ORIGINAL ARTICLE: GENETICS

DNA copy number variations are important in the complex genetic architecture of müllerian disorders

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Objective: To clinically and genetically investigate women with müllerian disorders, including Mayer-Rokitanksy-Kuster-Hauser (MRKH) syndrome.
Design: Two-year prospective clinical and laboratory study.
Setting: Not applicable.
Patient(s): Thirty-five women over 16 years of age with a müllerian disorder, including MRKH.
Intervention(s): Women were recruited from specialist gynecology clinics or identified from the Scottish Disorders of Sex Development Register (www.sdsd.scot.nhs.uk/index.html). Associated abnormalities were detected by clinical examination, imaging studies, and biochemical analyses. Chromosomal microduplications and microdeletions were detected by array comparative genomic hybridization (CGH) and validated by fluorescence in situ hydridization.
Main Outcome Measure(s): Identification of associated congenital and biochemical abnormalities and identification of regions of genomic imbalance using array CGH.
Result(s): Associated congenital anomalies were common, present in 25/35 (71%) of affected women, particularly renal and skeletal abnormalities, which were present in 15/35 (43%) and 17/35 (49%) women, respectively. Using array CGH, novel or recurrent regions of genomic imbalance were identified in 4/11 (36%) women with MRKH and in 5/24 (21%) women with other müllerian abnormalities.
Conclusion(s): Additional congenital abnormalities and regions of genomic imbalance are common in women with müllerian disorders, including MRKH. Recurrent microdeletions and microduplications associated with MRKH implicate specific possibly causative genes. The investigation of women with müllerian disorders should be thorough, and array CGH should be considered, given the potential highly significant familial implications of a chromosomal abnormality. (Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.)
Key Words: Müllerian, MRKH, CNV, array CGH

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The identification of abnormalities of the female reproductive tract is important as they are associated with a range of gynecological and obstetric problems such as infertility, recurrent miscarriage, malpresentation, intrauterine growth retardation, and premature delivery (1–4). The overall prevalence of these disorders may be as high as 6%, but they are even more common in certain groups of women, being present in 7% of females with infertility and 18% of those with recurrent miscarriage (5–7).

The classification of müllerian abnormalities is complex. The American Society of Reproductive Medicine (formerly the American Fertility Society) provided a classification based on

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uterine structure that is currently the most widely accepted and used (8). This divides müllerian disorders anatomically into seven different groups: (I) hypoplasia/agenesis, (II) unicornuate, (III) bicornuate, (IV) didelphus, (V) septate, (VI) arcuate, and (VII) diethylstilbestrol (DES) drug related. More recently, an embryological-clinical classification was described, which divides female reproductive tract anomalies into six groups and may be more useful in guiding appropriate treatment (9). These groups are [1] unilateral genitourinary hypoplasia or agenesis, [2] uterine duplicity with blind hemivagina and renal agenesis, [3] isolated or common uterine/uterovaginal anomalies, [4] accessory uterine masses and other gubernaculum dysfunctions, [5] urogenital sinus anomalies, and [6] malformative combinations. Women with partial or complete agenesis of the uterus and vagina, known as Mayer-Rokitanksy-Kuster-Hauser (MRKH) syndrome (OMIM 277000), are divided into two groups: typical (type I) and atypical (type II), the latter being associated with the presence of other congenital anomalies. MRKH occurs in one in 4,500 female births and is frequently not identified until affected women present in their late teens with primary amenorrhea and normal secondary sexual development (10). A third group that overlaps clinically with MRKH II present with müllerian hypoplasia-aplasia, renal abnormality, and cervicothoracic somite dysplasia syndrome (MURCS). The prevalence of anomalies associated with müllerian disorders is not well defined. In women with MRKH II, skeletal malformations including spinal segmentation, hemivertebrae, scoliosis, abnormal ribs, and pelvic and digital anomalies are reported in 10%–85% of cases. Renal abnormalities such as horseshoe kidney, ectopic kidney, duplicated system, and renal agenesis may be present in one-to two-thirds of women (10–15).

