall, it has been estimated that DSD occurs in approximately 1 in 4500–5500 newborns [12–14].

While the rate of molecular diagnosis is higher in 46, XX DSD patients compared to 46, XY DSD patients (64% vs. 12% respectively), the majority, if not all, of those are diagnosed with CAH [11,15,16]. The remaining 46, XX DSD patients often do not know the genetic etiology of their conditions, and historically, such patients have been diagnosed using phenotypic presentation. Indeed, geneticists have begun focusing on patient comorbidities and malformative syndromes with documented DSD for clues into the genetic origin of atypical genital development [17,18].

Copy Number Variations. Chromosomal microarray analysis (CMA) has been utilized to uncover copy number variants (CNVs) that are potentially significant in the manifestation of DSD. For example, previous studies have shown that duplications of *SOX9* [19,20] and *NR0B1* [21,22] are relevant in the development of 46, XX and 46, XY DSD, respectively. Throughout the years, an abundance of CNV data has been published and stored in various public databases such as DECIPHER [23]. Utilization of CNV data from these resources allowed for the characterization and delineation of the smallest regions of overlap (SROs) that often result in the identification of candidate genes that may have clinical relevance.

In this retrospective study, we queried the DECIPHER database for genomic regions containing recurrent duplications and/or deletions in patients with atypical development of female external and internal genital structures. From these data, we constructed a whole genome map of SROs and further investigated such regions for candidate genes that may be associated with various forms of atypical female genitalia. We also expanded our phenotype analysis to include other non-genital anomalies that may be comorbid with atypical female sex development.

To the best of our knowledge, this is the first study to create a whole genome CNV map of atypical female genitalia and to identify corresponding candidate genes. Data from this study can be utilized for downstream research, and findings from all above-mentioned efforts may provide a better understanding of the genetic etiology of atypical female genitalia, facilitate diagnosis and genetic counseling, and improve patient management. Lastly, our data will further contribute to the development of a whole genome map for DSD overall.

2. Materials and Methods

2.1. Data Source and Collection

We searched DECIPHER's open-access database (accessed 16 June 2020) to identify patients with documented "abnormality of the female genitalia" (thereafter referred to as atypical female genitalia when possible). Additionally, we categorized the phenotypes by whether they affected internal genitalia, external genitalia, or both. Some patients were categorized as "male" because they exhibited phenotypes such as hypospadias, cryptorchidism, and male "pseudohermaphroditism" (Supplementary Table S1).

2.2. Delineation of SROs

Using the chromosome map function "Browser" in DECIPHER, recurrent and overlapping copy number variable regions across the entire genome were identified. Within each CNV cluster ($\geq$2 CNVs), an SRO map was created. Singular CNVs were disregarded. SROs were further classified as unabridged or extrapolated (Supplementary Figure S1). Unabridged SROs are defined by one patient's full CNV and are completely contained within the confines of CNVs from all other patients in a given interval. Extrapolated SROs are not defined by one complete CNV—instead, CNVs from two individual patients establish the boundaries of the SRO (i.e., one CNV establishes the beginning of the SRO while another establishes the end).

Locus and size of each SRO were further determined using the UCSC Genome Browser (https://genome.ucsc.edu/; accessed on 16 June 2020) [24]. Chromosomal locations for each SRO were manually entered into Chromosome Analysis Suite 4.1 (ChAS; Affymetrix, Inc., Santa Clara, CA, USA) to generate the SRO CNV map. Gene content was determined by accessing each patient profile and their respective gene list. For extrapolated SROs, we obtained gene content by organizing genes by location and manually counting those, including non-protein coding, within the boundaries of the SRO. Based on DECIPHER data, genes were categorized as candidates for atypical female genitalia if they displayed a high likelihood of haploinsufficiency (%HI; 0.00–10.00) and/or the probability of loss-of-function (LoF) intolerance (pLI; 0.90–1.00).

2.3. Definition and Identification of Candidate Genes

In this study, we defined a candidate gene as one that has yet to undergo functional studies that prove causality between it and the development of AFG. The initial list of candidates contained hundreds of genes. To further narrow our list of candidate genes, we focused on small SROs ($\leq$500 kb) that contained five or fewer haploinsufficient and/or LoF intolerant genes. We investigated whether these genes had previously been associated with DSD or the development/function of the reproductive system. PubMed was searched to identify studies that had associated the candidate genes with atypical development of the female genitalia. We searched using the following terms: (gene name) and (disorders of sexual development OR DSD OR intersex). A similar approach was used with Google. To confirm each gene's novelty status, we accessed their individual OMIM phenotypes through their respective DECIPHER profiles. Candidate regions are SROs that contain no transcribed genes and may hold regulatory sequences, such as promoters, enhancers, silencers, etc.

2.4. Comorbidities

Within SROs, similarities in patients' DSD phenotypes were assessed. Phenotypes were classified based on the structure/organ affected. Certain phenotypes, such as uterine neoplasm, primary amenorrhea, etc., received individual categories. The proportion of patients that exhibited the same phenotypes of abnormal female genitalia as the reference patient, whose CNV determined the SRO, was noted. For extrapolated SROs, of the two CNVs defining its boundaries, the patient who had the fewest number of overall phenotypes was selected as the reference patient. DSD phenotypes not exhibited by the reference patient but exhibited by non-reference patients were also recorded. An analogous

process was used for non-DSD comorbidities which were listed in order of prevalence. Atypical female genitalia phenotypes were then grouped by affected region and assessed for recurring comorbidities.

3. Results

Genotypic and Phenotypic Characterization. We identified 300 patients (access date: 16 June 2020) with a spectrum of phenotypic findings including, but not limited to, aplasia/hypoplasia of reproductive structures, endocrine dysfunction, neoplasms, and cysts (Supplementary Table S1). There were 191 patients that only exhibited 1 DSD phenotype, while 108 exhibited two or more. One patient did not have any apparent DSD phenotypes. Additionally, 137 patients had DSD phenotypes that solely affected the internal genitalia while 94 patients had DSD phenotypes that only affected the external genitalia (Figure 1). Both internal and external genitalia were atypical in 25 patients. Very few patients (n = 5) exhibited atypical male phenotypes. 39 patients were classified as unspecified due to ambiguity in their reported phenotypes. Most of the patients (n = 264) that exhibited atypical female genitalia had a 46, XX sex chromosome complement, while 23 individuals were 46, XY (Figure 1). The remaining 13 did not have a known sex (n = 9) or were classified as "other" (n = 4). Patients exhibited a variety of phenotypes, with abnormality of the labia, abnormality of the uterus, and abnormality of the female genitalia being the most common (Figure 2).

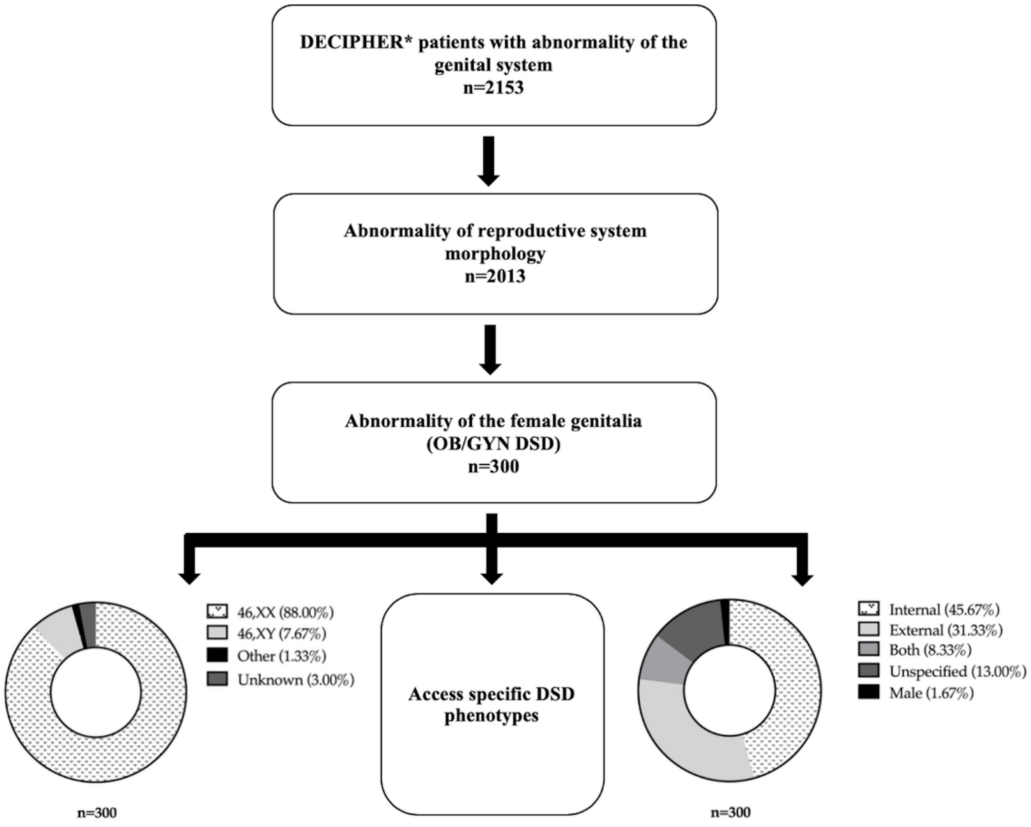

Figure 1. Methodology for identifying and categorizing patients with atypical female genitalia. * Access date: 16 June 2020.

