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Prenatal ultrasound finding of atypical genitalia: Counseling, genetic testing and outcomes

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Abstract

Objective: To report uptake of genetic counseling (GC) and prenatal genetic testing after the finding of atypical genitalia on prenatal ultrasound (US) and the clinical and genetic findings of these pregnancies.

Methods: A retrospective cohort study (2017–2019) of atypical fetal genitalia in a large expert center for disorders/differences of sex development. We describe counseling aspects, invasive prenatal testing, genetic and clinical outcome of fetuses apparently without [group 1, $n = 22$ (38%)] or with [group 2, $n = 36$ (62%)] additional anomalies on US.

Results: In group 1, 86% of parents opted for GC versus 72% in group 2, and respectively 58% and 15% of these parents refrained from invasive testing. Atypical genitalia were postnatally confirmed in 91% (group 1) and 64% (group 2), indicating a high rate of false positive US diagnosis of ambiguous genitalia. Four genetic diagnoses were established in group 1 (18%) and 10 in group 2 (28%). The total genetic diagnostic yield was 24%. No terminations of pregnancy occurred in group 1.

Conclusions: For optimal care, referral for an expert fetal US scan, GC and invasive diagnostics including broad testing should be offered after prenatal detection of isolated atypical genitalia.

Key points

What's already known about this topic?

- Prenatal genetic counseling (GC) and testing has a high diagnostic yield in multiple congenital anomalies and enables expecting parents to make well-informed choices about their pregnancy.

What does this study add?

- We present pre- and postnatal data on fetuses with atypical genitalia with and without concurrent anomalies on ultrasound (US). Our study emphasizes why isolated atypical genitalia on US should prompt referral to an expert prenatal (disorders or differences of sex development) center for offering extensive prenatal GC and invasive prenatal testing.

1 | INTRODUCTION

Guidelines for disorders or differences of sex development (DSD), defined as congenital conditions with atypical chromosomal, gonadal, and/or anatomical sex development,[1] do not include information on prenatal detection of atypical genitalia, although atypical genitalia are frequently detected or suspected in prenatal ultrasound (US) screening.[2–5] For genital anomalies, the frequency of additional anomalies is largely unknown. It was estimated to be 30% for males with hypospadias in a study that included fetal growth restriction (FGR) as an additional anomaly[6] and 41% overall in retrospective cohorts of neonatal ambiguous genitalia.[7] These were retrospective cohort studies in DSD-centers; the frequency of additional anomalies in the prenatal setting remains unknown.

The expanding options for prenatal genetic testing for congenital anomalies, including Whole Exome Sequencing (WES) followed by targeted analysis of gene panels, have led to an increase in prenatal genetic diagnoses.[8–17] Offering pretest GC with the option of invasive prenatal testing in case of fetal US anomalies has therefore become even more important. However, offering invasive prenatal testing in pregnancies once isolated atypical genitalia are observed is not common practice in every prenatal center.[3,5] Prenatal specialists, genetic counselors and the laboratory need to be aware of each other's workflow, policies and limitations such as turn-around-time, whether or not variants of unknown clinical significance (VUS) are reported in the prenatal setting, possibilities and limitations of both the US examination and the genetic tests. Then parents can be accurately informed about both the options of genetic testing and their pros, cons and limitations[18,19] while aware of the fact that prenatal US cannot detect all facets of the genital phenotype as is possible in the postnatal situation. A well-documented informed consent is necessary. The variable severity of genetic conditions adds to the complexity for parents to make a well-informed choice about invasive testing in the absence of other congenital anomalies on an expert US exam indicating a possible serious condition.[20,21] However, a genetic diagnosis may help parents in the decision whether or not to opt for a termination of pregnancy (TOP). Moreover, the possibly incomplete prenatal phenotype necessitates a thorough postnatal follow up. This study aims to evaluate the implementation of pretest GC and invasive testing, the frequency and accuracy of detection of additional anomalies at prenatal US and the yield of genetic testing.

2 | MATERIALS AND METHODS

2.1 | Patient identification and selection

We retrospectively included pregnant women with atypical genitalia seen in the fetus on at least one expert US exam referred to our expert center in our university hospital between January 2017 and December 2019. Atypical fetal genitalia were not always the reason for referral. The search terms used were: atypical genitalia; ambiguous genitalia; abnormal genitalia; hypospadias. Group 1 consisted of fetuses with apparently isolated atypical genitalia with or without a soft marker or FGR (defined as fetal growth <10th centile).[22] Group 2 consisted of fetuses with at least one other structural anomaly, with or without FGR. Assignment in group 1 or 2 was based on information at the time of atypical genitalia detection. Gestational age at inclusion varied as this depended on the moment of referral; mostly after routine mid–trimester screening around 20 weeks or after a third trimester US exam for growth assessment.

2.2 | Ultrasound examination

In addition to a complete expert US scan, group 1 fetuses received a dedicated 2D-US and 3D-US for detailed and systematic evaluation of the urogenital tract performed by one of three experienced sonographers. Targeted imaging of the urogenital tract assessed the following structures: phallus (i.e., dimensions, presence of blunted tip of the phallus, curvature, visualization of the corpora and their curvature, if possible the meatus, presence of extra tissue around the phallus); labio-scrotal folds (i.e., fusion and the presence or absence of a raphe) and, if the examination was performed after 28 weeks, the presence and location of the testes. Also the presence or absence of a uterus by measurement of the bladder—rectum distance and by visualization of a mass bulging into the bladder, the anal rectal sphincter complex, bladder, kidneys and the insertion of the umbilical cord were examined. 3D-US was used as a complementary tool to 2D-US when fetal position was favorable and good quality images could be obtained. The three-orthogonal-plane display was used for anatomic structural relations. The genitalia were also visualized in surface rendering display.

The medical records were retrospectively reviewed to assess the presence of other anomalies, whether or not GC had been performed and, if parents had opted for invasive prenatal testing, which genetic tests had been performed and finally, the postnatal clinical and genetic findings.

2.3 | Counseling process

Routine pre-test counseling for invasive testing and chromosomal microarray analysis (CMA) and the possibility to be referred to a clinical geneticist was provided by the expert sonographer or consultant obstetrician. A clinical geneticist provided more extensive pre-test counseling including possible diagnosis, information on the option of WES-based targeted gene panel analysis and informed consent on types of pathogenic variants that are to be reported, from the moment this test became an option in the course of 2018. This counseling is referred to as genetic counseling (GC).

Parents with fetuses likely to survive without major structural anomalies, necessitating extensive care, were expected to be followed-up principally by the DSD team. They received global information on sexual determination and differentiation to help them understand the fetal US findings regarding the atypical genitalia. Furthermore, information was provided on pre- and postnatal clinical management including the possibility that sex assignment after birth will sometimes be delayed until results of diagnostic evaluations are available. Information was also provided on the possibility that the genitalia might appear normal and no further investigation would be necessary.

Psychological counseling was offered to answer any questions parents might have, to help them cope with the uncertainties they faced, and discuss with them if and how they would like to disclose the child's condition to family and friends.

After pre-test counseling performed by an expert sonographer or consultant obstetrician parents could opt for amniocentesis followed by CMA with or without proceeding to GC. All parents were informed about the option of further genetic testing and if parents were interested in this they were referred to a clinical geneticist. WES-based targeted gene panel analysis was offered only after extensive GC, including discussing incidental findings (IF) and written consent from 2018 onwards. Parents expecting a child in group 1 had the option for analysis of a smaller DSD panel, containing genes known to be involved in isolated and syndromic forms of DSD,[23] thus reducing the chance of IF. In pregnancies with multiple congenital anomalies (MCA), a broader multiple congenital anomalies/intellectual disability (MCA/ID) panel was generally offered (see supplemental data S4, S5 and S6 for details).

2.4 | Prenatal genetic testing

In the Netherlands, all pregnant women are offered non-invasive prenatal testing, but the X and Y- chromosomes are not included in this analysis. If parents of our cohorts wished prenatal genetic testing, amniocentesis was performed either at 20–22 weeks, after the mid trimester expert US scan, or occasionally at 32 weeks, when risk of fetal loss is lower if the couple did not have the intention to terminate pregnancy in case of an adverse result but wanted to have genetic testing/results nonetheless. Blood from both parents was obtained. Which prenatal genetic tests were performed depended on the choices of the parents and on the anomalies found. Most frequently CMA[24] was performed, but when indicated also QF-PCR (Quantitative fluorescence polymerase chain reaction) of chromosomes 13, 18, 21, X and Y and/or karyotyping (see supplemental data S1 for details). In addition, WES was performed on DNA extracted from the amniotic samples and parental blood, followed by a trio analysis (MCA/ID panels) or by singleton analysis (small panel, such as DSD). All panels were WES based. Maternal cell contamination of the fetal DNA was excluded (see supplemental data for details).