The genetics of müllerian disorders is poorly understood. MRKH was originally thought to be sporadic, but familial cases have since been reported (13, 16, 17), which suggests the possibility of autosomal dominant inheritance with incomplete penetrance and variable expressivity (18). Few genes have been directly implicated in the pathogenesis of these conditions. *WNT4* mutations have been identified in some women with a distinct form of MRKH who have clinical and biochemical features of hyperandrogenism (19–21). A similar phenotype is displayed by the *wnt* knockout female murine model that is virilized owing to the production of excess androgen with a depleted number of ovarian follicles (22).

Mutations in *HNF1β* have been detected in approximately one-fifth of those women who possess both müllerian and renal abnormalities (23). In addition to single gene involvement, specific chromosomal abnormalities including 22q11 and 17q12 have been found in patients with MURCS.

Moreover, five studies have used array-based comparative genomic hybridization (array CGH) to identify submicroscopic regions of genomic imbalance in patients with müllerian aplasia (MRKH) (24–28). Recurrent, de novo chromosome alterations at 17q12, 22q11, 16p11.2, and 1q21.1 and sequence analyses have implicated *LHX1* and *TBX6* and have provided further evidence for *HNF1β* as a possible candidate gene for müllerian agenesis. Mutations in *LHX1* have been identified in two females with MRKH (29, 30) and also in a further five patients who were reported to have müllerian aplasia (28). In one of the MRKH cases, the mutation was a frameshift, which would be predicted to cause major disruption to the protein (29). A novel variant in one of the splice sites of *TBX6* has been detected in two patients, although pathogenicity could not be confirmed (28).

While additional congenital and biochemical abnormalities have been found in some women with MRKH, it is uncertain whether these may be present in association with other müllerian disorders. We report on the results of our investigation of women with a range of müllerian anomalies, including MRKH. The phenotypic data show that associated renal, skeletal, and other congenital anomalies are common in our cohort. Moreover, using oligonucleotide array CGH, we identify novel regions of genomic imbalance in women with MRKH and other müllerian disorders, further elucidating the complex genetic architecture of these conditions. These findings indicate that microdeletions and microduplications are frequently found in women with müllerian disorders. Women with MRKH who wish to explore the reproductive option of surrogacy should be counseled about the option of preimplantation genetic diagnosis given the potential risk for the inheritance of such a genomic imbalance by the offspring.

MATERIALS AND METHODS

Ethical approval for the multicenter study was provided by the West of Scotland Research Ethics Committee, and national research and development approval was obtained from National Research Scotland.

Clinical

Women were recruited from one of several specialist gynecology clinics or identified from the Scottish Disorders of Sex Development (DSD) Register (www.sdsd.scot.nhs.uk/index.html). Women over 16 years of age with a müllerian abnormality met the inclusion criteria, and the only exclusion criterion was lack of a capacity to provide informed consent for participation in the study. Data collected included age at diagnosis, growth parameters, the presence of clinical features of hyperandrogenism (acne requiring medical treatment or hirsutism), assessment of learning difficulties based on response to two questions (regarding ability to live independently and attendance at a mainstream school) since most had not had a formal IQ assessment, occupation, and family history of müllerian abnormalities or consanguinity.

Phenotyping of the müllerian abnormality was most commonly done by one of three gynecologists (depending on geographical region) with expertise in this area, the most common modality used being magnetic resonance imaging (MRI).

Women were examined by a clinical geneticist, and any dysmorphic features were recorded. A renal ultrasound scan and anterior-posterior X-ray of the thoracic and lumbar spine were arranged if such imaging had not been carried out previously.