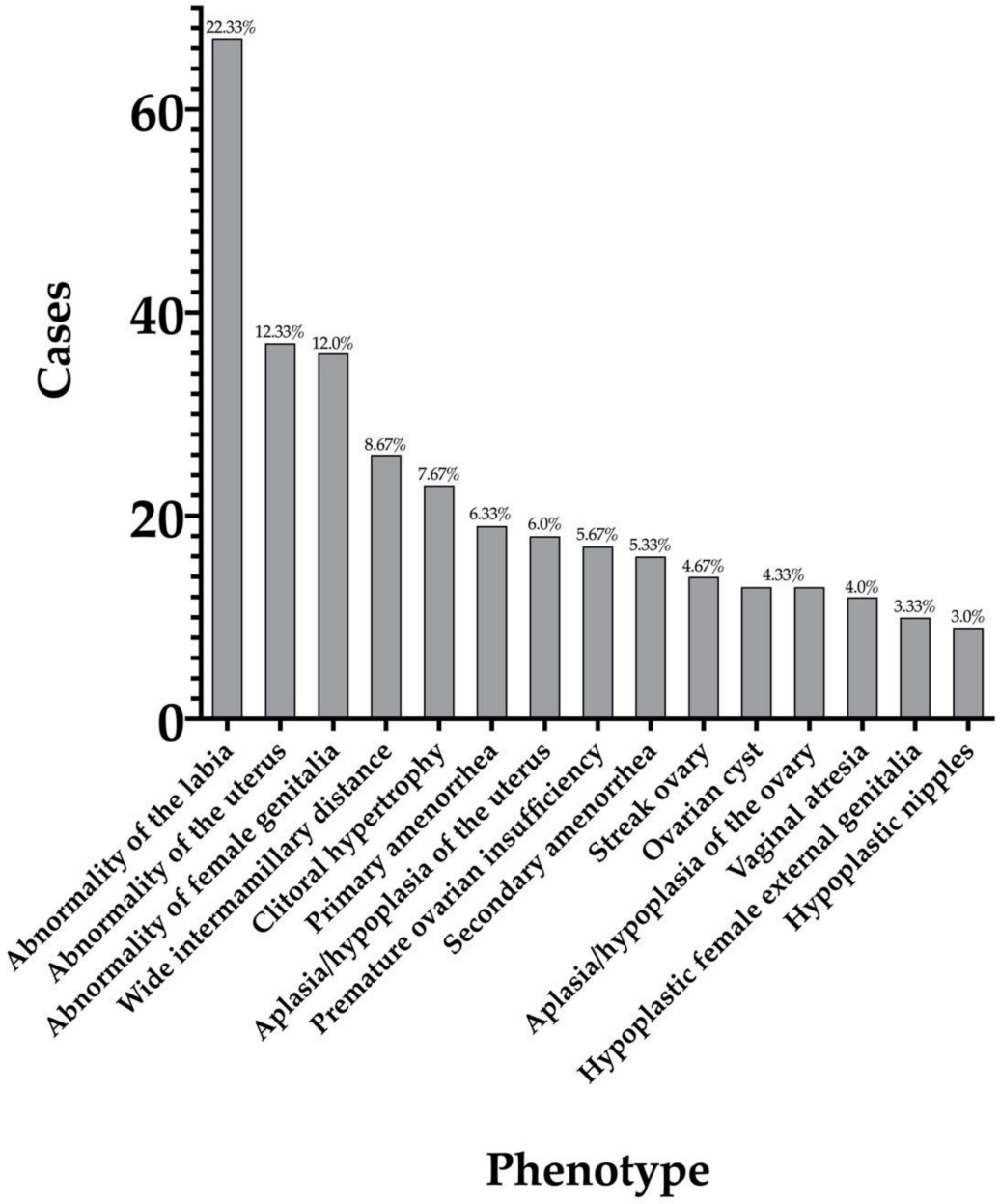

Figure 2. Most frequent DSD phenotypes seen in patients with atypical female genitalia.

3.1. Delineation of SROs

We were able to identify 56 CNV clusters across the genome. Within those clusters, we identified 180 SROs throughout 22 pairs of chromosomes (Figure 3). The X chromosome contained the highest number of SROs (n = 35) while chromosomes 14 and Y had the least number of SROs with one each (Figure 4). Chromosome 3 was the only chromosome that did not contain any SROs. The majority (64%) of SROs were extrapolated while 65 were unabridged (Supplementary Figure S1). SROs tended to consist of a combination of deletions and duplications, with 64% containing both. Of the remaining SROs, 33% only contained deletions while a mere 3% solely contained duplications (Figure 5). SROs ranged from 7.95 kb to 45 Mb in size. Most SROs were larger than 1 Mb (70.00%), with only 10.56% (n = 19) being less than 250 kb (Figure 5).

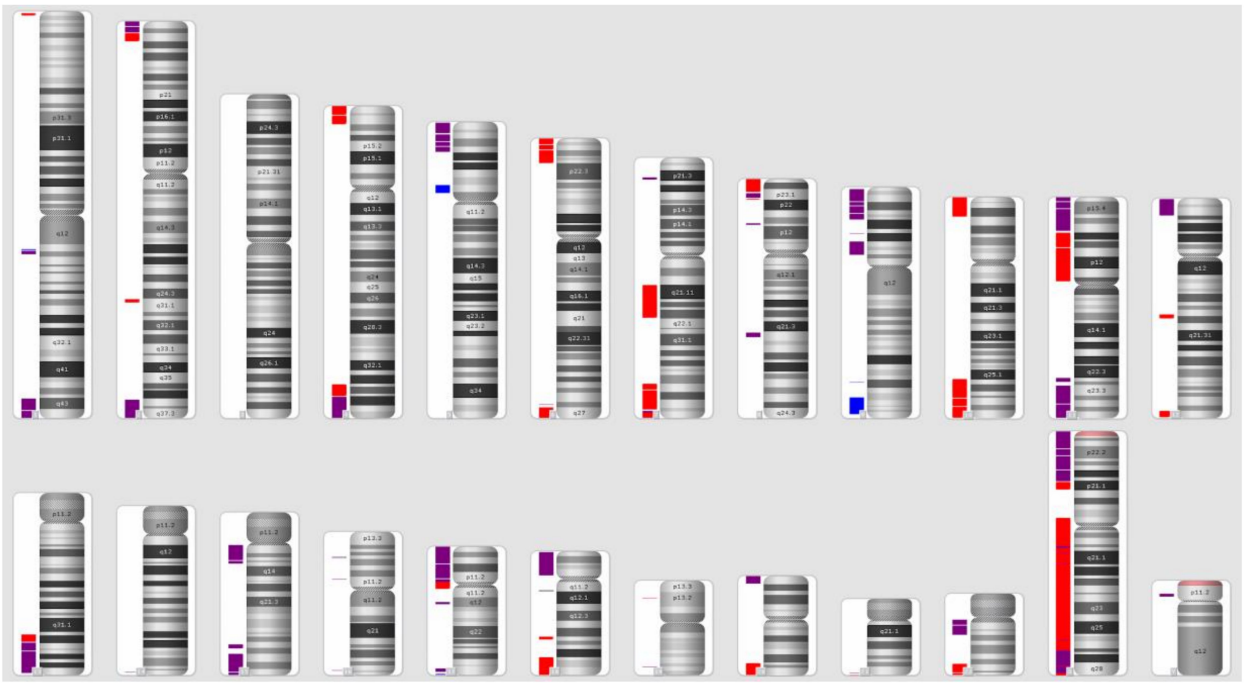

Figure 3. SRO Distribution across the Genome. Red bars indicate SROs composed entirely of deletions while blue bars indicate SROs composed solely of duplications. Purple bars are SROs that feature both deletions and duplications.

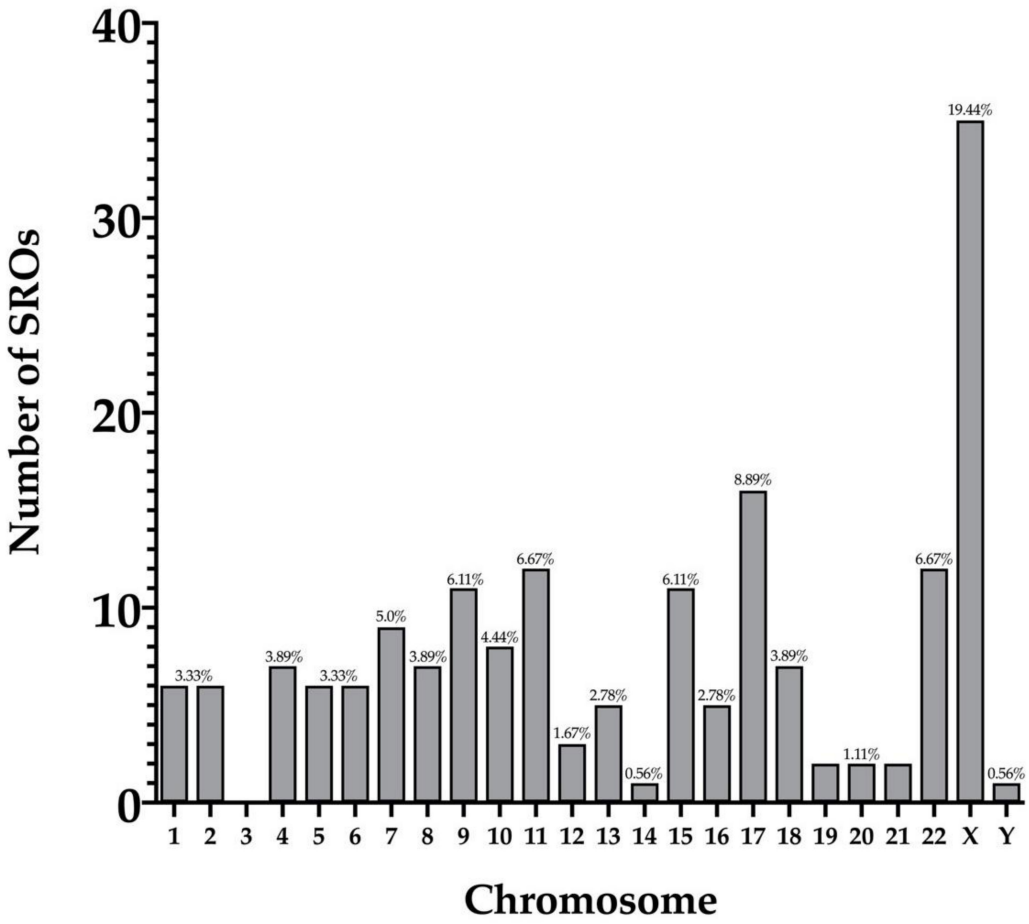

Figure 4. Number of SROs per chromosome.