2.5 | Disclosure of variants

CNVs (copy number variant) found by CMA were classified according to published recommendations[25] and all pathogenic variants were reported. Susceptibility CNVs and likely pathogenic CNV's were first discussed with the multidisciplinary prenatal team and reported when considered relevant.

Classification of the sequence variants found after WES was according to the international standard ACMG - America College of Medical Genetics criteria[26] and only likely pathogenic (class 4) and pathogenic variants (class 5) were reported in the prenatal setting. Variants of unknown clinical significance (VUS, class 3) were not reported by the laboratory and are also not reported in this paper. Besides (likely) pathogenic causative variants, (likely) pathogenic, early onset or treatable IF were reported to parents as indicated in the informed consent. In cases of doubt whether or not to report a variant, including IF, this was discussed in a multidisciplinary team and reported if considered relevant.

2.6 | Postnatal genetic testing

Fluorescent in situ hybridization for X-specific (CEPX/DXZ1, Vysis) and Y specific probes (CEPY/DYZ3, Vysis) in blood or buccal smear were performed to determine the chromosomal sex and to look for sex-chromosomal mosaicism. Karyotype and/or SNP-array were performed when indicated and not performed prenatally as described in the prenatal setting.[27] Likewise, gene panel analysis was performed or extended to a broader panel or WES analysis when indicated in case of additional anomalies. Re-analysis were performed only after additional GC and after obtaining informed consent. VUS in relevant genes were then reported in the postnatal setting; these variants are also reported in the current study. Chromosomal and molecular VUS were not regarded explanatory for the phenotype (for details see Tables S1 and S2).

Endocrine work-up was performed when indicated as proposed in DSD-guidelines[27,28] and low birth weight was defined following the fetal growth calculator.[29]

3 | RESULTS

3.1 | Uptake of prenatal genetic counseling and testing

Eighty-two percent (18/22) of group 1 fetuses and 66% (24/36) of group 2 fetuses were referred before the 24th week of gestation. Median gestational age at the first US examination was 21 + 4 weeks for both groups (range 20 + 2 – 33 + 6 weeks, group 1; 10 + 3 – 35 + 5 weeks, group 2). Figures 1 and 2 show examples of second and third trimester US examinations. Table 1 summarizes the uptake of GC and invasive testing for both groups. All fetuses that had gene panel analysis also had CMA.

3.2 | Pre- and postnatal genetic testing

Figure 3, Table 2 and Tables S1–S3 show details on the outcome of prenatal genetic testing. In group 1 there were only postnatal genetic diagnoses made in four (18%) children (Table 2). These were heterozygous (likely) pathogenic variants in *MAP3K1* and *NR5A1*, genes known to be involved in DSD, but also a homozygous *TSEN54* pathogenic variant unrelated to the atypical genitalia, causing pontocerebellar hypoplasia and a ring chromosome 18. In five (23% of group 1) cases no pre- or postnatal genetic testing was performed because of postnatal normal genitalia (once), buried penis not needing genetic testing (twice) or loss to follow-up in our DSD center (two cases).

In group 2 prenatal either an MCA panel (6), MCA-ID panel (2) or Noonan panel (1) was performed. A prenatal genetic diagnosis, at least partially explaining the fetal phenotype was made in 22% (8/36 fetuses, see for details Figure 1, Table 2, Table S1–S3). Non-CMA diagnoses were Cornelia de Lange syndrome and ARX-related ID/MCA syndrome. Postnatal genetic testing revealed a trisomy 21 and a second diagnosis of Cornelia de Lange syndrome.

In four (11%) cases no pre- or postnatal genetic testing was performed because of postnatal normal genitalia (once), intrauterine fetal demise (IUFD) (once) or loss to follow-up in our DSD center (twice).

The overall yield of pre- and postnatal genetic tests was 24% (14/58): 18% (4/22) in group 1 versus 28% (10/36), in group 2 (Figure 1), after excluding one IF, a child with an XXY genotype and normal genitalia at birth. Figure 3 shows the yield for the different tests.

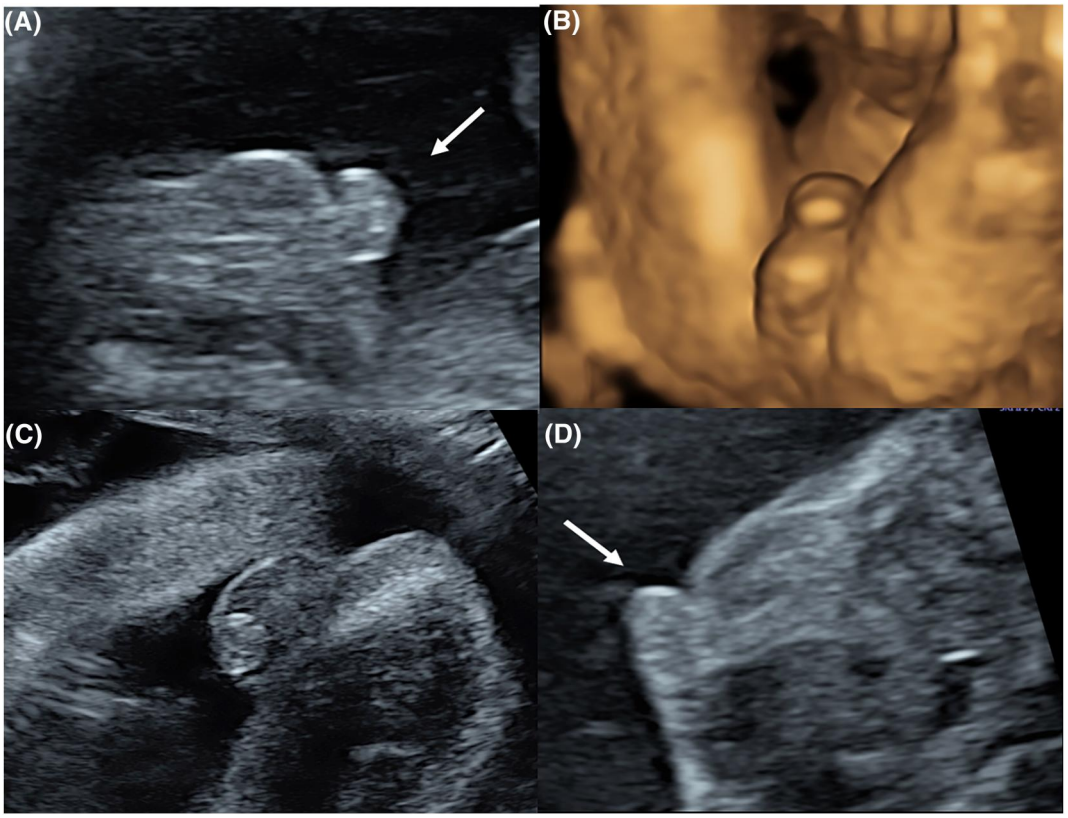

FIGURE 1 2D and 3D US of the genitalia in severe hypospadias in the second trimester. (A) 2D US in case 5 at 21 weeks of gestation demonstrating the ventrally curved and shortened phallus and blunt tip of phallus (arrow) in a nearly sagittal plane. (B) 3D US surface rendered view in case 5 of the curvature of the phallus. (C) 2D US in case 8 at 21 weeks of gestation demonstrating the blunt tip and shortened phallus in the axial plane. (D) 2D US in case 8 demonstrating the ventrally curved and shortened phallus and blunt tip of phallus (arrow) in the sagittal plane. US, ultrasound