Biochemical

Androstenedione, DHEAS, T, LH, FSH, and E_2 were measured in serum, and urine samples were collected for 24-hour steroid profile analysis. T, LH, FSH, and E_2 were measured by automated immunoassay using Abbott Architect analyzers. Androstenedione was measured by a manual RIA with extraction into hexane/ether (4:1), and DHEAS was measured by the Siemens Immulite analyser. Urine steroid profiles were assayed by gas chromatography mass spectrometry (GC-MS) of hydrolyzed urines derivatized with methoxyamine and silylated, using a Thermo Scientific Trace Ultra Gas Chromatograph and an ITQ 900 mass spectrometer (31) (Supplemental Appendix).

Molecular Cytogenetics

DNA was extracted from peripheral blood samples using standard methods and tested using CytoChip Oligo ISCA 8×60K v2 array (NCBI Build 36 and GRCh37), which targets 498 clinically significant regions and has a working backbone resolution of 170 kb. Regions of imbalance that were considered to be pathogenic were validated using an additional higher resolution array CGH platform: the CytoChip Oligo ISCA 4×180K, which has a working backbone resolution of 60 kb. In almost all cases, the presence of any detected duplications and deletions was also confirmed by fluorescence in situ hybridization. Array CGH data analysis was performed using BlueFuse Multi v2.3 or v2.5 software with CytoChip v2 algorithm. To identify and exclude known polymorphisms, any abnormal findings were compared with data held in the Database of Genomic Variants in Toronto and in the Decipher database at the Sanger Institute, United Kingdom.

RESULTS

Clinical

Thirty-five women with a karyotype of 46,XX were recruited, with a median age of 27.6 years (interquartile range [IQR], 21.3–36.5) and a median age at diagnosis of 19.1 years (IQR, 15.7–27.3). None of these women had consanguineous parents, and none were known to have any concomitant pathology that could bias any results obtained. A family history of müllerian abnormality was present in one case only, where two first cousins were affected, one with MRKH. We were unable to recruit the other cousin, who reportedly had a different müllerian abnormality. All of the women except one attended a mainstream school and were capable of independent living. Four had facial dysmorphism (patients 1, 2, 15, and 20; Table 1).

A third (11/35) were affected by MRKH (seven with MRKH I and four with MRKH II). Two-thirds (24/35) had other müllerian abnormalities including uterus didelphus (n = 9, one with vaginal atresia but normal uterus); bicornuate uterus (n = 7, one with partial vaginal atresia, one with endocervical atresia, and one with duplex cervix); unicornuate uterus (n = 3, two associated with MURCS); partial septate uterus (n = 2, one with transverse vaginal septum and absent lower third of vagina); two with complete septate uterus with complete vaginal septum and duplex cervix; and one with vaginal agenesis.

All 11 women with MRKH and two women with bicornuate uterus presented with primary amenorrhea. Other presenting features were abdominal pain, dyspareunia, hematocolpos, intermenstrual bleeding, weight loss, and infertility, and in three women a müllerian abnormality was identified at a routine health check. The method of diagnosis most commonly used was MRI (15/35), with other patients being diagnosed using a combination of the following: ultrasound, examination under anesthetic, vaginogram, laparoscopy, laparotomy, and computed tomography scan.

Associated structural abnormalities were identified in 71% of the women, with renal tract, skeletal, or other congenital anomalies being present in 25/35, including cloacal anomalies in 3/35 women (Table 1). Ovarian abnormality was identified in only one individual, who had polycystic ovaries in addition to a complete septate uterus, duplex cervix, and complete vaginal septum. In terms of metabolic parameters, the mean body mass index was normal at 24.2 kg/m^2. Acne requiring medication was present in eight women, and hirsutism in only one case; however, there was no biochemical hyperandrogenism, and urinary steroid metabolite profiles were normal. Serum gonadotropins and estrogen were normal, except in one woman, age 39, in whom they may have reflected a premature menopause (LH, 16.2 IU/L; FSH, 20.8 IU/L, E_2, 165 pmol/L) (Table 2).