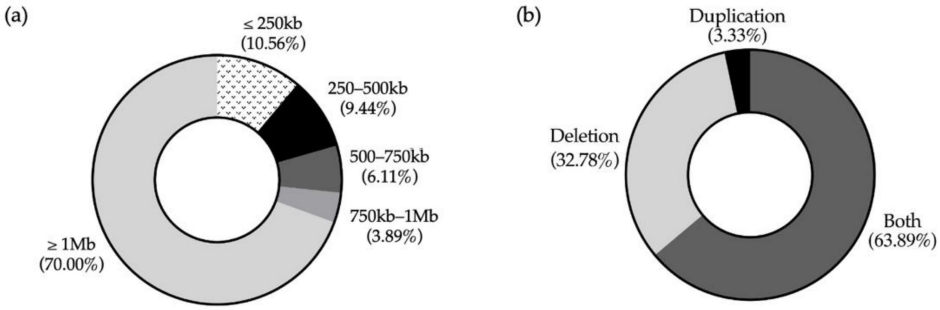

Figure 5. Characteristics of SROs. (**a**) Breakdown of SROs by size. (**b**) Breakdown of SROs by CNV content.

3.2. Previously Described Genes and Regions

There were 25 SROs that overlapped with genes or regions that are known to cause or are associated with atypical female genitalia (Table 1). Only one region (CNV 22q11.21), containing six adjacent SROs, overlapped with candidate genes identified in our analysis.

3.3. Candidate Genes and Regions

Within the 180 SROs, 335 genes displayed a high probability of haploinsufficiency while 660 genes were considered extremely loss-of-function (LoF) intolerant (Table 2). Applying the SRO size and gene quantity limitations, we were left with 22 candidates to further investigate. Although none of the genes were widely associated with DSD, a literature review indicated that almost half were potentially involved in the development and/or function of the reproductive system (Table 3). Most of these genes did not exist in regions that were linked to DSD. In exception, *GGNBP2*, *MAZ*, *SCARF2*, *MED15*, *UBE2L3*, and *MAPK1* overlapped with CNVs that are associated with MRKH syndrome types I and II [25,26]; however, none of these genes correlated to phenotypes/syndromes in Online Mendelian Inheritance in Man (OMIM). Additionally, six candidate genes had associated OMIM phenotypes, while the remaining 16 had no associated phenotypes. Of the six genes with OMIM phenotypes, only one was associated with a disorder that reported an individual patient with ambiguous genitalia [27].

Table 1. Genes Known to be Associated with DSD [1].

DSD Gene	Location	OMIM DSD Phenotype	Overlap with SRO	SRO	Notes
WNT4	1:22443798-22470462	Mullerian aplasia and hyperandrogenism, Serkal syndrome	No		
RSPO1	1:38076951-38100595	Palmoplantar hyperkeratosis with squamous cell carcinoma of skin and 46, XX sex reversal	No		
FOXL2	3:138663066-138665982	BPES type I, premature ovarian failure 3	No		
NR2F2	15:96869167-96883492	Congenital heart defects (multiple types, 4), 46, XX sex reversal 5	Yes	SRO097	
NR5A1	9:127243516-127269709	Premature ovarian failure 7, 46XY sex reversal 3, spermatogenic failure 8, 46XX sex reversal 4	No		
SOX3	X:139585152-139587225	Mental retardation, X-linked with isolated growth hormone deficiency; panhypopituitarism, X-linked	Yes	SRO171	
SOX9	17:70117161-70122561	Campomelic dysplasia with 46XY sex reversal	No		
SOX10	22:38366693-38383429	Waardenburg syndrome, type 2E	No		
SRY	Y:2654896-2655740	46XX sex reversal 1, 46XY sex reversal 1	No		
HSD3B2	1:119957554-119965658	CAH	No		
CYP21A2	6:32006042-32009447	CAH	No		
POR	7:75528518-75616173	Antley-Bixler syndrome, Disordered steroidogenesis due to P450 oxidoreductase	No		
CYP19A1	15:51500254-51630807	Aromatase excess syndrome, aromatase deficiency	No		
ESR1	6:151977826-152450754	Estrogen resistance	No		
GRα/NR3C1	5:142657496-142815077	Glucocorticoid resistance	No		
HOXA13	7:27233122-27239725	Hand-foot-uterus syndrome, Guttmacher syndrome	No		
FGF9	13:22245522-22278637	N/A	No		
CNV 17q12	-	MRKH, types I and II	Yes	SRO115	
CNV 1q21.1	-	MRKH, types I and II	Yes	SRO002-SRO003	
CNV 22q11.21	-	MRKH, types I and II	Yes	SRO135-SRO141	Overlaps with candidate genes *SCARF2* and *MED15* (SRO137), *UBE2L3* and *MAPK1* (SRO135)
CNV Xq21.31	-	MRKH, types I and II	Yes	SRO164-SRO166	

Table 1. *Cont.*

DSD Gene	Location	OMIM DSD Phenotype	Overlap with SRO	SRO	Notes
LHX8	1:75594119-75627218	N/A	No		
EIF2B3	1:45316450-45452282	Leukoencephalopathy with vanishing white matter	Yes	SRO003	Phenotype may lead to ovarian failure in female carriers
HFM1	1:91726323-91870426	Premature ovarian failure 4	No		
LMNA	1:156052364-156109880	Malouf syndrome	No		
EIF2B4	2:27587219-27593353	Leukoencephalopathy with vanishing white matter	No		Phenotype may lead to ovarian failure in female carriers
LHCGR	2:48859428-48982880	Precocious puberty, male-limited; Leydig cell hypoplasia, type I	No		
FSHR	2:49189296-49381676	Ovarian dysgenesis 1, ovarian hyperstimulation syndrome, ovarian response to FSH stimulation	No		
FIGLA	2:71004442-71017775	Premature ovarian failure 6	No		
HS6ST1	2:128994290-129076151	Hypogonadotropic hypogonadism 15 with or without anosmia	No		
DCAF17	2:172290727-172341562	Woodhouse-Sakati syndrome	No		
LARS2	3:45429998-45590913	Perrault syndrome 4	No		
EIF2B5	3:183852826-184402546	Leukoencephalopathy with vanishing white matter	No		Phenotype may lead to ovarian failure in female carriers
TP63	3:189349205-189615068	Limb-mammary syndrome, ADULT syndrome	No		
TACR3	4:104507188-104640973	Hypogonadotropic hypogonadism 11 with ot without anosmia	No		
HSD17B4	5:118788138-118972894	Perrault syndrome 1	No		
HARS2	5:140071011-140078889	Perrault syndrome 2	No		
MCM9	6:119134605-119256327	Ovarian dysgenesis	No		
GLI3	7:42000548-42277469	Pallister-Hall syndrome, hypothalamic hamartomas	No		
SEMA3A	7:83585093-84122040	Hypogonadotropic hypogonadism 16 with or without anosmia	No		

Table 1. *Cont.*

DSD Gene	Location	OMIM DSD Phenotype	Overlap with SRO	SRO	Notes
FEZF1	7:121941448-121950745	Hypogonadotropic hypogonadism 22 with or without anosmia	No		
NOBOX	7:144094333-144107320	Premature ovarian failure	Yes	SRO039	
FGF17	8:21899909-21906320	Hypogonadotropic hypogonadism 20 with or without anosmia	No		
CHD7	8:61591337-61779465	CHARGE syndrome, hypogonadotropic hypogonadism 5 with or without anosmia	No		
CYP11B1	8:143953772-143961262	CAH	No		
SOHLH1	9:13858253-138591374	Ovarian dysgenesis 5, spermatogenic failure 32	Yes	SRO052	
FGF8	10: 103529899-103535854	Hypogonadotropic hypogonadism 6 with or without anosmia	No		
C10ORF2	10: 102747124-102754158	Perrault syndrome 5	No		
WDR11	10: 122610687-122669036	Hypogonadotropic hypogonadism 14 with or without anosmia	Yes	SRO065	
SYCE1	10:135367404-135382876	Premature ovarian failure 12; spermatogenic failure 15	No		
FSHB	11:30252563-30256808	Hypogonadotropic hypogonadism 24 without anosmia	Yes	SRO072	
WT1	11:32409321-32457176	Frasier syndrome, Denys-Drash syndrome, Meacham syndrome	Yes	SRO073	
TAC3	12:57403784-57422667	Hypogonadotropic hypogonadism 10 with or without anosmia	No		
NUP107	12:69080514-69136785	Ovarian dysgenesis 6	No		
TBX3	12:115108059-115121969	Ulnar-mammary syndrome	No		
EIF2B1	12:124104953-124118313	Leukoencephalopathy with vanishing white matter	No		Phenotype may lead to ovarian failure in female carriers
REC8	14:24641062-24629463	N/A	No		
EIF2B2	14:75469614-75476292	Leukoencephalopathy with vanishing white matter	No		Phenotype may lead to ovarian failure in female carriers

Table 1. *Cont.*

DSD Gene	Location	OMIM DSD Phenotype	Overlap with SRO	SRO	Notes
PMM2	16:8882680-8943188	Congenital disorder of glycosylation, type Ia	No		
PSMC3IP	17:40724333-40729849	Ovarian dysgenesis 3	No		
CBX2	17:77751931-77761782	46XY sex reversal 5	No		
KISS1R	19:917287-921015	Precocious puberty central 1, hypogonadotropic hypogonadism 8 with or without anosmia	No		
CLPP	19:6361463-6368919	Perrault syndrome 3	No		
LHB	19:49519237-49520338	Hypogonadotropic hypogonadism 23 with or without anosmia	No		
MKKS	20:10381657-10414870	McKusick-Kaufman syndrome, Bardet-Biedl syndrome 6	No		
MCM8	20:5931298-5975852	Premature ovarian failure 10	No		
FLRT3	20:14303634-14318262	Hypogonadotropic hypogonadism 21 with anosmia	No		
SMC1B	22:45739944-45809500	N/A	Yes	SRO144	
BMP15	X:50653784-50659607	Ovarian dysgenesis 2	No		
DIAPH2	X:95939662-96859996	Premature ovarian failure 2A	Yes	SRO166	

[1] [3,4,26,27].