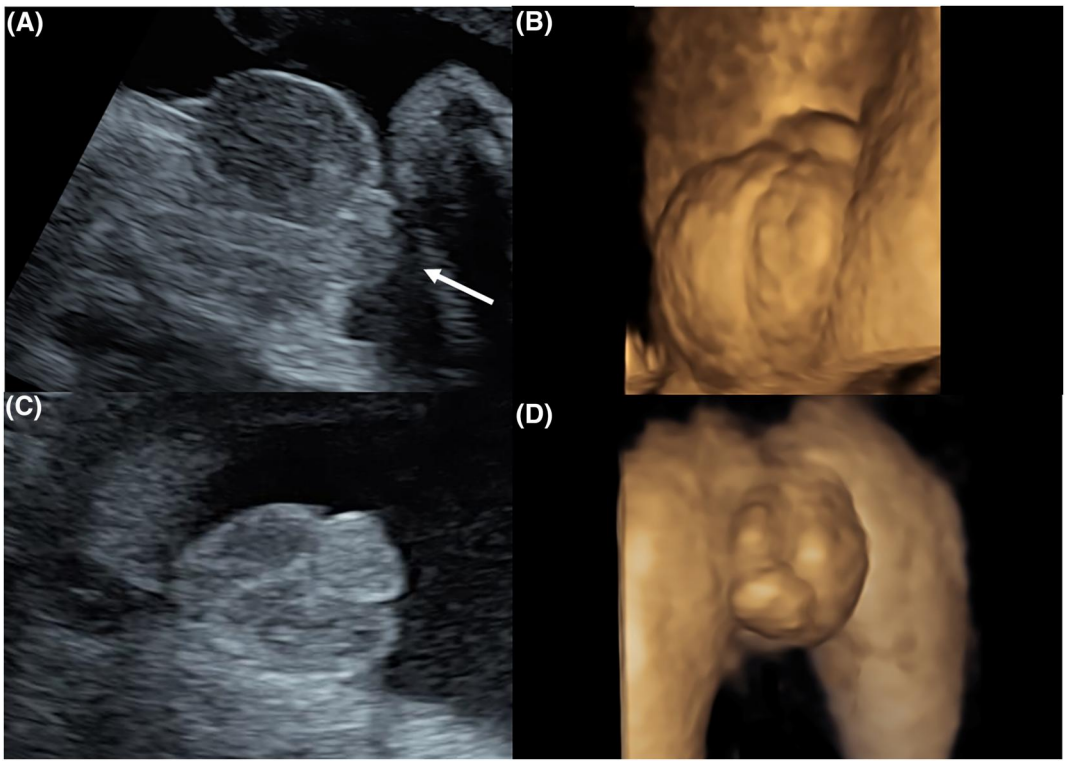

FIGURE 2 2D and 3D US of the genitalia in severe hypospadias in the third trimester. (A) 2D US in case 25 at 31 weeks of gestation demonstrating the ventrally curved and shortened phallus, blunt tip of phallus (arrow) in the sagittal plane. (B) 3D US surface rendered view in case 25 showing the scrotum in distal hypospadias and the ventral curvature of the shortened phallus. (C) 2D US in case 22 at 30 weeks of gestation demonstrating the shortened phallus interposed between the scrotal walls in the axial plane. (D) 3D US surface rendered view in case 22 showing the partial bifid scrotum in penoscrotal hypospadias and the ventral curvature of the shortened phallus. US, ultrasound

TABLE 1 Uptake of prenatal GC and invasive testing in isolated and non-isolated atypical genitalia

Feature	Isolated atypical genitalia (group 1) 22 (38%)	Non-isolated atypical genitalia (group 2) 36 (62%)
Prenatal genetic counseling[a]	19/22 (86%)	26/36 (72%)
Prenatal invasive testing with genetic counseling[a]	8/19 (42%)	22/26 (85%)
Prenatal invasive testing, no genetic counseling[a]	1/22 (5%)	4/36 (11%)
Prenatal genetic counseling[a], no invasive testing	11/19 (58%)	4/26 (15%)
No prenatal genetic counseling[a], no testing	2/22 (9%)	6/36 (17%)
Prenatal priority panel analysis offered[b]	4/8 (50%)	8/22 (36%)
Prenatal panel analysis performed	3/4 (75%)	8/8 (100%)

Abbreviations: GC, genetic counseling; WES, whole exome sequencing.

[a]GC by a geneticist, not the pretest counseling performed by the expert sonographer or consultant obstetrician.

[b]Cases after 2017 only, if parents declined prenatal invasive testing or already decided on TOP, panel analysis was not offered as prenatal priority test. All gene panels were WES-based.

3.3 | Postnatal clinical findings in group 1 and 2

Table 3 summarizes and compares the clinical outcome of groups 1 and 2. There was no TOP in group 1, but one extremely premature discordant twin died within 2 weeks after birth.

Atypical genitalia were confirmed postnatally in 91% (20/22, group 1) versus 64% (23/36, group 2). Overall, atypical genitalia were correctly identified prenatally in 80% (43/54) with known postnatal genital phenotype. Four fetuses (18%) that initially presented with isolated atypical genitalia had additional anomalies, either at a subsequent US (1) or at postnatal examination (3): bladder extrophy (case 2), cleft palate (case 16), proximal implantation of the thumb and heart defect (case 39) and hypoplastic cerebellum (case 51). In group 2 the additional anomalies were not confirmed in four cases