Genetic

In 35 women with müllerian abnormalities, we identified nine regions of genomic imbalance that were likely to be clinically significant (Table 3). Parental samples were obtained and analyzed in eight out of these nine cases, confirming that four aberrations were de novo, while three were paternally and one was maternally inherited. Clinical abnormalities were not known to be present in the parents from whom these aberrations were inherited. Four regions of genomic imbalance were identified in women with MRKH, including two overlapping microduplications, of 0.257 Mb and 4.6 Mb, at chromosome 1q21. These microdeletions both overlap with the common 1q21 microdeletion syndrome region and also with two previously described aberrations found in other women with MRKH (Fig. 1) (23, 25) In addition, a duplicated region at chromosome 17q12, identified in a woman with MRKH I overlaps with a region previously reported to be deleted in one woman affected by MRKH I and in another affected by MRKH II; see the study of Ledig et al. (27) Of note, in the current study, five regions of possible clinical significance at 1q44 (0.32 Mb), 4q35.2 (1.1 Mb), 13q21 (0.41 Mb), Xp22.3 (0.33 Mb), and 1q21 (0.257 Mb) were found in women with müllerian abnormalities other than MRKH.

DISCUSSION

Recurrent regions of microdeletion and microduplication are identified in 15% of patients with learning disability of unknown cause, and array CGH is now used routinely for the clinical investigation of these conditions (32). The technique has also allowed the identification of critical genes that are involved in congenital malformation syndromes (33).

TABLE 1

Reproductive tract abnormalities.

Patient	Diagnosis	Presentation	Renal abnormality	Skeletal abnormality	Other abnormality	Dysmorphic features	CNV
1	Unicornuate uterus (MURCS) (3.A.2)	Routine	Crossed fused renal ectopia	Klippel-Feil, spinal scoliosis	R deaf, sprengal	Facial asymmetry with facial scoliosis	1q44 del
2	MRKH I (3.C)	PA	N	N	N	N	1q21.1 del
3	MRKH I (1.2)	PA	R agenesis, L kidney cyst, urethral stenosis	Partial sacralization sixth LV, scoliosis, degree of OA	N	N	N
4	Uterus didelphus, bifid upper vagina, double cervix (3.A.3)	Other	N	Short sacrum, spinal dysrhaphism from S1	Imperforate anus, urogenital sinus, rectovaginal septum	N	N
5	Bicornuate uterus, endocervical atresia (3.A.4)	PA + recurrent abdominal pain	N	Mild scoliosis, minor fusion abnormality of L5	N	N	N
6	Bicornuate uterus, partial VA (3.A.4)	Abdominal pain, weight loss	N	N	N	N	N
7	MRKH I (3.C)	PA	N	N	R TMJ dysfunct, R strabismus	N	N
8	Subseptate uterus (2.4)	Abdominal pain	R agenesis	N	Imperforate anus, double IVC	N	N
9	MRKH II (3.C)	PA	R pelvic K, L cross-fused ectopia	Slight scoliosis	R Duane anomaly	N	N
10	MRKH II (3.C)	PA	R hypoplasia	Spina bifida occulta	Congenital dislocation of hip	N	N
11	Complete septate uterus, bicollis, longitudinal vaginal septum, imperforate hymen (3.A.5 and 5)	Recurrent abdominal pain	N	Spina bifida occulta	N	N	N
12	Complete septate uterus, bicollis, complete vaginal septum, polycystic ovaries (3.A.5)	Oligomenorrhea, acne, hirsuitism	NK	NK	N	N	N
13	Uterine didelphus, vaginal and urethral atresia, fistula between upper one-third of vagina and back of bladder (2.1+5)	Distended bladder on antenatal scan	Solitary left kidney, high bladder	Scoliosis of the lumbar spine to the right. There are six lumbar vertebrae.	Imperforate anus, ano-rectal atresia, L strabismus, lumbar naevus	N	N
14	MRKH I (3.C)	PA	N	Sacral linear density	Inverted nipples	N	N
15	MRKH II (MURCS) (1.2)	Other	R dysplastic kidney (nephrectomy)	Abnormal T10 vertebra - butterfly, hypoplasia of first, second R ribs and first rib L; mild scoliosis	Absent R thumb, rib abnormality, R sprengal abnormality	Short stature (height <third c.), hypertelorism coarse facial features	N

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TABLE 1

Continued.