Table 2. List of SROs.

SRO	Locus	Del, Dup, or Both	Size (kb)	Genes	Names of Genes w/HI (≤10)	Names of Genes w/pLI (≥0.9)
SRO001	1p36.33-36.32	Del	1598.83	63	-	*GABRD*, *GNB1*, *PANK4*, *SKI*, *UBE2J2*
SRO002	1q21.1-21.2	Both	1356.86	29	*GJA5*	-
SRO003	1q21.1	Dup	1062.73	18	-	*ANKRD34A*, *PIAS3*
SRO004	1q21.2	Both	1365.84	10	-	-
SRO005	1q44	Both	4590.14	103	*HNRNPU*	*AHCTF1*, *HNRNPU*, *KIF26B*, *ZNF496*
SRO006	1q43-44	Both	7279.55	8	*AKT3*	*AHCTF1*, *AKT3*
SRO007	2p25.3	Both	3152.14	11	-	*MYT1L*
SRO008	2p25.3-25.1	Both	3933.81	12	-	*RNF144A*, *RPS7*
SRO009	2p25.1-24.3	Del	5100.00	29	*CPSF3*, *ID2*, *YWHAQ*	*RNF144A*, *ASAP2*, *ADAM17*, *ROCK2*
SRO010	2q31.1	Del	2268.94	20	*TLK1*, *GAD1*, *SSB*, *PPIG*, *BBS5*	*TLK1*, *UBR3*, *PPIG*, *LRP2*
SRO011	2q37.1-37.3	Both	11,223.73	165	*ALPP*, *ALPPL2*, *ECEL1*, *GBX2*, *HDAC4*, *PSMD1*, *TWIST2*	*AGAP1*, *ATG16L1*, *ATG4B*, *DIS3L2*, *GIGYF2*, *HDAC4*, *HDLBP*, *ILKAP*, *INPP5D*, *KIF1A*, *NCL*, *PPP1R7*, *PSMD1*, *UBE2F*
SRO012	2q37.1	Del	0.002	1	-	-
SRO013	4p16.3	Del	4371.93	83	*FGFR3*, *MAEA*	*ADD1*, *CPLX1*, *CTBP1*, *FAM193A*, *HTT*, *PCGF3*, *WHSC1*
SRO014	4p16.3-16.2	Del	1400.00	14	*MSX1*	*CRMP1*
SRO015	4p15.33-12	Del	34,458.19	85	-	*WDR1*, *KIAA0232*, *PPP2R2C*, *JAKMIP1*, *CRMP1*
SRO016	4q35.1-35.2	Both	7804.23	82	*CASP3*, *TENM3*	*CDKN2AIP*, *TENM3*
SRO017	4q34.3-35.1	Both	5400.00	12	*TENM3*	*TENM3*
SRO018	4q34.1-34.3	Del	5899.58	28	*VEGFC*, *GALNTL6*, *HAND2*	*GPM6*, *HMGB2*
SRO019	4q33-34.1	Del	1500.00	9	-	*CLCN3*
SRO020	5p15.2	Both	151.00	0	-	-
SRO021	5p15.33-15.31	Both	6549.11	46	-	*EXOC3*, *KIAA0947*, *PAPD7*, *SLC9A3*, *SLC6A3*, *TERT*, *TRIP13*
SRO022	5p15.31-15.2	Both	4555.85	25	*CTNND2*, *ADCY2*	*CTNND2*, *MARCH6*, *CCT5*, *ADCY2*
SRO023	5p15.2-15.1	Both	2817.92	15	-	*TRIO*
SRO024	5p15.1-14.3	Both	3417.24	18	-	-
SRO025	5p13.1-12	Dup	5210.14	49	*NNT*, *GHR*, *DAB2*, *RICTOR*	*NNT*, *PAIP1*, *HMGCS1*, *ZNF131*, *PTGER4*, *RICTOR*

Table 2. *Cont.*

SRO	Locus	Del, Dup, or Both	Size (kb)	Genes	Names of Genes w/HI (≤10)	Names of Genes w/pLI (≥0.9)
SRO026	6p25.2-25.1	Both	358.45	6	*PRPF4B*	*PRPF4B*
SRO027	6p25.3-25.2	Del	3789.82	36	*FOXC1*, *GMDS*	*TUBB2B*, *TUBB2A*, *GMDS*, *FOXC1*
SRO028	6p25.1	Del	2846.73	21	*NRN1*	*CDYL*
SRO029	6p25.1-23	Del	8137.02	41	*BMP6*, *TFAP2A*, *EDN1*	*RREB1*, *DSP*, *TFAP2A*, *HIVEP1*, *RANBP9*
SRO030	6q26	Both	465.00	3	*PARK2*	-
SRO031	6q26-27	Del	6567.36	53	*DLL1*, *TBP*	*DLL1*, *MLLT4*, *PDE10A*, *PSMB1*
SRO032	7p21.3	Both	1639.52	8	-	*THSD7A*
SRO033	7q21.11-21.3	Del	8291.93	66	*ABCB1*, *CDK14*, *CDK6*, *COL1A2*, *DMTF1*, *FZD1*, *GRM3*, *KRIT1*, *SRI*	*ANKIB1*, *CDK6*, *COL1A2*, *DBF4*, *DMTF1*,
SRO034	7q11.23-21.11	Del	8520.97	36	*SEMA3A*, *HGF*, *GNAI1*, *MAGI2*	*SEMA3A*, *PCLO*, *CACNA2D1*, *HGF*, *GNAI1*, *MAGI2*
SRO035	7q21.3	Del	3687.10	47	*SHFM1*, *DLX6*, *DLX5*, *TAC1*	*DLX6*, *LMTK2*
SRO036	7q36.2-36.3	Both	1257.04	13	*SHH*	*SHH*, *RBM33*, *PAXIP1*
SRO037	7q36.3	Del	3338.25	24	*MNX1*	*NCAPG2*, *UBE3C*
SRO038	7q35-36.2	Del	6376.72	93	*KCNH2*, *NOS3*, *CDK5*, *SMARCD3*, *RHEB*, *EZH2*, *CUL1*, *CNTNAP2*	*ACTR3B*, *KMT2C*, *PRKAG2*, *RHEB*, *AGAP3*, *SLC4A2*, *KCNH2*, *ZNF777*, *EZH2*, *CUL1*
SRO039	7q34-35	Del	5878.83	144	*CNTNAP2*,	*FAM131B*
SRO040	7q33-34	Del	3840.78	65	*BRAF*	*BRAF*, *TMEM178B*, *MKRN1*, *KDM7A*, *HIPK2*, *UBN2*, *KIAA1549*, *TRIM24*
SRO041	8p23.1	Both	204.79	2	-	-
SRO042	8p23.1	Both	3134.75	45	-	*XKR6*
SRO043	8p23.1	Del	802.03	33	-	-
SRO044	8p23.3-23.1	Del	8091.64	104	*ANGPT2*	*ANGPT2*, *CSMD1*, *DLGAP2*
SRO045	8p21.2-21.1	Both	1085.85	20	*PBK*	*PTK2B*
SRO046	8q21.3	Dup	468.05	5	*NECAB1*	-
SRO047	8q22.1	Both	3012.69	31	*CCNE2*	*ESRP1*, *INTS8*, *KIAA1429*
SRO048	9p24.3	Both	155.65	4	-	-