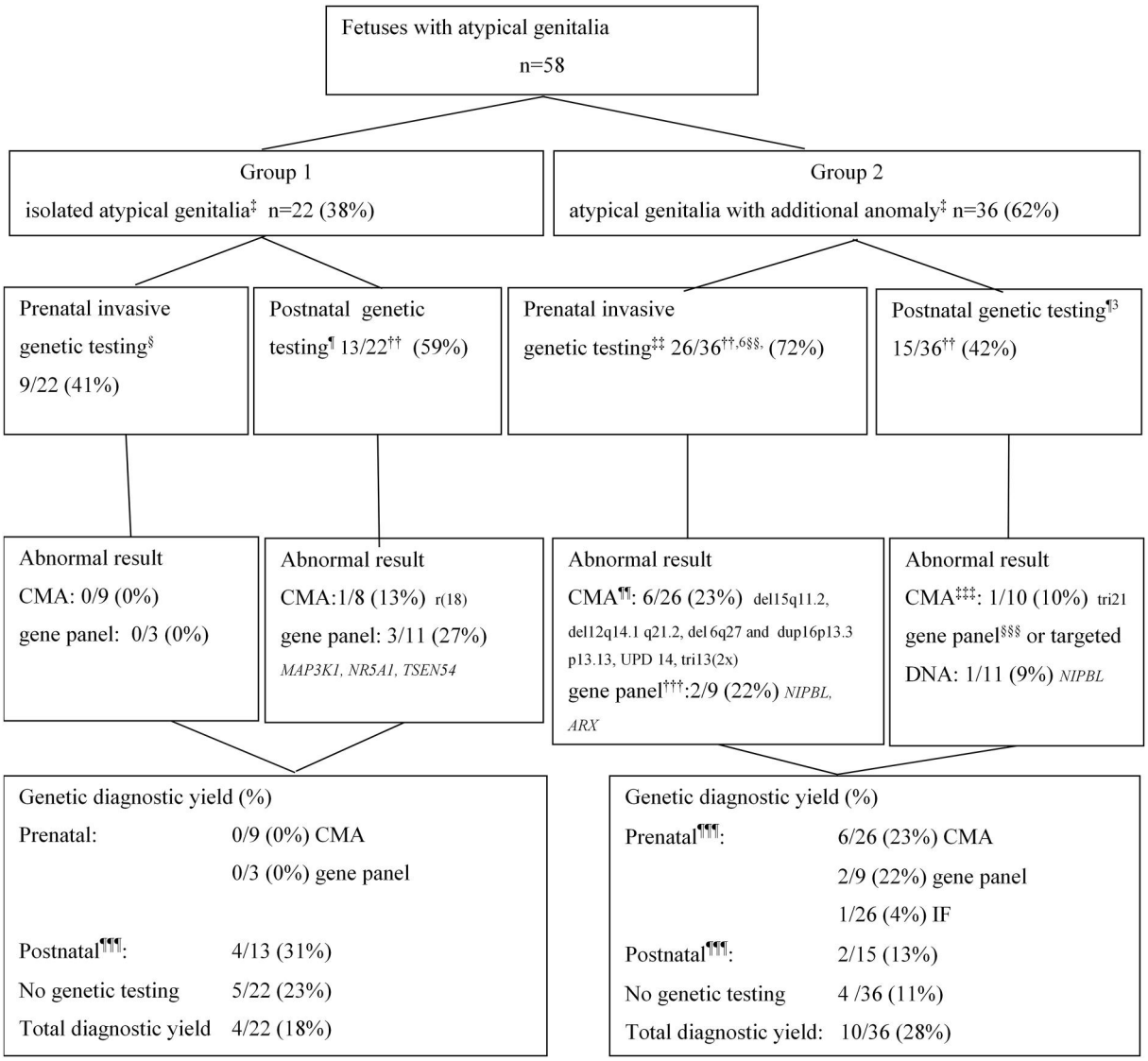

FIGURE 3 Comparison of genetic counseling and results of group 1, isolated atypical genitalia, and group 2, atypical genitalia with additional anomalies†. † for complete overview of who got which test see Table S3. The same individual could have had analysis of a gene panel prenatally and a broader panel or whole exome sequencing postnatally; ‡ situation at referral; § CMA, gene panel optional; ¶ variable: CMA or karyotyping and FISH for identification of the sex chromosomes, gene panel analysis or full exome; †† includes cases with additional genetic testing in probands who also had prenatal genetic testing; ‡‡ CMA, gene panel optional, but if high suspicion chromosomal anomaly also QF-PCR and karyotyping; §§ once a Noonan panel was performed for large nuchal translucency, prior to ultrasound detection of atypical genitalia; ¶¶ in three cases only targeted array or QF-PCR was performed, no CMA (cases 4, 53, 55); ††† in two cases (14, 56) targeted DNA analysis was performed, no gene panel analysis; ‡‡‡Mostly this was FISH for the sex chromosomes and/or karyotyping; §§§ twice there was targeted DNA-analysis (cases 34, 52); ¶¶¶ explaining atypical genitalia and/or additional anomalies. CMA, chromosomal microarray analysis; FISH, fluorescent in situ hybridization; UPD, uniparental disomy

(11%). These were short tubular bones (2×), pyelectasia, aortic coarctation (cases 13, 15, 30, 46).

FGR or low birthweight (<p10) was not classified as an additional anomaly, but was frequently present in both groups. The percentage of twin pregnancies was high, especially in group 1, 23% (5/22). Endocrine testing was performed in 67% (14/21 surviving cases) and was abnormal in two: case 8 with insufficient androgen production and case 22 with partial gonadal dysgenesis. In group 2 this was performed in 7 of 17 surviving infants (41%) and abnormal in two (case 10 and 44).

An overview of all pregnancies, genetic testing and genetic and clinical outcomes is presented in Tables 4 and 5.

4 | DISCUSSION

To our best knowledge this is the largest study of clinical and genetic aspects including broad genetic testing with CMA and gene panel analysis of a prenatal cohort of fetuses with atypical genitalia. It provides the following important findings:

TABLE 2 (Likely) pathogenic cytogenetic and molecular genetic results in isolated atypical genitalia group (group 1)

Nr	Postnatal clinical findings	Pre-and postnatal genetic findings	Class	Interpretation in relation to atypical genital
6	Hypospadias	DSD-panel: Heterozygous -NM_005921.1(MAP3K1):c.152G>C, p.(Gly51Ala) dn PS2 and PM2	4	Likely pathogenic
8	Hypospadias	DSD panel: Heterozygous -NM_004959.4(NR5A1):c.938G>A, p.(Arg313His) dn PS1, PS2, PM1, PM2, PP2	5	Pathogenic
39	Hypospadias in one twin with postnatally additional anomalies	Karyotyping and CMA: 46,XY,r(18).arr[hg19]18p11.32 (11358_701192)x1, 18p11.32p11.21(705519_15165362)x3, 18p22.1q23(65573535_78010620)x1	5	Pathogenic, ring chromosome 18 with a deletion at 18p22.1q23
51	Hypospadias, later with brain and cardiac anomaly	MCA-panel: Homozygous - NM_207346.2 (TSEN54):c.919G>T, p.(Ala307Ser) mat, pat PS4, PM2, PM3, PP1, PP5	5	Pathogenic, pontocerebellar hypoplasia unrelated to hypospadias

Abbreviations: CMA, chromosomal micro array analysis; dn, de novo; DSD, disorders/differences of sex development; mat, maternal; MCA, multiple congenital anomalies; Nr, case number; pat, paternal; PS2, PM2 etc., ACMG criteria[26] used to decide on pathogenicity of a variant.

1. Atypical genitalia are in nearly 2/3 of cases associated with other anomalies (62%).
2. When prenatal apparently isolated atypical genitalia were detected, the majority of parents opted for GC (86%), but not all opted for invasive prenatal genetic testing with the aim to find the genetic cause of the atypical genitalia or related syndromes (58%).
3. Accuracy concerning additional anomalies was high, but in 18% fetuses with apparently isolated atypical genitalia, additional findings were diagnosed postnatally. On the other hand, in 11% additional anomalies in group 2 were not confirmed at birth.
4. There were less false-positive diagnosis of genital anomalies in group 1 (9%) who received targeted US of the urogenital system in addition to an extended US exam than in group 2 (28%) after postnatal follow-up.
5. The diagnostic yield of genetic testing was 24%, in both groups combined, 18%, in group 1% and 28%, in group 2, although not in all instances explaining the atypical genital. The genetic findings that were considered diagnostic have previously been reported in the literature in association with a similar phenotype.[30–39]
6. No parents terminated pregnancy for apparently isolated atypical genitalia.

In our DSD-team experience it is not exceptional that parents of children with apparently isolated atypical genitalia report that during the prenatal period there was uncertainty or confusion about the genitalia on prenatal US. Not all of these parents are referred to a specialized center for expert sonography, GC or invasive testing as is common practice if extra-genital anomalies are diagnosed prenatally. Nevertheless, the high acceptance of GC in this group, suggests that most parents wish to receive information on the possible DSD and discuss diagnostic options. This underlines the need to refer all patients with atypical genitalia for GC and to offer further genetic testing. Because our study was retrospective we were not able to investigate the motives to decline or proceed to pre-test GC or testing. In the isolated group the information on postnatal management of atypical genitalia may have been a motive to opt for testing. Alternatively, parents in this group may have found it more difficult to decide for or against genetic testing and this may have been a motive to come for GC.

On the one hand, the chance that there is an underlying genetic condition even in the absence of signs of a serious condition on expert US exam[20,21,40] may not outweigh any small risk of fetal loss due to a prenatal invasive procedure. On the other hand, there is a risk of an underlying genetic condition even in the absence of signs of a serious condition on expert US exam.[20,21,40]

While the risk of a syndromic disorder in isolated structural fetal anomalies is lower, it is well known that fetal phenotype is incomplete and that isolated anomalies can be part of a genetic syndrome as well.

Our data illustrate this by showing postnatal genetic diagnoses in 19% of the fetuses in the isolated atypical genitalia group (cases 6, 8, 39, 51). This considerable diagnostic yield of genetic testing in group 1 after birth illustrates the potential benefit of GC and offering prenatal invasive testing with CMA and broad panel analysis, regardless of the presence of additional anomalies.

TABLE 3 Number, frequency of pre- and postnatal characteristics in isolated atypical genitalia (group 1) and atypical genitalia with additional anomalies (group 2)

Feature	Isolated atypical genitalia (group 1) 22 (38%)	Non-isolated atypical genitalia (group 2) 36 (62%)	Total 58 (%)
Postnatal genital phenotype			
Atypical	20 (91%)	23 (645%)	43 (74%)
Hypospadias[a]	17 (77%)	15 (42%)	32 (55%)
Buried penis	2 (10%)	1 (3%)	3 (5%)
Micropenis	0	1 (3%)	1 (2%)
Other	1 (5%)	6 (17%)	7 (12%)
Normal	2 (9%)	9/32 (28%)	11 (19%)
Unknown	0	4 (11%)	4 (7%)
Genotype			
XY	17 (77%)	27 (75%)	44 (76%)
XX	0	3 (8%)	3 (5%)
Other	0	1 (3%): XXY	1 (2%)
Unknown	5 (24%)	5 (14%)	10[§§] (17%)
Pre/postnatal MCA discrepancy[b]	4 (18%)	4 (11%)	9 (16%)
TOP/IUFD/ED	0/0/1 (5%)	12/3/4 (53%)	20 (34%)
FGR[c]	8 (36%)	16 (44%)	24 (41%)
Twin pregnancy	5 (23%)	2 (6%)	7 (12%)
Endocrine findings			
Abnormal[d]	2 (9%)	2 (6%)	4 (7%)
Normal[e]	12 (55%)	5 (14%)	17 (29%)
Not tested	8 (36%)	29 (81%)	37 (64%)

Abbreviations: ED, early death; FGR, fetal growth restriction; IUFD, intra uterine fetal demise; MCA, multiple congenital anomalies; TOP, termination of pregnancy.