Patient	Diagnosis	Presentation	Renal abnormality	Skeletal abnormality	Other abnormality	Dysmorphic features	CNV
16	Bicornuate uterus (3.A.4)	PA	R kidney scarred	Scoliosis thoracic-lumbar at L1	Imperforate anus, hydrocephalus, deaf, L strabismus	Long face, hypotelorism, prominent nose	N
17	Partial septate uterus, TVS, absent lower third of vagina (3.A.5)	Dyspareunia	N	N	Clinodactyly bilaterally	N	4q35.2 del
18	Uterus didelphus, complete vaginal septum (3.A.3)	Infertility	NK	NK	Imperforate anus, L clinodactyly	Skin syndactyly of hands	N
19	MRKH I (3.C)	PA	N	N	Skin syndactyly, sacral pit, pre-auric tag, R hearing impairment	Facial scoliosis	7q31.2 del
20	Unicornuate uterus (MURCS) (1.2)	Pregnancy	Renal agenesis	N	Asymmetry of face, hand, and breast	N	N
21	Vaginal atresia (3.B.1)	Other	Bilateral hydronephrosis	Absent lower sacrum, abnormal segmentation	Imperforate anus, recto-vaginal fistula, TOF, PA dilatation	N	13q21 del
22	Uterus didelphus, double cervix, single ext os (2.1)	Abdominal pain	R cystic, nonfunctioning kidney (nephrectomy)	NK	N	N	N
23	MRKH I (3.C)	PA	N	N	N	N	15q26.3 dup
24	Bicornuate uterus, single cervix and vagina (3.A.4)	Oligomenorrhea	N	Spina bifida occulta	N	N	N
25	Uterine didelphus, complete vaginal septum, blind ending accessory vagina (2.1)	Loin and groin pain, hematocolpos	Absent R kidney	Thoracic scoliosis to the L	N	N	N
26	Uterine didelphus, complete vaginal septum (2.1)	Foul discharge, R hematocolpos	L agenesis	N	N	N	N
27	MRKH I (3.C)	PA	N	N	N	N	N
28	Uterine didelphus, complete vaginal septum, (2.1)	Abdominal pain, dyspareunia, unilateral R hematocolpos	R renal agenesis	Very mild lumbar scoliosis, concave to R	Proximally placed fourth and fifth toe	N	N
29	Bicornate uterus, single cervix (3.A.4)	Infertility	NK	NK	N	N	N
30	Uterine didelphus, double cervix (3.A.3)	Routine	N	NK	N	N	Xp22.3 dup
31	MRKH II (3.C)	PA	R renal cyst	N	Left third nipple	N	17q12 del

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TABLE 1

Continued.

Patient	Diagnosis	Presentation	Renal abnormality	Skeletal abnormality	Other abnormality	Dysmorphic features	CNV
32	Bicornuate uterus, double cervix, vaginal septum (3.A.4)	Pregnancy, dysmenorrhea	N	Slight angulation at lumbosacral junction	NF1	N	N
33	Uterus didelphus, double cervix and vagina (3.A.3)	Menorrhagia	N	N	N	N	1q21.1 dup
34	Bicornuate uterus (3.A.4)	Pregnancy	N	Minimal scoliosis	N	N	N
35	Transverse vaginal septum (3.B.2)	Oligomenorrhea	N	NK	N	N	N

Note: Description of reproductive tract abnormalities using both the American Society of Reproductive Medicine's classification and below (bold) an embryological-clinical classification as suggested by Acien and Acien (9). The associated renal, skeletal, and other congenital abnormalities, dysmorphic features, and CNV are presented. N = normal; PA = primary amenorrhea; NK = not known; R = right; L = left; del = deletion; dup = duplication; LV = lumbar vertebrae; OA = osteoarthritis; VA = vaginal atresia; TMJ = temporomandibular joint; IVC = inferior vena cava; K = kidney; (c) = centile; TOF = tracheoesophageal fistula.