Table 2. *Cont.*

SRO	Locus	Del, Dup, or Both	Size (kb)	Genes	Names of Genes w/HI (≤10)	Names of Genes w/pLI (≥0.9)
SRO049	9p24.3-24.1	Both	5468.51	46	*SMARCA2*, *RFX3*, *GLIS3*, *JAK2*	*UHRF2*, *CDC37L1*, *RFX3*, *SMARCA2*
SRO050	9p24.1-23	Both	2483.79	10	*PTPRD*	*PTPRD*
SRO051	9p23	Both	2341.97	3	-	-
SRO052	9p23	Both	2758.03	10	*NFIB*	*NFIB*
SRO053	9p23-22.3	Both	2146.47	20	*NFIB*, *ZDHHC21*, *PSIP1*	*NFIB*, *PSIP1*
SRO054	9p22.3-21.3	Both	3653.53	21	*BNC2*, *SH3GL2*, *ADAMTSL1*, *RPS6*	*RPS6*, *BNC2*
SRO055	9p21.1	Both	604.19	1	*LINGO2*	-
SRO056	9p13.3-12	Both	8272.95	166	*VCP*, *RNF38*, *PAX5*, *NPR2*, *GNE*	*VCP*, *UBE2R2*, *UBAP1*, *TLN1*, *TESK1*, *SHB*, *RUSC2*, *RNF38*, *PAX5*, *NOL6*, *CNTFR*, *CLTA*
SRO057	9q33.1	Dup	320.79	4	*ASTN2*, *PAPPA*	*ASTN2*
SRO058	9q33.3-34.3	Dup	10,616.44	203	*SPTAN1*, *SET*, *RXRA*, *PBX3*, *MED27*, *LMX1B*, *ABL1*	*ZER1*, *ZBTB43*, *ZBTB34*, *WDR5*, *TSC1*, *STXBP1*, *SPTAN1*, *SETX*, *SET*, *RXRA*, *RPL7A*, *RAPGEF1*, *RALGPS1*, *PRRC2B*, *PRDM12*, *PPP2R4*, *OLFM1*, *NUP188*, *NTNG2*, *LRRC8A*, *GOLGA2*, *ENG*, *DNM1*, *COL5A1*, *CAMSAP1*, *BRD3*, *ABL1*
SRO059	10p15.3-15.1	Del	6499.99	62	-	*DIP2C*, *GTPBP4*, *KLF6*, *LARP4B*, *RBM17*, *ZMYND11*
SRO060	10p15.1-14	Del	5600.00	34	*CELF2*, *GATA3*	*UPF2*, *CELF2*, *GATA3*, *TAF3*, *SFMBT2*
SRO061	10q26.2	Both	150.61	1	-	-
SRO062	10q26.2-26.3	Del	7000.59	38	*EBF3*	*EBF3*, *PPP2R2D*, *INPP5A*, *FAM196A*
SRO063	10q26.1-26.2	Del	1143.31	9	-	-
SRO064	10q26.13	Del	3868.30	42	*BUB3*, *FGFR2*	*FGFR2*, *HMX3*, *ZRANB1*
SRO065	10q25.3-26.13	Del	4831.70	48	*TIAL1*, *EMX2*	*HSPA12A*, *PDZD8*, *EMX2*, *RAB11FIP2*, *CACUL1*, *EIF3A*, *TIAL1*, *MCMBP*
SRO066	10q25.1-25.3	Del	6900.00	64	*ATRNL1*, *GFRA1*, *ADRB1*, *TCF7L2*, *VTI1A*, *SHOC2*, *PDCD4*, *SMC3*, *SMNDC1*, *MXI1*,	*ATRNL1*, *FAM160B1*, *ADD3*, *SMC3*, *RBM20*, *SHOC2*, *TCF7L2*, *TDRD1*, *ABLIM1*
SRO067	11p15.1	Both	1076.48	9	*NAV2*, *NELL1*	*NAV2*
SRO068	11p15.4	Both	2055.28	97	*APBB1*, *ILK*	*APBB1*, *DCHS1*, *FAM160A2*, *TRIM3*
SRO069	11p15.5-15.4	Both	2783.42	102	*TH*, *INS-IGF2*, *HRAS*	*AP2A2*, *BRSK2*, *CD81*, *MUC5B*, *PSMD13*

Table 2. *Cont.*

SRO	Locus	Del, Dup, or Both	Size (kb)	Genes	Names of Genes w/HI (≤10)	Names of Genes w/pLI (≥0.9)
SRO070	11p15.4	Both	2366.15	104	*RRM1, STIM1, RHOG*	*RRM1, PGAP2, NUP98*
SRO071	11p15.4-15.1	Both	12,676.07	156	*LMO1, IPO7, SBF2, CTR9, EIF4G2, TEAD1, PTH, RRAS2, COPB1, SOX6, C11orf58, PIK3C2A, KCNJ11, ABCC8, MYOD1, KCNC1, TPH1, CTF2H1, TSG101, NAV2*	*NAV2, SPTY2D1, GTF2H1, KCNC1, SOX6, PSMA1, COPB1, RRAS2, FAR1, ARNTL, TEAD1, USP47, EIF4G2, CTR9, WEE1, IPO7, ST5, RPL27A, EIF3F*
SRO072	11p14.3-13	Del	9200.00	37	*MPPED2, BDNF, SLC17A6*	*KCNA4, MPPED2*
SRO073	11p13-12	Del	5400.00	47	*CSTF3, LMO2, CAT, PDHX, CD44, IMMP1L, ELP4, PAX6, RCN1, WT1, EIF3M*	*FJX1, CAPRIN1, FBXO3, CSTF3, QSER1, PAX6, WT1*
SRO074	11p12-11.12	Del	15,150.78	137	*RAG2, LRRC4C, API5, ALX4, PHF21A, AMBRA1, F2, CKAP5. PSMC3, CELF1*	*TRAF6, LRRC4C, API5, TTC17, PRDM11, MAPK8IP1, PHF21A, CREB3L1, CHRM4, AMBRA1, ATG13, CKAP5, NR1H3, SPI1, PSMC3, CELF1, FNBP4*
SRO075	11q24.3-25	Both	4351.73	22	*NTM, OPCML*	*VPS26B*
SRO076	11q24.2-24.3	Both	4237.86	34	*KIRREL3, ETS1, FLI1*	*ZBTB44, KIRREL3, FLI1, ARHGAP32*
SRO077	11q23.3-24.2	Both	11,362.14	225	*KIRREL3, CHEK1, STT3A, PKNOX2, HSPA8, PVRL1, CBL, H2AFX, HMBS, TRAPPC4, DDX6, ARCN1, BACE1, TAGLN, PAFAH1B2, CADM1*	*KIRREL3, PKNOX2, MSANTD2, GRAMD1B, HSPA8, TBCEL, ARHGEF12, C2CD2L, HMBS, BCL9L, DDX6, ARCN1, KMT2A, DSCAML1, RNF214, PAFAH1B2, SIK3,*
SRO078	11q23.1-23.2	Both	2764.10	52	*CRYAB, NCAM1*	*SIK2, NCAM1*
SRO079	12p13.33-13.2	Both	10,062.99	197	*CHD4, GAPDH, ENO2, CCND2, CD4, FOXM1, NTF3, PHB2, ERC1, ATN1, CACNA1C*	*NTF3, USP5, CLSTN3, PRMT8, CCND2, TNFRSF1A, LPCAT3, ZNF384, FOXJ2, PHC1, ATN1, NOP2, PTPN6, CACNA1C, CHD4, KDM5A, WNK1*
SRO080	12q15-21.1	Del	2517.45	18	*CNOT2, TRHDE*	*ZFC3H1, CNOT2, KCNMB4*
SRO081	12q24.33	Del	4092.91	41	*POLE, RAN*	*EP400, SFSWAP, TMEM132D, ULK1, RAN*
SRO082	13q34	Both	1174.90	56	-	*COL4A1, CUL4A, ARHGEF7, MYO16, IRS2*
SRO083	13q32.3-33.3	Both	9271.11	76	*ZIC2, FGF14, EFNB2, ARGLU1*	*TM9SF2, ZIC2, FGF14, TPP2, EFNB2, FAM155A, TNFSF13B*
SRO084	13q31.3-32.3	Both	5436.94	68	*DCT, MBNL2, HS6ST3*	*HS6ST3, IPO5, DOCK9*
SRO085	13q31.3	Both	591.95	3	*GPC6*	-
SRO086	13q31.2-31.3	Del	4689.28	32	*GPC6*	-
SRO087	14q32.33	Both	29.28	0	-	-

Table 2. *Cont.*

SRO	Locus	Del, Dup, or Both	Size (kb)	Genes	Names of Genes w/HI (≤10)	Names of Genes w/pLI (≥0.9)
SRO088	15q11.2	Both	179.10	9	-	-
SRO089	15q11.2	Both	118.16	29	-	-
SRO090	15q13.1-13.2	Both	1334.43	7	*TJP1*	*TJP1*
SRO091	15q11.1-11.2	Both	2123.17	46	-	-
SRO092	15q11.2	Both	2788.12	40	-	*MAGEL2*, *CYFIP1*, *NIPA2*
SRO093	15q11.2-13.1	Both	3656.33	72	*GABRB3*, *UBE3A*	*HERC2*, *GABRA5*, *GABRB3*, *UBE3A*
SRO094	15q13.2-13.3	Both	1971.35	48	-	*OTUD7A*
SRO095	15q26.3	Both	403.94	7	-	-
SRO096	15q26.3	Both	1303.08	29	-	*ASB7*, *SNRPA1*
SRO097	15q25.3-26.3	Both	11,733.12	117	*FURIN*, *NR2F2*, *IGF1R*, *MEF2A*	*ACAN*, *ZNF710*, *IQGAP1*, *FURIN*, *CHD2*, *NR2F2*, *IGF1R*, *MEF2A*
SRO098	15q25.2-25.3	Both	2641.57	53	*CPEB1*, *SEC11A*	*ZNF592*, *CPEB1*, *BNC1*
SRO099	16p13.11	Both	205.81	3	-	-
SRO100	16p13.11	Both	8.99	1	-	-
SRO101	16p13.11	Both	572.98	10	*MYH11*	*KIAA0430*
SRO102	16p11.2	Both	469.06	28	*CDIPT*, *MAPK3*	*TAOK2*, *MAZ*
SRO103	16q24.2	Both	692.95	6	-	*JPH3*, *ZCCHC14*
SRO104	17p13.3	Both	235.24	5	*PAFAH1B1*	*PAFAH1B1*
SRO105	17p13.1	Both	19.17	9	*SENP3*	*SENP3*, *EIF4A1*
SRO106	17p13.3	Both	2326.50	39	*YWHAE*, *CRK*, *PITPNA*, *PRPF8*, *HIC1*, *SMG6*, *MNT*	*MNT*, *SMG6*, *RTN4RL1*, *PRPF8*, *PITPNA*, *CRK*, *YWHAE*, *NXN*
SRO107	17p13.3-13.1	Both	4913.54	129	*SENP3*, *POLR2A*, *C1QBP*, *ENO3*, *ARRB2*, *UBE2G1*, *PAFAH1B1*	*POLR2A*, *ZBTB4*, *NLGN2*, *RABEP1*, *DERL2*, *PITPNM3*, *DLG4*, *PHF23*, *CTDNEP1*, *YBX2*, *NEURL4*, *C17orf85*, *ANKFY1*, *PELP1*, *MINK1*, *CAMTA2*, *PAFAH1B1*, *CLUH*, *RAP1GAP2*
SRO108	17p13.1-12	Both	3464.84	66	*TP53*, *NTN1*	*FXR2*, *KDM6B*, *CHD3*, *RPL26*, *NDEL1*, *MYH10*, *NTN1*
SRO109	17p12-11.2	Both	5300.00	26	*NCOR1*, *MAP2K4*	*MAP2K4*, *ARHGAP44*, *PMP22*, *NCOR1*
SRO110	17p11.2	Both	541.36	6	*UBB*	-