[a]Once a likely epispadias changed to likely hypospadias on subsequent ultrasound.

[b]Fetuses thought to have isolated atypical genitalia prenatally but were found to have additional anomalies postnatally, or fetuses thought to have MCA prenatally which was not confirmed postnatally; MCA was detected at subsequent ultrasound at 30 w in one.

[c]Prenatal fetal growth restriction FGR <10th centile, or BW < p10.

[d]Not within the range fitting the chromosomal sex and age.

[e]Within the range fitting the chromosomal sex and age.[f]

[f]§§ - Case 55 had NIPT without sexchromosomes, followed by targeted SNP array for chromosome 16 and WES MCA

The extragenital anomalies in 62% of patients were usually the reason for referral and exceed the 30%–41% in previous postnatal studies.[6,7] It emphasizes the importance of thorough US-screening for additional anomalies possibly associated with serious congenital conditions. Some bias cannot be ruled out, as pregnant women are more likely to be referred to a specialized center when atypical genitalia are accompanied by additional anomalies.

We found a significant rate of false positive diagnoses of atypical genitalia. In our study additional targeted US of the genitalia in the isolated group resulted in less false positive diagnoses, namely 9% versus 28% in the group without such targeted US. This high percentage of false positives is in line with other studies.[41] Excluding cases 1, 2, 32, 55, 57 and 58 with a normal genital at a subsequent prenatal US, would lower the false positive rates, but these cases were included in the study groups so we feel they should not be omitted. These false positives cannot be taken lightly and therefore in the pretest counseling a normal genital at birth was always mentioned as a possible outcome. A dedicated US scan for detailed and systematic evaluation of the urogenital tract in addition to the expert US scan is of importance to lower the rate of false positive diagnoses. When the genitalia appeared normal at birth, the pediatrician would do a routine examination and follow up as indicated depending on eventual non-genital anomalies.

Prenatal diagnosis of an atypical genital causes parental distress,[40] which may be a reason to decline GC or testing. Finney et al. reported on negative feelings that may be experienced.[3] Apart

TABLE 4 Group 1—Isolated group: phenotype, diagnostics and outcome[a]

Nr	Year pregnancy	Prenatal US	Prenatal genetic counseling	Results prenatal genetic testing[a]	Postnatal genital phenotype	Endocrine investigations	Relevant[g] postnatal genetic results	Other	FGR
1.	2017	Hypospadias[b] Family[c]	Yes	-	Normal	-	None	None	Unknown
3.	2018	Hypospadias[d], maternal DM	Yes	-	Epispadias, scrotal testes	-	CMA: Normal XY profile Full exome: Normal	Bladder extrophy Non-isolated	No
5.	2018	Hypospadias	No	NIPT only	Proximal hypospadias	-	None	None	No
6.	2017	Atypical genital, ambiguous and asymmetric FGR	Yes	CMA: Normal XY profile	Penoscrotal hypospadias, low inserted penis, bifid scrotum, ventral chordae	Normal	DSD-panel: Heterozygous: *MAP3K1* dn likely pathogenic variant	None	Yes
8.	2017	Hypospadias	Yes	-	Scrotal hypospadias, scrotal testes	Normal sertoli cell markers, in hCG-test insufficient increase of testosterone	Karyotype: 46,XY FISH buccal smear: XY DSD panel: Heterozygous *NR5A1* pathogenic variant,	None	No
12.	2017	Ambiguous genital	No	-	Buried penis	Normal	None	None	No
16.	2017	Hypospadias	Yes	CMA normal XY profile	Scrotal hypospadias, scrotal testes	Normal	FISH buccal smear: XY MCA-panel/full exome: no relevant variants: Methylation analysis and MLPA Silver Russell syndrome: Normal	Cleft palate, motor delay, retrognathia Non-isolated	No
19.	2018	Hypospadias FGR, Twin, concordant	Yes	CMA: Normal XY profile both DSD panel: Child 2: Normal	Penoscrotal hypospadias, scrotal left testes, inguinal hernia at right testes position unclear	Normal	None[b]	None	Yes, both twins

(Continues)

TABLE 4 (Continued)

Nr	Year pregnancy	Prenatal US	Prenatal genetic counseling	Results prenatal genetic testing[a]	Postnatal genital phenotype	Endocrine investigations	Relevant[g] postnatal genetic results	Other	FGR
21.	2018	Atypical genital, possible hypospadias Twin discordant	No	CMA: Normal XY profile, both	Typical male genitalia	-	None	Unknown	Unknown
22.	2018	Hypospadias	Yes	-	Penoscrotal hypospadias, partial bifid, asymmetric scrotum, scrotal testes, at surgery enlarged utriculus with a cervix indication at the tip.	Low AMH, normal inh B, normal androgen synthesis: Partial gonadal dysgenesis	FISH blood [100] XY; Karyotype: 46,XY; DSD panel: Heterozygous: *GATA4*, class 3 variant	Utriculus with cervix	No
23.	2018	Ambiguous genital	Yes	-	Perineal hypospadias, tissue extending caudally and extra opening. Bifid shawl scrotum, scrotal testes, no raphe	Normal	FISH blood [100] XY; Karyotype: 46,XY; DSD panel: Heterozygous:	Possibly slightly anterior anus	No
25.	2018	Epispadias[e]	Yes	NIPT only	Proximal shaft hypospadias	Normal	Karyotype: 46,XY	None	No
31.	2018	Hypospadias discordant twin	Yes	CMA: Normal XY profile both DSD panel: Normal	Hypospadias in one	-	DSD panel heterozygous: *ZFPM2*, class 3 (affected child)	24 + 6 weeks, ED 13/20 days, NEC both	No, neither
33.	2019	Hypospadias FGR	Yes	-	Penoscrotal hypospadias, scrotal testes	-	None	None	Yes
36.	2019	Hypospadias genital	Yes	-	Penoscrotal hypospadias scrotal testes	Normal	FISH buccal smear [100]: XY Karyogram: 46,XY DSD panel: *HSD17B3*:pathogenic variant and VUS;	None	Yes
39.	2019	Hypospadias Twin discordant	Yes	-	Scrotal hypospadias Partial bifid scrotum. Left scrotal testis, right scroto-inguinal testis	Normal	CMA: XY profile, 18p11.32 del, 18p11.32p11.21dup, 18p22.1q23 del karyotype: 46,XY,ring18 FISH blood: Ring 18	Proximal thumb implant, heart defect, feeding problems Non-isolated	No
40.	2019	Hypospadias	Yes	CMA: Normal XY profile	Proximal hypospadias Scrotal testes, underdeveloped corpora cavernosa	-	None	None	No

TABLE 4 (Continued)

Nr	Year pregnancy	Prenatal US	Prenatal genetic counseling	Results prenatal genetic testing[a]	Postnatal genital phenotype	Endocrine investigations	Relevant[g] postnatal genetic results	Other	FGR
42.	2019	Hypospadias, FGR, clomid	Yes	NIPT only	Buried and webbed penis Meatus not visualized, scrotal testes	Normal	None	None	No
47.	2017	Ambiguous genital, choroid plexus cyst	Yes	QF-PCR: Normal CMA: Normal XY profile	Penoscrotal hypospadias, scrotal testes	Normal	FISH buccal smear: XY profile DSD panel: Normal	Choroid plexus cyst	No
49.	2018	Hypospadias, mild in one Twin	Yes	CMA: Normal XY profile	1: Likely hypospadias, meatus not visualized[f], skin deficit ventral shaft, scrotal testes 2: Meatus not visualized[f], scrotal testes	Normal	None	None	No
50.	2019	Hypospadias	Yes	-	Low glandular meatus with dysplasia of ventral penis shaft, short skin and curving, scrotal testes	Normal	CMA: Normal XY profile FISH buccal smear [100]: XY DSD panel: Heterozygous VUS, maternal: *DHH*; *DMRT2*	None	No
51.	2019	Hypospadias Later cardiac + cerebellar anomaly	Yes	CMA: Normal XY profile DSD-panel: Normal	Hypospadias, scrotal testes	-	MCA-panel: homozygous *TSEN54*, pathogenic variant *MAP3K1*: VUS, maternal	Ponto-cerebellar hypoplasia, ASDII Not isolated	Yes

Abbreviations: -, not performed; AMH, anti-Müllerian hormone; ASD II, atrial septum defect type II; CMA, chromosomal microarray analysis; DM, diabetes mellitus; dn, de novo; DSD, disorders/differences of sex development; ED, early death; FGR, fetal growth restriction: FGR <10th centile, or BW < p10; FISH, fluorescent in situ hybridization; hCG-test, human chorionic gonadotrophin-test; inh B, inhibin B; MCA, multiple congenital anomalies; MLPA, multiple ligation-dependant probe amplification; NEC, necrotizing enterocolitis; NIPT, noninvasive prenatal testing; Nr, case number; US, ultrasound; VUS, variant of unknown clinical significance.