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Recently, array CGH was used in five studies to investigate women with MRKH (uterine aplasia), identifying five clinically significant regions: 1q21.1, 7p14.3, 16p11.2, 17q12, and 22q11.21-q11.23 and possibly implicating several genes (*LHX1*, *BBS9*, *HNF1β*, and *TBX6*) that may be involved in its pathogenesis (24, 25, 27, 28). In this study, we carried out a clinical and genetic investigation of 35 women with a spectrum of müllerian disorders, including MRKH. Using array CGH we identified regions of microdeletion and microduplication in 26% (9/35) of cases with five regions of copy number variation (CNV) associated with müllerian abnormalities other than MRKH.

In patient 2, who had MRKH type I, a de novo 4.6-Mb microdeletion was identified that overlaps with the 1q21 deletion syndrome region and includes the TAR (thrombocytopenia absent radius) locus (OMIM 27400). The proband had short stature (height <0.4th centile), mild facial dysmorphism, and microcephaly (occipito-frontal circumference <0.4th centile) but no learning difficulty, skeletal, or other congenital anomaly and no known platelet or bleeding disorder. An overlapping, paternally inherited 0.257-Mb microduplication at 1q21.1 was identified in patient 33 (Fig. 1). The proband, who was affected with a duplicated uterus, cervix, and vagina, had no facial dysmorphism, learning difficulty, skeletal abnormality, or known platelet disorder and had a normal head circumference.

The variability of the 1q21.1 microdeletion and microduplication syndromes is well characterized, although these syndromes are statistically significantly correlated with the clinical features of microcephaly and macrocephaly, respectively. Additional features include developmental delay, neuropsychiatric abnormality, facial dysmorphism, and congenital malformations. Incomplete penetrance and variable expression are features of CNV in this region, and parental inheritance is frequently found. In a series of 36 probands with CNV in the 1q21.1 region (21 with microdeletion and 15 with microduplication), none were reported to have müllerian abnormalities (34). However, Cheroki et al. described a patient with MRKH in whom two maternally inherited microduplications, at 1q21.1 and 22q11, were identified (25). The former region included the TAR locus, although that patient (like our patient 2) had no clinical evidence of TAR syndrome and the carrier parent was unaffected. More recently, Ledig et al. identified a smaller, 398.5-kb deletion in the same region in a proband with MRKH type II and TAR syndrome (27). Uterine anomalies have previously been reported, albeit infrequently, with TAR syndrome, which is autosomal recessively inherited and is associated with a common 200-kb microdeletion encompassing at least 11 genes (34–37). Interestingly, in three out of four families the TAR microdeletion is inherited from an unaffected parent, suggesting that the 1q21.1 microdeletion region includes susceptibility loci with incomplete penetrance and variable expressivity for skeletal and müllerian disorders.

The 1q21.1 microduplication (identified in patient 33) is the smallest CNV in this region reported to date in association with müllerian abnormality. The region, in common with the previously reported 1q21.1 aberrations, includes 13 genes,

TABLE 2

Serum reproductive hormone results for women with müllerian abnormalities.