Table 2. *Cont.*

SRO	Locus	Del, Dup, or Both	Size (kb)	Genes	Names of Genes w/HI (≤10)	Names of Genes w/pLI (≥0.9)
SRO111	17p11.2	Both	3418.41	95	*COPS3*	*COPS3*, *ALKBH5*, *GID4*, *MPRIP*, *RAI1*
SRO112	17p11.2-11.1	Both	2043.96	13	-	-
SRO113	17p11.1-q11.1	Del	3112.87	1	-	-
SRO114	17q11.1-11.2	Del	1600.00	39	*WSB1*, *NLK*	*NLK*, *FOXN1*
SRO115	17q12	Both	1132.85	12	*AATF*, *ACACA*, *HNF1B*, *LHX1*, *TADA2A*	*SYNRG*, *HNF1B*, *ACACA*
SRO116	17q12	Both	263.86	6	-	*GGNBP2*
SRO117	17q25.3	Both	334.18	4	-	-
SRO118	17q25.3	Dup	642.63	19	-	*FASN*, *CSNK1D*
SRO119	17q25.3	Both	2454.63	21	*ACTG1*	*NPLOC4*
SRO120	18p11.32-11.31	Both	2900.00	24	*TYMS*, *USP14*, *YES1*	*USP14*, *THOC1*, *SMCHD1*, *COLEC12*
SRO121	18p11.31-11.1	Both	12,500.90	122	*PTPRM*, *DLGAP1*	*DLGAP1*, *PTPRM*, *ANKRD12*, *PPP4R1*, *GNAL*, *PTPN2*
SRO122	18q11.2	Both	789.00	6	*KCTD1*, *AQP4*	*KCTD1*
SRO123	18q23	Del	4843.15	26	*MBP*	*ZNF236*, *ZNF516*
SRO124	18q22.1-23	Del	6421.99	27	-	*TSHZ1*, *SOCS6*, *ZNF407*
SRO125	18q21.31	Del	1634.63	15	*NEDD4L*, *TXNL1*	*WDR7*, *ONECUT2*, *NEDD4L*
SRO126	18q21.2-21.31	Del	684.32	0	-	-
SRO127	19p13.2	Del	140.94	9	-	*ILF3*
SRO128	19q13.42	Both	14.95	1	-	-
SRO129	20p13	Both	4992.00	105	*SNRPB*, *FKBP1A*, *CSNK2A1*	*ATRN*, *CENPB*, *CSNK2A1*, *PTPRA*, *SCRT2*, *SNPH*, *TBC1D20*
SRO130	20q13.31-13.33	Del	7598.93	139	*BMP7*, *GNAS*	*ZNF512B*, *YTHDF1*, *TCFL5*, *TAF4*, *SYCP2*, *SS18L1*, *RGS19*, *RAE1*, *PSMA7*, *PMEPA1*, *PHACTR3*, *MYT1*, *MRGBP*, *LSM14B*, *KCNQ2*, *GMEB2*, *GID8*, *EEF1A2*, *DIDO1*, *ADRM1*
SRO131	21q22.3	Both	75.21	1	-	-
SRO132	21q22.3	Del	103.63	3	*S100B*	-
SRO133	22q11.11	Both	280.84	26	-	-
SRO134	22q11.22	Both	74.42	6	-	-

Table 2. *Cont.*

SRO	Locus	Del, Dup, or Both	Size (kb)	Genes	Names of Genes w/HI (≤10)	Names of Genes w/pLI (≥0.9)
SRO135	22q11.21-11.22	Both	400.00	14	*UBE2L3*, *MAPK1*	*MAPK1*
SRO136	22q11.21	Both	807.70	31	*CRKL*	*HIC2*
SRO137	22q11.21	Both	290.00	12	-	*MED15*, *SCARF2*
SRO138	22q11.21	Both	970.85	27	*DGCR8*, *RANBP1*	*DGCR8*, *RTN4R*
SRO139	22q11.21	Both	874.38	26	*TBX1*, *CDC45*	*SEPT5*, *CLDN5*, *UFD1L*, *HIRA*
SRO140	22q11.21	Both	971.53	10	-	*PEX26*, *MICAL3*, *CECR2*
SRO141	22q11.1-11.21	Both	1875.45	51	-	*CECR2*
SRO142	22q11.22-12.1	Both	3371.48	140	*SMARCB1*, *BCR*	*BCR*, *GNAZ*, *SMARCB1*
SRO143	22q13.33	Del	1201.52	40	-	*BRD1*, *MAPK8IP2*, *PIM3*, *PLXNB2*, *SBF1*, *SHANK3*
SRO144	22q13.2-13.33	Del	5799.67	54	*PPARA*	*GRAMD4*, *CELSR1*, *FBLN1*, *PHF21B*, *SULT4A1*
SRO145	Xp22.33	Both	558.00	0	-	-
SRO146	Xp22.33	Both	61.70	1	-	-
SRO147	Xp22.33	Both	1138.89	7	-	-
SRO148	Xp22.33	Both	343.81	1	-	-
SRO149	Xp22.33	Both	546.49	4	-	-
SRO150	Xp22.33-22.31	Both	3748.32	9 *	-	*NLGN4X*
SRO151	Xp22.31-22.2	Both	4754.29	30 *	*MID1*	*GPR143*, *TBL1X*, *MID1*, *KAL1*, *ARHGAP6*, *CLCN4*, *WWC3*
SRO152	Xp22.2	Both	828.04	4	-	*ARHGAP6*, *MSL3*
SRO153	Xp22.2	Both	3584.97	24 *	*MID1*	*FRMPD4*, *ACE2*, *FANCB*, *MOSPD2*, *TLR7*, *GLRA2*, *PIGA*, *OFD1*, *GEMIN8*, *PRPS2*
SRO154	Xp22.2	Both	1415.85	23 *	*RBBP7*	*ZRSR2*, *TXLNG*, *RBBP7*, *SYAP1*
SRO155	Xp22.1-22.1	Both	7600.00	73 *	*NHS*, *RPS6KA3*, *PTCHD1*, *PHEX*, *CNKSR2*, *ZFX*, *SH3KBP1*	*RPS6KA3*, *NHS*, *CNKSR2*, *PHEX*, *SCML2*, *CDKL5*, *SH3KBP1*, *ZFX*, *MBTPS2*, *KLHL15*, *PDHA1*, *PPEF1*, *PCYT1B*, *EIF1AX*, *GPR64*, *RS1*, *SCML1*, *EIF2S3*, *SMS*, *PTCHD1*
SRO156	Xp22.11-21.3	Both	2906.29	24 *	*POLA1*	*POLA1*, *ARX*, *PCYT1B*
SRO157	Xp21.3-21.1	Both	4742.52	21 *	*DMD*, *IL1RAPL1*	*NR0B1*, *GK*, *IL1RAPL1*, *DMD*

Table 2. *Cont.*

SRO	Locus	Del, Dup, or Both	Size (kb)	Genes	Names of Genes w/HI (≤10)	Names of Genes w/pLI (≥0.9)
SRO158	Xp21.1	Del	5184.76	23 *	*DMD*	*DMD*, *CXorf22*
SRO159	Xq13.2-13.3	Both	1330.05	31	-	*KIAA2022*, *RLIM*, *SLC16A2*
SRO160	Xq13.3-21.1	Del	2992.29	24 *	*ATRX*, *FGF16*	*ATRX*, *ABCB7*, *ATP7A*, *MAGT1*, *MAGEE1*
SRO161	Xq21.1	Both	427.85	6	*PGK1*	*ATP7A*
SRO162	Xq21.1	Del	6380.50	49 *	-	*BRWD3*, *CYLC1*, *MAGT1*, *TBX22*, *RPS6KA6*
SRO163	Xq21.1	Both	359.98	3	-	-
SRO164	Xq21.1-21.31	Del	1877.83	16 *	*DACH2*	*CHM*, *ZNF711*
SRO165	Xq21.31	Del	1060.65	2	-	-
SRO166	Xq21.31-26.2	Del	45,338.26	478 *	*STAG2*, *UBE2A*, *MID2*, *RAP2C*, *COL4A5*, *RAB9B*, *CUL4B*, *AMMECR1*, *PCDH19*, *AIFM1*, *PAK3*, *THOC2*, *BTK*, *DIAPH2*, *IL1RAPL2*, *XIAP*, *GRIA3*, *DCX*, *PLP1*, *TENM1*, *GPC3*	*STAG2*, *CUL4B*, *COL4A5*, *TENM1*, *THOC2*, *ARMCX4*, *SMARCA1*, *OCRL*, *BCORL1*, *ALG13*, *BTK*, *IGSF1*, *UTP14A*, *GRIA3*, *PCDH19*, *CENPI*, *TRPC5*, *WDR44*, *CSTF2*, *ZNF280C*, *GPC3*, *AIFM1*, *MORC4*, *NKAP*, *RGAG1*, *LRCH2*, *GLA*, *AMOT*, *NKRF*, *DIAPH2*, *NRK*, *ATG4A*, *PLS3*, *SLC25A14*, *GPRASP2*, *PAK3*, *RPS6KA6*, *TAF7L*, *UPF3B*, *IL1RAPL2*, *ACSL4*, *RBM41*, *IL13RA1*, *HNRNPH2*, *DCAF12L1*, *CXorf56*, *TMEM164*, *RBMX2*, *SLC25A5*, *SEPT6*, *PLP1*, *GPC4*, *COL4A6*, *ZBTB33*, *PRPS1*, *XIAP*
SRO167	Xq26.2	Both	747.60	11	*GPC3*	*GPC3*
SRO168	Xq26.2-26.3	Del	1584.34	37 *	*HPRT1*, *PHF6*	*DDX26B*, *PHF6*, *HPRT1*, *MOSPD1*
SRO169	Xq26.3-27.1	Del	3800.81	38 *	*FGF13*, *ZIC3*, *CD40LG*	*ARHGEF6*, *SLC9A6*, *F9*, *HTATSF1*, *ZIC3*, *BRS3*, *FHL1*, *MCF2*
SRO170	Xq27.1	Both	729.17	4 *	-	*ATP11C*
SRO171	Xq27.1-27.3	Both	6562.57	55 *	*SOX3*	-
SRO172	Xq27.3-28	Both	3575.07	42 *	*FMR1*, *AFF2*	*IDS*, *AFF2*
SRO173	Xq28	Both	2144.49	33 *	-	*MTM1*, *MTMR1*
SRO174	Xq28	Both	2306.72	73 *	*MECP2*, *DKC1*, *FLNA*	*FAM58A*, *RPL10*, *EMD*, *GAB3*, *NSDHL*, *G6PD*, *OPN1LW*, *ARHGAP4*, *FAM50A*, *MPP1*, *IRAK1*, *HAUS7*, *ATP6AP1*, *GDI1*, *SLC6A8*, *ABCD1*, *DKC1*, *TKTL1*, *ATP2B3*, *F8*, *L1CAM*, *HCFC1*, *FLNA*
SRO175	Xq28	Del	7.95	1	-	*F8*
SRO176	Xq28	Del	431.69	9	-	*F8*