[a]For official annotation see Table 2 and Tables S1 and S2.

[b]Proband normal genital at subsequent US.

[c]Family history positive for hypospadias.

[d]Familial epispadias.

[e]Hypospadias at subsequent US.

[f]Due to narrow preputium meatus could not be inspected.

[g]By relevant we mean (likely) pathogenic genetic findings and VUS in genes associated with (part of) the phenotype.

TABLE 5 Group 2 atypical genital with additional anomalies: phenotype, diagnostics and outcome[a]

Nr	Year of pregnancy	Prenatal US findings and information	Prenatal genetic counseling	Relevant results prenatal genetic testing[a]	Postnatal genital	Endocrine investigation	Relevant postnatal genetic results[a]	Other (new and prenatally observed)	FGR
2.	2018	Hypospadias[b], pyelectasia, dilated bladder	Yes	QF-PCR: Normal CMA: Normal XY profile	Normal	-	Craniosynostosis panel	Hydronephrosis, Craniosynostosis	No
4.	2018	Ambiguous genital, holoprosencephaly, AVSD, double urinary system at left, rocker-bottom feet	Yes	QF-PCR/targeted karyotype: Trisomy13, XY	Unknown, after diagnosis not examined again	-	None	ED 33 + 3 w	Unknown
7.	2017	Atypical genital, hypospadias, double inlet LV	Yes	QF-PCR: Normal CMA:15q11.2 del. maternal,XY Noonan panel analysis: Normal	Glandular hypospadias, Scrotal testes	-	None	Cardiac anomaly ED 6 m	Yes
9.	2017	Atypical genital, hypospadias, short femur, cardiac anomaly, dysmorphism	Yes	Late amniocentesis QF-PCR: Normal CMA: Normal XY profile	Glandular hypospadias with important skin deficit ventrally, non-midline raphe, scrotal testes	-	None	ASDII	No
10.	2017	Atypical genital, no uterus, scrotal like structure with raphe and small phallus, anus not well visualized, edema of hands, feet and legs, echogenic intestine	Yes	QF-PCR: Normal CMA: Normal XY profile DNA *CFTR*: Normal MCA panel: No relevant variant	Micropenis, scrotal testes	Low Inh B, normal AMH in hCG test: insufficient testosterone response	FISH: XY DSD panel: Normal	Edema lower limbs	No
11.	2017	Ambiguous genital, corpus callosum agenesis	No	CMA late: normal XY profile	Perineal hypospadias with important ventral curving and partial penoscrotal transposition, large inguinal hernia at right, scrotal testes	Normal	DSD panel: Normal	Corpus callosum agenesis	No
13.	2017	Atypical genital, proximal hypospadias, short tubular bones	No	-	Penoscrotal meatus with dysplasia extending to more proximal of meatus, buried aspect of hypospadias in suprapubic fat, pre-mature non-scrotal testes, spontaneously descending	-	46,XY	Isolated	Yes

TABLE 5 (Continued)

Nr	Year of pregnancy	Prenatal US findings and information	Prenatal genetic counseling	Relevant results prenatal genetic testing[a]	Postnatal genital	Endocrine investigation	Relevant postnatal genetic results[a]	Other (new and prenatally observed)	FGR
14.	2017	Atypical genital, proximal hypospadias, aorta stenosis, right ventricle hypertrophy, SUA, short femur, vermis defect/large fossa posterior cyst	Yes	Late amniocentesis: CMA: Normal XY profile DNA-analysis *SOX9*: No mutation	Penoscrotal meatus with dysplasia extending to more proximal, with partial bifid scrotum, scrotal testes	Normal	FISH buccal smear: XY MCA panel: Normal Full exome: VUS hemizygous *KLHL15*	Coarctatio, aortae pelvic kidney, hydrocephalus, motor delay	No
15.	2017	Atypical genital, buried penis/micropenis SUA, pyelectasia	Yes	CMA: Normal XY profile MCA panel: Normal QF-PCR: Normal	Buried penis, scrotal testes	-	MCA panel no relevant variant	Macrosomia, kidneys: Normal Isolated	No
17.	2018	Hypospadias, bilateral mild ventriculomegaly, cerebellar hypo-plasia, nuchal fold, prefontal skin edema, cardiac defect, absent gallbladder, syndactyly both hands with cleft right hand	Yes	CMA: dn: 6q27 del and 16p13.3p13.13dup 46,XY,der(6)t(6;16)(q27;p13.13) paternal	Unknown	-	Unknown	Unknown TOP	No
18.	2018	Atypical genital, no testes visualized, curved phallus, impression of bifid scotum, nuchal fold, polyhydramnios, omphalocele, twisted axis of the heart, enlarged v.cava and v umbilicalis, discordant twin	Yes	QF-PCR: Fetus 2: Normal CMA: Normal XY profile	Glandular hypospadias, no palpable testes, asymmetric scrotum with skin surplus at right	-	None	Umbilical hernia, anterior anus, tracheaschisis no surgical options. ED 1 day	No
20.	2018	Hypospadias, lemon and banana sign, possible spina bifida, CLP, microcephaly, micrognathia, heart displaced to right, multicystic kidneys, empty bladder, polydactyly left hand	Yes	CMA: arr(13)x3; XY profile	Normal	-	None	Horseshoe kidney with cysts, TGV, CLP, TOP	No

(Continues)

TABLE 5 (Continued)

Nr	Year of pregnancy	Prenatal US findings and information	Prenatal genetic counseling	Relevant results prenatal genetic testing[a]	Postnatal genital	Endocrine investigation	Relevant postnatal genetic results[a]	Other (new and prenatally observed)	FGR
24.	2018	Atypical genital, epispadias-bladder extrophy, spectrum	Yes	CMA normal XY profile	Abnormal, ambiguous	-	None	Anal atresia, OEIS, bladder extrophy, TOP	Yes
26.	2018	Atypical genital, hypospadias, short tubular bones, small thorax	Yes	CMA: Normal XY profile MCA panel: *NIPBL*: dn pathogenic variant	Hypospadias	-	None	Cornelia de Lange syndrome, dysmorphism TOP	Unknown
27.	2018	Atypical genital, not further specified, hydrocele, other not specified	No	NIPT only	Unknown	-	None	None	No
28.	2018	Ambiguous genital, pelvic kidneys, short tubular bones	Yes	QF-PCR: Normal CMA: Normal XX profile	Extra skinfold over clitoris	Normal	FISH buccal smear: Normal	Short stature	Yes
29.	2017/18	Ambiguous genital, constriction rings, clubfeet, maternal alcohol abuse and possible cocaine abuse	No	QF-PCR: Normal CMA: Normal XX profile	Ambiguous, prominent phallus, small dimple no evident meatus. Prominent labioscrotal walls, no posterior fusion, introitus visible, no palpable gonads	-	None	Dysmorphism, clubfeet, constrictions TOP	No
30.	2018	Ambiguous genital, coarctatio aortae	Yes	-	Penoscrotal hypospadias, partial bifid scrotum, scrotal testes	Normal	Karyotype: 46,XY DSD panel: Class 3 variant: *HOXA13*maternal, I	No coarctation, Isolated	No
32.	2018	Ambiguous genital Hydrops, ascites, hydrothorax, contractures	No	-	Normal female	-	none	Edema, contractures, retrognathia, IUFD	Yes

TABLE 5 (Continued)