Patient	LH	FSH	E_2	T	SHBG	Free androgen index	DHEAS	Androstenedione
1	NK	NK	NK	NK	NK	NK	NK	NK
2	12.5	5.6	173	2	107	1.9	3.8	8.8
3	16.2	20.8	165	1.3	88	1.5	1.8	4
4	NK	NK	399	2.7	76	3.6	1.9	4.5
5	7.8	5.3	101	2.1	28	7.5	5.4	6.3
6	4.2	6.3	162	1.7	54	3.1	2.8	7.1
7	6	4.8	488	2	83	2.4	4.3	11.8
8	4.3	4.2	130	3	86	3.5	4.6	5.6
9	NK	NK	158	1.4	NK	NK	5.9	5.4
10	5	3.2	606	2.1	138	1.5	7.2	8.6
11	NK	NK	NK	NK	NK	NK	NK	NK
12	10	5.7	189	7.4	47	15.7	8.5	7
13	NK	NK	128	2.8	69	4.1	1.5	7.2
14	2.7	2.9	336	2.3	66	3.5	6.0	7.9
15	10.1	6.8	424	2.7	34	7.9	6.8	7.9
16	5.3	5.1	200	3.0	90	3.3	2.0	6.4
17	<0.5	<0.5	<70	2.1	378	0.6	5.1	5.9
18	NK	NK	98	2.8	24	11.7	2.2	4.9
19	19.3	29.6	345.0	0.9	99	0.9	1.0	2.4
20	9.1	4.8	281	1.2	140	0.9	3.4	3.5
21	3.2	4.1	159	1.7	67	2.5	3.2	4
22	4.9	3.6	151	1.8	58	3.1	6.8	6.2
23	4.3	5.8	107	3.1	24	12.9	8.2	11.5
24	NK	NK	NK	NK	NK	NK	NK	NK
25	NK	NK	NK	NK	NK	NK	NK	NK
26	4.4	6.4	<70	1.6	214	0.7	2.8	4.8
27	3.5	8.0	159.0	1.4	46.0	3.0	6.9	4.6
28	NK	NK	NK	NK	NK	NK	NK	NK
29	11.6	13.6	222	1.4	72	1.9	4.6	3.8
30	6.5	18.6	194	0.9	91	1	3.5	3.2
31	<0.5	2.8	130	2	30	6.7	5.4	3.9
32	5.7	6.7	167	1.7	45	3.8	3.3	7.1
33	<0.5	3	87	2.1	20	10.5	5.4	4.6
34	3	1.8	375	2.2	81	2.7	10.8	5.7
35	0.6	<0.5	6,844	3.6	275	1.3	5.6	7

Note: NK = analysis not possible.

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seven of known function (Fig. 1). Overexpression of one of the genes, *TXNIP* (OMIM 606599), resulted in reduced cardiac hypertrophy in response to mechanical strain, which led Yoshioka et al. to suggest a dual function as an antioxidant and signaling protein (38). It is involved in transcriptional repression, is highly expressed in human cervical cells (GeneCards database: www.genecards.org), and may play a critical role in modifying the early development of the female reproductive tract.

In patient 31 with MRKH I, a 17q12 microdeletion (of 1.9 Mb) was identified that overlapped with two deleted regions previously reported in women with MRKH (24, 27). The region includes two candidate genes for müllerian disorders, *LHX1* and *HNF1β*. The *lhx* knockout murine model has absence of the uterus and oviducts (30). The *HNF1β* gene has also been implicated by the finding of mutations in nine of 108 affected women in the study by Oram and colleagues (39). Moreover, *HNF1β* is a transcription factor with a role in the control of gene expression during development of the murine genitourinary system and pancreas (23). Mutation of *HNF1β* is associated with maturity-onset diabetes of the young type 5 (OMIM 137920), a form of monogenic diabetes characterized by the presence of renal cysts. Sequencing of *HNF1β* in women with MRKH identified no mutation in the gene, but a missense mutation in *LHX1* has been detected in one patient with MRKH (27), as well as in five further patients, reported to have müllerian aplasia (28), and a frameshift mutation has been reported in a patient with MRKH and unilateral renal agenesis (29). Patient 31 had normal stature, mild learning difficulties, an accessory nipple, and a single renal cyst with no known diabetes.