Table 2. *Cont.*

SRO	Locus	Del, Dup, or Both	Size (kb)	Genes	Names of Genes w/HI (≤10)	Names of Genes w/pLI (≥0.9)
SRO177	Xq28	Del	281.08	19 *	-	-
SRO178	Xq28	Del	348.63	10 *	-	-
SRO179	Xq28	Del	42.80	1 *	-	-
SRO180	Yp11.2	Both	1944.43	56 *	-	-

* Initially, the total number of genes in these SROs was unable to be calculated due to formatting errors in the list of genes within a patient CNV. This only occurred for some variants located on sex chromosomes. This issue has since been resolved. These totals reflect the data in DECIPHER on 11 June 2022. Gene content may have changed since the original generation of this table, but likely not significantly.

Table 3. Candidate Genes.

Candidate Gene	Location	Locus	%HI	pLI	Associated with Reproductive System	OMIM Phenotype	Atypical Female Genitalia Assoc. with OMIM
SENP3	17:7465192-7475287	17p13.1	9.88	0.83	Yes	-	-
EIF4A1	17:7476024-7482323	17p13.1	18.71	1.00	Yes	-	-
F8	X:154064063-154255215	Xq28	30.72	1.00	No	Hemophilia A	None
PAFAH1B1	17:2496504-2588909	17p13.3	2.20	1.00	Yes; in cattle and boar	Lissencephaly Type 1	None
ILF3	19:10764937-10803093	19p13.2	21.20	1.00	No	-	-
S100B	21:48018875-48025121	21q22.3	6.81	0.04	No	-	-
UBE2L3	22:21903736-21978323	22q11.21	3.65	0.87	Possibly; may interact with sex hormones	-	-
MAPK1	22:22108789-22221970	22q11.21-22q11.22	0.43	1.00	Possibly; interacts with MAP3K1	-	-
GGNBP2	17:34900737-34946278	17q12	10.96	-	Yes; testes development	-	-
PARK2	6:161768452-163148803	6q26	-	0.00	No	Ovarian cancer (somatic), lung cancer, juvenile Parkinson disease (type 2)	None
PRPF4B	6:4021501-4065217	6p25.2	3.37	1.00	No	-	-
PAPPA	9:118916083-119164601	9q33.1	6.99	0.16	Yes; ovarian function and fertility in mice	-	-
NECAB1	8:91803778-91971636	8q21.3	7.98	0.03	No	-	-

Table 3. *Cont.*

Candidate Gene	Location	Locus	%HI	pLI	Associated with Reproductive System	OMIM Phenotype	Atypical Female Genitalia Assoc. with OMIM
ASTN2	9:119187504-120177348	9q33.1	2.18	0.72	No	-	-
CDIPT	16:29869678-29875057	16p11.2	9.02	0.13	No	-	-
MAPK3	16:30125426-30134827	16p11.2	1.14	0.04	Yes; hyperactivity associated with impaired fertility	-	-
TAOK2	16:29984962-30003582	16p11.2	27.93	1.00	No	-	-
MAZ	16:29817427-29823649	16p11.2	44.56	0.93	Yes; TF necessary for GU system development	-	-
MED15	22:20850200-20941919	22q11.21	24.40	1.00	No	-	-
SCARF2 [1]	22:20778874-20792146	22q11.21	70.16	-	Yes; on individual academic center's DSD panel	Van den Ende-Gupta syndrome	One case of ambiguous genitalia [27]
ATP7A	X:77166194-77305892	Xq21.1	30.06	1.00	No	Spinal muscular atrophy (distal, X-linked), Occipital horn syndrome, Menkes disease	None
PGK1	X:77320685-77384793	Xq21.1	2.45	0.77	No	Phosphoglycerate kinase 1 deficiency	None

[1] Although SCARF2 had low likelihood of haploinsufficiency and did not have a pLI listed, we opted to include it in our review as to not miss a potentially significant gene.

3.4. Gene-Desert SROs

Four SROs did not contain any genes, including one that was considered for candidate genes (SRO087). They ranged from 29.28 kb to 684.32 kb. 75% of these SROs were extrapolated and consisted of both deletions and duplications. The most common DSD phenotype seen in these patients were abnormalities of the external genitalia (50%).

3.5. Comorbidities

The majority of our DSD patient population exhibited additional phenotypes unrelated to the reproductive system. Intellectual disability (47%), short stature (35%), micrognathia (27%), microcephaly (24%), and low-set ears (23%) were frequently encountered (Supplementary Table S2); however, certain female reproductive organs presented with unique comorbidities. Not infrequently, abnormality of the pinna, hypertelorism, atrial septal defect, short neck, and premature birth were seen with abnormalities of the uterus, while anal atresia and hypertelorism were common in patients with abnormalities of the vagina. Patients with abnormalities of the ovaries tended to be small for gestational age and have hypertelorism, while patients with abnormalities of the breasts and nipples tended to have muscular hypotonia. Comorbidities in patients with labial abnormalities and clitoral abnormalities aligned with those of the overall patient population, and there was only one patient with an abnormality of the fallopian tubes, so we could not determine the significance of the present comorbidities.

4. Discussion

Human sexual development requires a complicated synchronization of many biological elements that affect female reproductive structures and endocrine function. Dysregulation of a single gene or gene networks can lead to the atypical development and function of the reproductive system. Historically, clinicians relied on visible phenotypes to diagnose patients with 46, XX DSD. As advanced genomic technologies evolved, diagnoses of various forms of DSD have accelerated, resulting in a wealth of CNV data. Such data are used for further investigation of candidate genes and regions as well as downstream functional studies to elucidate clinical relevance.

4.1. DECIPHER

The database proved to be instrumental in the creation of our SRO map and our search for novel candidate genes. DECIPHER is unique in that persons and institutions can freely access its data and submit genetic information that is available for proceeding use. This quality makes it invaluable not only for research of DSD but also for research of all genetic variations. Additionally, DECIPHER's inclusion of sex chromosome information that is more expansive than "male" and "female" takes away one of the obstacles faced by DSD researchers while also being affirmative to patient sex identity [28].

4.2. Delineation of SROs

It has already been shown that small CNVs are potentially involved in the development of DSD [29–31]. Therefore, we focused on SROs less than 500 kb in size because they are often overlooked when assessing the genome for candidate genes. Although many SROs were large in size (>500 kb) and therefore outside our range of focus, these data can be used in future investigations. With further analysis, these larger CNVs can be partitioned into smaller regions that eventually pinpoint the etiology of various AFG.

4.3. Candidate Genes and Regions

Clinical medicine has often excluded small CNVs in the genomic analysis of DSD. Over the past decade, the identification of small deletions and duplications has improved diagnostics given that approximately 30% of all DSD patients cannot be classified as sex chromosome, 46, XY, or 46, XX DSD [32]. Furthermore, 19–21% of 46, XX individuals receive a molecular diagnosis [33,34]. Previous studies have shown that small CNV analysis can

reveal genes/regions that are relevant in sex development [29,30]. For instance, a 5.2 kb region upstream of *SOX9* was found to contain regulatory elements for sex development in both 46, XX and 46, XY individuals [31]. Our study further demonstrates the promise of small (<500 kb) CNVs when performing genetic testing for patients with atypical female genitalia. We would argue that size does not matter, but gene content does.

We identified 36 small SROs (<500 kb) that contained 22 candidate genes with %HI < 10% and pLI scores > 0.90 (Table 3). The majority of these genes did not overlap with genes or regions that were previously documented in cases of 46, XX DSD. Identification of these regions further endorses the importance of deletions and duplications in sexual development, and given the use of HI and pLI as predictive tools, our data can steer scientists towards relevant genes for functional studies. Some SROs (SRO102, SRO115, SROs135-141) overlapped with CNVs that have been identified as potentially pathogenic [25,26,35]; however, studies don't often address these novel genes, and the candidates have yet to be associated with DSD phenotypes in OMIM. For example, *GGNBP2* is located within the region 17q12, which is commonly associated with MRKH types I and II [36–38], but remains to be further explored as a candidate gene despite its role in testes morphology and spermatogenesis [39]. Other dosage-sensitive genes housed within common CNVs have also yet to be thoroughly investigated. *MAZ*, located in region 16p11.2, is a dosage-sensitive transcription factor that is responsible, in part, for genitourinary development [40]. While one CNV study briefly mentions *MAZ* in its methodology [37], it exists in the periphery of the study's focus, and the literature is otherwise devoid of information on *MAZ*'s role in GU development.