Nr	Year of pregnancy	Prenatal US findings and information	Prenatal genetic counseling	Relevant results prenatal genetic testing[a]	Postnatal genital	Endocrine investigation	Relevant postnatal genetic results[a]	Other (new and prenatally observed)	FGR
34.	2019	Atypical genital, initially female, later proximal hypospadias, FGR hydrops, clubfeet, complex cardiac defect, anemia; maternal diabetes.	Yes	QF-PCR: Normal CMA: VUS 3p24.3p24.2 deletion dn, XY profile MCA/ID panel: Normal	Ambiguous	-		Full eyebrows, tight nose, lips and eyelids IUFD	Yes
35.	2019	Atypical genital, hypospadias, short tubular bones, maternal diabetes	Yes	-	Distal shaft meatus, dysplasia extending to scrotum, penoscrotal hypospadias, scrotal testes	-	None		Yes
37.	2019	Hypospadias/ambiguous genital, serious FGR, anhydramnios	No	QF-PCR: Normal CMA: Normal XX profile	Ambiguous	-	None	Dysmorphism, cleft palate, low-set, dysplastic ears, IUFD 17+6w	Yes
38.	2019	Atypical genital, suggestive of micropenis, absent gall bladder, hernia diaphragmatica	No	CMA: XY profile	Normal	-	None	Hernia diaphragmatica	Yes
41.	2019	Ambiguous genital, holoprosencephaly, ventriculomegaly, plexus choroideus cyst	Yes	QF-PCR: Normal CMA: Normal XY profile MCA panel: *ARX* pathogenic variant, maternal	Hypospadias, bifid scrotum	-	None	Fam. Stickler syndrome not present TOP	No
43.	2019	Hypospadias, median CLP, heart (VSD and narrow aorta)	Yes	CMA: XY profile;12q14.1q21.2 deletion dn,	Hypospadias	-	None	Complex heart defect, accessory adrenal, CLP, possible poly-microgyria, TOP	Yes
44.	2019	Micropenis with bifid scrotum, AVSD, absent gall bladder, maternal use of psychofarmaca	No	-	Hypospadias with bifid scrotum, scrotal testes	Low basal testosterone and sertolicell-markers, (age 4 months), no hCG test	Karyotype:47,XY,+21	AVSD	No

(Continues)

TABLE 5 (Continued)

Nr	Year of pregnancy	Prenatal US findings and information	Prenatal genetic counseling	Relevant results prenatal genetic testing[a]	Postnatal genital	Endocrine investigation	Relevant postnatal genetic results[a]	Other (new and prenatally observed)	FGR
45.	2019	Buried penis or micropenis: Corpus callosum agenesis,	No	-	Normal	-	None	Corpus callosum agenesis	No
46.	2019	Hypospadias, short tubular bones	Yes	NIPT only	Penoscrotal hypospadias, scrotal testes	Normal	QF-PCR: Normal Karyotype:46,XY DSD panel: Normal	Pre-auricular tag Isolated	Yes
									No
48.	2017	Hypospadias, omphalocele, maternal hypothyreoidism	Yes	QF-PCR: Normal CMA: Normal XY profile	Midshaft hypospadias with torsion and curving, scrotal testes	-	FISH buccal smear: XY DSD panel: Normal	Umbilical hernia	No
52.	2017	Atypical genital, hypospadias, wide metopic suture, frontal edema, micrognathia, syn-/oligodactyly right hand, small stomach, FGR, ICSI pregnancy	Yes	QF-PCR: Normal CMA: Normal XY profile	Abnormal	-	*NIPBL*: Pathogenic variant, dn	Cornelia de Lange syndrome, oligodactyly right hand; syndactyly 2–3, anterior anus, four left lung lobes TOP	Yes
53.	2017	Atypical genital, micropenis or hypospadias, cardiac defect rocker bottom feet, bilateral multiple choroid plexus choroid cysts, nuchal fold, retrognathia, bell-shaped thorax	Yes	14q11.2q32.33 homozygous, UPD14, paternal Karyotype: 46,XY	Unknown	-	None	Ogata Kagami syndrome, no examination TOP	Unknown
54.	2018	Ambiguous genital, only one iliac bone, external genitalia could not be well evaluated, sacral agenesis, fusion of feet, nuchal fold, abdominal cyst, not typical for an enlarged bladder	Yes	CMA: Normal XY profile MCA panel: No relevant variant	Genital in membrane, hypospadias	-	None	Sirenomelia, ear tags, abnormal upper limbs, fused feet, renal and sacral agenesis, hemi-vertebrae, abnormal lung lobulation, atresia of esophagus, TOP	Yes

TABLE 5 (Continued)

Nr	Year of pregnancy	Prenatal US findings and information	Prenatal genetic counseling	Relevant results prenatal genetic testing[a]	Postnatal genital	Endocrine investigation	Relevant postnatal genetic results[a]	Other (new and prenatally observed)	FGR
55.	2018	Atypical genital, unilateral renal agenesis	Yes	NIPT: Indicative for trisomy 16, Targeted SNP-array: 16p13.2q24.2 homozygous, UPD16paternal[a] MCA panel: Panel: Normal,	Normal	-	None	Short long bones, right renal agenesis TOP	Yes
56.	2019	Atypical genital, sacral anomalies, FGR, echogenic intestine	yes	CMA normal XY profile DNA-analysis: *CFTR*: Normal	Normal	-	None	Pre-dysmature, sacral anomalies	Yes
57.	2019	Hypospadias, anencephaly; discordant twin	No	-	Normal	-	CMA: Normal XY profile	Anencephaly, child, ED 1d	Yes
58.	2019	Atypical genital, oligohydramnios, double collecting system, intestinal obstruction, IVF pregnancy	Yes	CMA: XXY[c] pattern DNA-analysis *CFTR*: Normal; MCA/ID panel: Normal	Normal	-	Full exome: No relevant variant	Anal atresia, sacral dimple, flexed digit 2–4 left hand, right clubfoot, renal cysts, echogenic intestine, stomach hypoplastic, normal brain, TOP	No

Abbreviations: -, not performed; AMH, anti-Müllerian hormone; ASD II, atrial septum defect type II; AVSD, atrioventricular septal defect; CLP, cleft lip and palate; CMA, chromosomal microarray analysis; del, deletion; dn, de novo; DSD, disorders/differences of sex development; dup, duplication; ED, early death; FGR, fetal growth restriction: FGR <10th centile, or BW < p10; FISH, fluorescent in situ hybridization; hCG-test, human chorionic gonadotrophin-test; ICSI, intracytoplasmatic sperm injection; inh B, inhibin B; IUFD, intra-uterine fetal demise; IVF, in vitro fertilization; LV, left ventricle; MCA, multiple congenital anomalies; Nr, case number; OEIS, omphalocele-extrophy vesica-imperforate anus-spina bifida; SUA, single umbilical artery; TGV, transposition of the great vessels; TOP, termination of pregnancy; UPD, uniparental disomy; US, ultrasound; VSD, ventricle septum defect; VUS, variant of unknown clinical significance.

[a]For official annotation see Table 2 and Tables S1 and S2.

[b]Child normal genital at 30 weeks US.

[c]Considered an incidental finding.

from the worries that parents may have with any kind of prenatal US diagnosis, in case of atypical genitalia additional distress may be related to psychosocial aspects of DSD such as unease because of the atypical appearance of the genital and body, reduced fertility, impaired sexual functioning and challenges in psychosexual development. Counseling to reinforce parental coping abilities can be helpful to reduce discomfort related to these psychosocial aspects.

All genetic diagnoses in our cohort can nowadays be made prenatally, if parents opt for prenatal invasive testing with both CMA and panel analysis of MCA/ID related genes. CMA is routinely offered in pregnancies with US anomalies and this prenatal panel analysis, is increasingly becoming common practice in several countries,[8–17] although offered for variable indications. De Koning et al.[42] have previously shown how prenatal exome testing may affect parental decision making and may support pre- and perinatal management. Diderich et al.[8] argued that especially in pregnancies with a milder phenotype, priority prenatal testing using this technique may affect decision-making, particularly whether or not to opt for a TOP. It can also provide guidance for obstetric and postnatal management, such as resuscitation, intubation or emergency surgery.[42] In this study in only 36% of pregnancies panel analysis was offered, due to the fact that prenatal priority panel testing was not yet available at the beginning of the study period and sometimes couples were referred during later stages of pregnancy. Although the chance of an IF is generally estimated to be low[43,44] the impact can be very high. The homozygous pathogenic variant in *TSEN54* would have been an IF, if detected prenatally before the brain anomaly was visualized, but it might have been of great importance for the parents. While information on possible genetic syndromes may induce unnecessary worries in parents expecting a child with a possible mild phenotype, not informing on such syndromes may lead to no prenatal genetic testing and parents being confronted postnatally with a child with a serious lifelong condition. Thus, careful counseling on the pros and cons of extensive genetic testing is essential and we recommend this be provided by a clinical geneticist.