Within regions 22q11.21 and 16p11.2 exist two genes, *MAPK1* and *MAPK3,* respectively, that have the potential to become more explicitly associated with DSD. *MAPK1* and *MAPK3* function as kinases downstream of *MAP3K1*, which has an established role in DSD. Increased phosphorylation of *MAPK1* and *MAPK3* due to gain-of-function mutations in *MAP3K1* leads to upregulation of *FOXL2* and *FST* [41,42], additional genes with roles in ovary formation [43–45]. It is also known that *MAPK1/3* plays an important role in luteinizing hormone signal transduction during ovulation [46]. The dosage-sensitivity of *MAPK1* and *MAPK3* further supports evidence that CNVs in these regions lead to the development of ambiguous genitalia.

SCARF2 presents an interesting case: Despite its presence on an individual academic center's DSD panel [47], there is no evidence explicitly stating the gene's role in development of AFG. However, like *GGNBP2*, it is located in a region (22q11.21) that commonly exhibits CNVs that are associated with ambiguous genitalia and MRKH types I and II [26,35]. Currently, it is known that mutations in *SCARF2* cause the rare, autosomal recessive disorder Van Den Ende-Gupta syndrome (VDEGS; OMIM: 600920) which is mainly characterized by skeletal and craniofacial phenotypes [48]; however, a single case of VDEGS reportedly exhibited ambiguous genitalia [27]. Interestingly, VDEGS maps to distal 22q11.2, which contains the critical region responsible for DiGeorge syndrome (OMIM: 188400). Similar to VDEGS, patients with DiGeorge syndrome have distinct abnormal facies but with added cardiac defects, thymic hypoplasia, and hypocalcemia [49]. Less frequently, 46, XX patients may exhibit genitourinary anomalies such as absent uterus or uterine didelphys [50,51]. While DiGeorge syndrome's distinguishing phenotypes can be attributed to a haploinsufficiency of TBX1 [49], the genetic etiology of the genitourinary phenotypes is not well understood. Further investigation into the function of *SCARF2* may resolve the association between CNV 22q11.2 and abnormalities of female genitalia while also shedding light as to why 46, XX patients with VDEGS and DiGeorge syndrome exhibit genital anomalies.

Outside of identified but unexplored CNVs, there still exist genes that are prime for inquiry. PAPPA is an insulin-like growth factor binding protein (IGFBP) protease that increases bioavailable insulin growth factor which, in turn, promotes the development of a dominant ovarian follicle [52,53]. Predictably, when *PAPPA* is knocked out, follicular development and ovarian function in female murine models is disrupted, resulting in decreased fertility [54] and suggesting that the gene plays a significant role in the ovary's

functional integrity. Nonetheless, to our knowledge, *PAPPA* is not yet under consideration as a potential candidate gene for 46, XX DSD.

SENP3 and *EIF4A1* are jointly transcribed and eventually spliced into their respective, individual transcripts. Despite their different functions at the molecular level, they both seem to play a unique role in fertility. During meiosis, SENP3, a deSUMOylation protease, is required for the G2-M transition. Downregulation of the gene disrupts spindle assembly and germinal vesicle breakdown, which prevents the first polar body extrusion and, therefore, proper oocyte maturation [55]. Unlike *SENP3*, it is suggested that *EIF4A1* plays a critical role post-fertilization. As a translation initiation factor, *EIF4A1* produces proteins that are necessary for early embryonic cell division. Sans *EIF4A1*, there is inadequate growth of the blastocyst, leading to implantation failure and decreased fertility [56]. Additionally, it should be noted that point mutations in the *EIF4B* family transcription factor family can result in gonadal dysgenesis and amenorrhea in 46, XX individuals [57]. *SENP3* and *EIF4A1* flock the point of fertilization and seem to play significant roles in oocyte integrity. The potential of these genes leads us to suggest further investigation into their effects on female reproductive system development.

4.4. Gene-Desert SROs

SROs lacking expressed genes should also not be ignored, for they may contain DNA sequences that are necessary for the regulation of genes involved in the development of the female reproductive tract. In 2011, Benko et al. identified a 78 kb non-coding regulatory region upstream of *SOX9*. A few years later, this sex-determining region was whittled down to approximately 5.2 kb [31,58]. Similarly, rearrangements in the regulatory region of *SOX3* have been found to be associated with sex reversal in 46, XX males [59]. Despite the landscape being devoid of genes, non-coding regions of the genome hold unfound potential in understanding human sex development.

4.5. Comorbidities

Although anomalies affecting every organ system were revealed, the most common comorbidities were intellectual disability, short stature, and micrognathia. Interestingly, we found that intellectual disability and other developmental delays occurred notably less frequently in other studies, and when separated by sex, 46, XX patients were found to have no incidences of short stature [11,17]. The prevalence of these comorbidities in our patient population is important to note, given that DSD diagnosis has historically relied on clinical presentation. Turner syndrome is often the first diagnosis considered in female-presenting DSD patients when short stature, intellectual disability, and cardiac anomalies are present; karyotypes are typically used to diagnose this condition [60,61]. Turner patients also tend to exhibit smaller mandible size, along with retrognathia and mandibular posterior rotation [62–64]. Additionally, most individuals with alpha-thalassemia X-linked intellectual disability (ATRX) syndrome have comorbid undifferentiated streak gonads [18]. Furthermore, MRKH phenotypes can occur in Silver-Russel syndrome, which is characterized by abnormalities of the skeletal system such as short stature and micrognathia with narrow chin [65–67]. Given the concurrence of these phenotypes, clinicians should perhaps approach DSD with a wider scope and consider other whole genome technologies, such as CMA, when assessing patients and performing targeted analysis of DSD genes and regions.

Although our study focused on patients with atypical female genitalia who were presumably 46, XX, a small proportion (7.67%) of patients were 46, XY. These patients displayed a wide range of DSD phenotypes, but most common were abnormalities of the ovary, uterus, and labia. Five of these patients had what would be considered male phenotypes such as cryptorchidism, hypospadias, and male DSD ("pseudohermaphroditism"). The remaining 18 patients only exhibited female DSD phenotypes. Interestingly, despite the fair number of patients with 46, XY phenotype, we found only one SRO on chromosome Y, and it contained no genes with high likelihood of haploinsufficiency or loss-of-function intolerance. Regardless of the presence of candidate genes, it is nonetheless necessary to

follow-up with these patients. 46, XY females with gonadal dysgenesis are at higher risk of developing germ cell tumors (e.g., gonadoblastoma, dysgerminoma, Sertoli cell tumor, etc.) [68,69]. Endocrine, gastrointestinal, and congenital comorbidities are also frequent, and these patients should be assessed and counseled accordingly [70].

4.6. Limitations and Recommendations

The generation of our SRO map depended on the properties of the CNVs reported in the DECIPHER database. Despite our expectations, many of the deletions and/or duplications found in patients were large, which in turn led to massive SROs outside the scope of this study. There are a few likely reasons for the skewed presentation of size; the current standard for CMA tends to disregard smaller CNVs potentially leading to underreporting. Additionally, DECIPHER collects data from institutions internationally which utilize different array technologies and techniques when collecting patients' genetic information. It is for these reasons that we present our SRO map not as a complete and conclusive coordinate system but as a guide towards regions of the genome that are promising for future study. Furthermore, we would argue that these larger regions often contain an overabundance of data that is difficult to sieve through. Larger CNVs must be resolved at finer detail, and we suggest that subsequent studies focus on smaller regions in order to pinpoint significant candidate genes.

Not infrequently, DECIPHER was lacking in data regarding the haploinsufficiency and loss of function effects of genes. Therefore, it is possible that potential candidate genes were missed given our method of selection. Regardless, our study provides adequate fodder for inquiry, allowing scientists to utilize our data to predict gene HI and pLI in future functional studies.

When combing the literature for previously established associations with atypical female genitalia, it was impossible to perform a complete and total search on each candidate gene. There is a slight possibility that we overlooked a study that tied a candidate gene to DSD, especially if the study indicated a CNV region but didn't address the genes within. However, given our search protocol, we believe we adequately assessed the literature for existing evidence.

It is still necessary to discuss an apparent disconnect between studies on specific genes and those on CNVs. We found quite a few studies that investigated large CNVs that are associated with the manifestation of MRKH [36–38], yet there existed no follow-up studies probing potential candidate genes found in those regions. These CNV regions proved valuable in better understanding the etiology of the disorder; nonetheless, it is important to resolve the genes in said regions as to understand why certain CNVs lead to pathological states.

Our final recommendation regards neither genes nor CNVs. Instead, it addresses DECIPHER's classification system. While analyzing the database, we came across DSD phenotypes that included the term "hermaphroditism". Only a few patients were cataloged using this terminology; nonetheless, using "hermaphroditism" to describe patients is both unhelpful and pejorative. Many intersex patients consider the term outdated and stigmatizing [14], and clinically, "hermaphroditism" is problematic given that it focuses on gonadal anatomy and does not consider other aspects of atypical genital development nor gender identity [9,71,72]. Classifications centered on "hermaphroditism" should be removed from DECIPHER, and instead, clinicians should record phenotypes that are specific to organ morphology and dysfunction [28]. This follows for other classifications such as general "abnormality of the female genitalia" and "ambiguous genitalia, female". Since these classifications provide no insight into the patient's biology or lived experience, we recommend a more affirmative term such as "atypical" instead of "abnormal". Understandably, it may be impossible to dispose of these classifications altogether, depending on the extent of data submitted; nevertheless, physicians should be encouraged to enter more detailed phenotypes when adding patient information into DECIPHER.

5. Conclusions

This study successfully identified small regions of overlap in patients with atypical development of female genitalia. Our findings suggest that CMA can be used to identify smaller, clinically significant CNVs which have historically been disregarded. The genes within these regions have an untapped potential which, upon further investigation, may provide a better understanding of the genetic etiology of the development of atypical female genitalia. We are hopeful that this study will inspire exploration of these candidate genes and lead to better diagnosis and management of individuals with AFG.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/reprodmed3020014/s1, Figure S1: Examples of Clusters, Table S1: DSD Characteristics of Patients with AFG, Table S2: Common Comorbidities in Patients with AFG.

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