The high rate of twin pregnancies in the current study is in agreement with the previously reported association between hypospadias and twin pregnancies.[45] We also confirmed the known association between FGR and hypospadias,[45] with a high incidence of FGR or low birth weight in both groups (see Table 3).

4.1 | Limitations of the study

As mentioned above, our study included only fetuses diagnosed with atypical genital in our center or referred to us and therefore the frequency of additional structural anomalies may be overestimated. The retrospective single-center design may potentially have led to selection bias as well.

It is also important to realize that routine rapid prenatal gene panel analysis was not available in our center before 2018, so in the first study year patients who had already decided on continuation or TOP based on the US findings and those that were referred to the clinical geneticist too late in pregnancy for TOP, were not routinely offered this prenatal WES. Not in all cases of unexplained TOP or IUFD, parents came for additional GC and testing. Therefore, in the studied period, not all couples had the same prenatal options. Prenatal gene panels gradually became more readily available from 2018 to 2019 and the flow was adjusted and optimized. This may have influenced the percentage of prenatal diagnoses and the percentage of couples opting for GC.

VUS are not routinely reported prenatally, because these are not considered actionable and incomplete phenotypic information does not always allow for clinical validation of such findings. Therefore, it is important to realize that repeating the genetic evaluation of the child postnatally, after renewed counseling, should be considered, allowing the laboratory to reanalyze and report eventual VUS in case a particular diagnosis is suspected.

4.1.1 | General limitation

Prenatal evaluation of the genital area is not easy. Normal genitalia were present in 20% (11/54) of cases from whom information on the postnatal genital phenotype was available, in line with the 21% reported in literature.[41] There is also a difference in specificity of a targeted US scan for detailed systematic evaluation of the urogenital tract as offered as extra investigation for fetuses in group 1 and an expert advanced US scan used for all fetuses. This may have contributed to the differences between the two groups.

5 | SUGGESTIONS FOR FUTURE STUDIES AND MANAGEMENT

The impact of prenatal US or genetic findings, including IF, on parents and their motivation to choose or decline GC and testing merit further studies. Although we have not systematically assessed the main concerns of the parents, several parents stated they were concerned not only about health, but also about their child's psychosocial wellbeing. Frequently parents asked about surgical reconstruction options during prenatal counseling. Whether or not changes in the availability of postnatal treatment options, including early surgery, will affect the uptake of prenatal counseling, testing and continuation of the pregnancy, needs to be studied.[46] Studies from other centers or prospective multicentre studies reporting on their policy in case of prenatal detection of atypical genitalia, prenatal phenotypes, systematic clinical and genetic follow-up would be of a great value, make numbers more robust and may possibly indicate reasons for differences in choices. In addition, it will be important to evaluate the diagnostic yield over time, as both US techniques and genetic testing possibilities will continue to evolve which may affect diagnostic yield and the rate of false positives. It has been argued that prenatal diagnostic procedures and management may lead to unnecessary

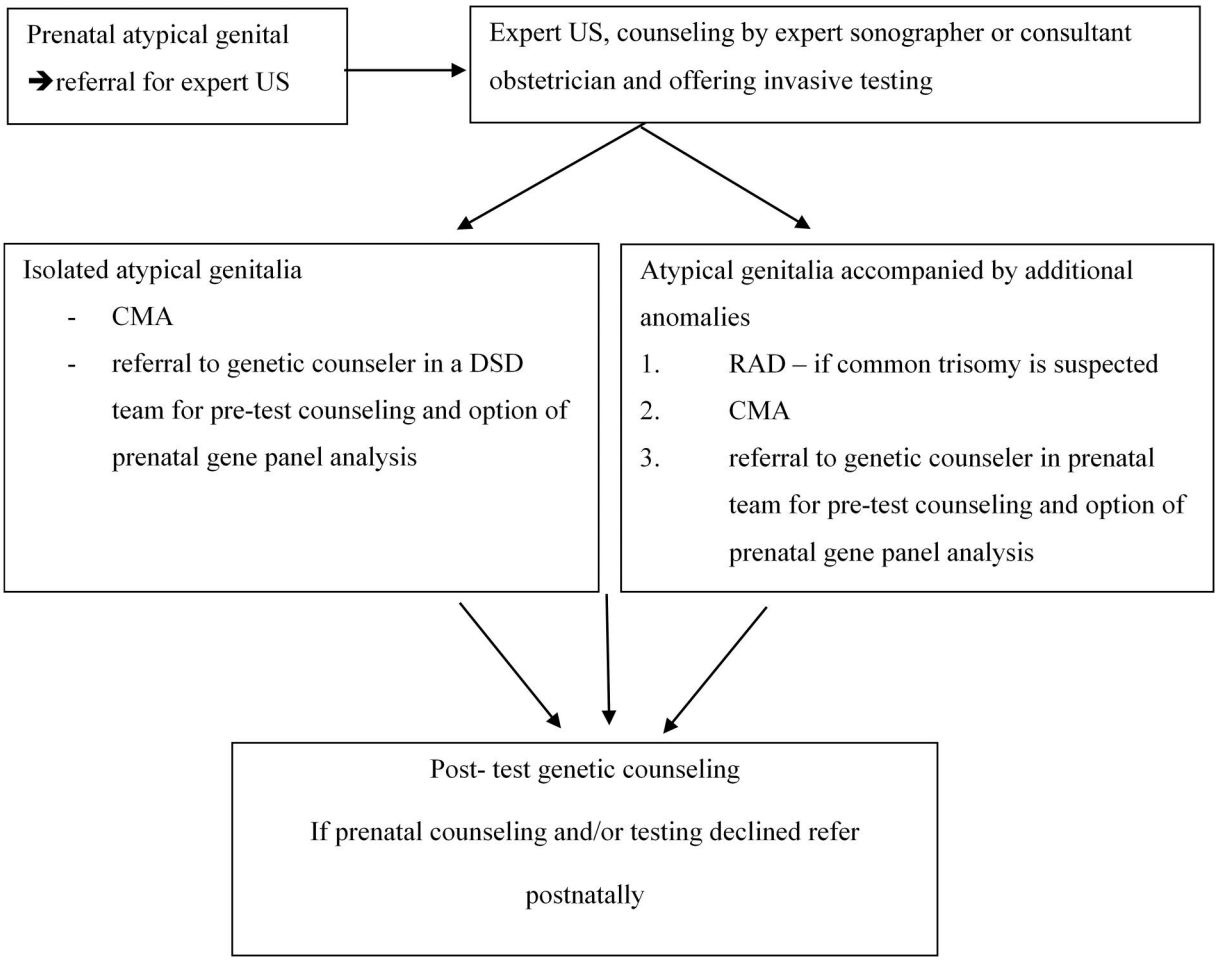

FIGURE 4 Suggested flow chart after detection of prenatal atypical genitalia. The group with isolated atypical genitalia is more likely to indeed have an isolated anomaly and may therefore benefit more from a specific counseling by the DSD team focused on postnatal management of atypical genitalia. CMA, chromosomal microarray analysis; DSD, disorders or differences of sex development; RAD, rapid aneuploidy detection; US, ultrasound

medicalization of DSD[47] or TOP, induced by parental fear of being unable to cope with consequences of raising a child with a congenital condition. We advise careful and extensive GC by a clinical geneticist and offering invasive testing in all prenatal cases (Figure 4). In this study, prenatal diagnostic testing did not lead to termination of any of the pregnancies with isolated fetal genital anomalies.

6 | CONCLUSION

Upon US diagnosis of fetal atypical genitalia, expert US screening and invasive diagnostic testing, including CMA and gene panel analysis, should be offered in the context of counseling by a clinical geneticist, irrespective of the presence of concurrent US anomalies. The aim of such testing is to provide genetic information to the prospective parents -to find or rule out as much as possible an underlying genetic cause with possible serious consequences, which can guide further pregnancy management and parental decision making.

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CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest to this work.

DATA AVAILABILITY STATEMENT

Our institution does not allow for sharing raw or processed DNA data.

ETHICS STATEMENT

For this study no ethical committee needed to be involved as no new test has been performed. We only looked retrospectively at the diagnostic outcomes, which were already communicated to participants and which were anonymized and counted for this study.

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SUPPORTING INFORMATION

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