We identified two regions of CNV in patients with MRKH type I that had not been previously reported. The paternally inherited 15q26.3 microduplication of 0.54 Mb found in patient 23 lies within the 15q26 region previously associated with an overgrowth syndrome encompassing six genes, including *MEF2A* (myocyte-specific enhancer factor 2A) (40). This transcription factor is expressed in smooth muscle and has a key role in the development and differentiation of postsynaptic dendritic neurones (41, 42). Mutations in this transcription factor linked the gene to the development of coronary artery disease, but larger studies have not confirmed this (43). The patient had normal growth parameters and no cardiac

FIGURE 1

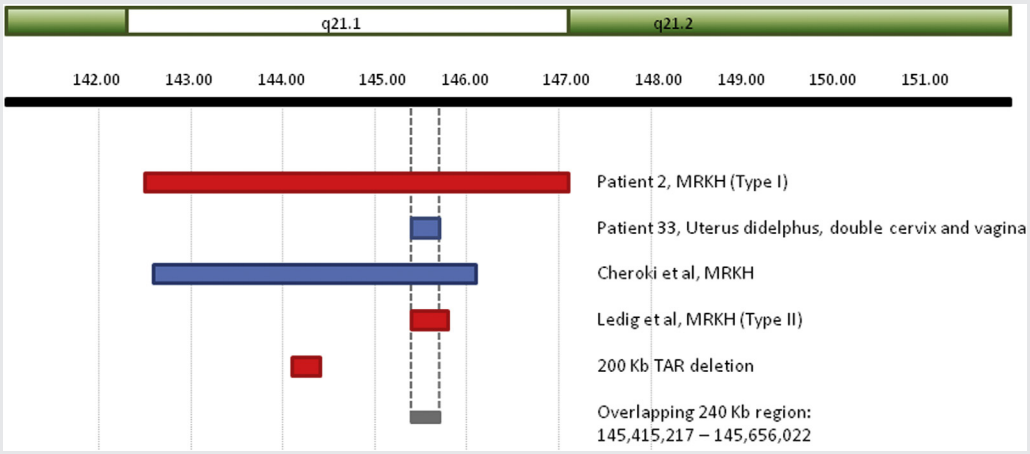

Diagram illustrating region of chromosome 1 at 1q21.1 and 1q21.2 (*green bar, top*) with size of region indicated in megabases (Mb; *black bar, below*). The 4.6-Mb microdeletion at 1q21.1 found in patient 2 (*red bar*) and the 0.257-Mb, 1q21.1 microduplication (*blue bar*) found in patient 33 are shown. The common 0.2-Mb deletion found in TAR syndrome and CNVs previously described by Ledig et al. (27) (0.398 Mb, microdeletion) and Cheroki et al. (25) (2.7 Mb, microduplication), which lie in the same region, are illustrated as *red and blue bars* (*below*). The region of overlap, in common with the two 1q21.1 CNVs found in this study and with those previously described, spanning approximately 240 kb, at nucleotides 145, 415, 217–145, 656, 022, is also indicated.

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problems or associated congenital anomalies. The other novel region, a 7q31.31 (1.76 Mb) microdeletion identified in patient 19, spanned six genes (including OMIM 609772, 610731, 607288). One of these, *CTTNBP2* (OMIM 609772), has previously been suggested to be a possible autism-susceptibility gene (44), but no autistic spectrum traits were detected in patient 19.

Furthermore, five regions of CNV (1q44, 4q35.2, 13q21, Xp22.3, and 1q21.1) were found in patients with müllerian abnormality other than MRKH, two of whom had unicornate uterus in association with MURCS. The de novo 1q44 loss, identified in patient 6, encompasses 12 olfactory receptor genes, although the patient had no reported problems with perception of odor, while the (paternally inherited) 13q21 microdeletion in patient 21 (with vaginal atresia) did not include any known genes and is of uncertain significance. Patient 17, with a partial septate uterus, transverse vaginal septum, and absent lower third vagina, was found to have a maternally inherited 4q35.2 microdeletion of 1.32 Mb encompassing three genes not known to be disease causing or to be expressed in müllerian tissue. Moreover, a de novo 0.33-Mb microduplication in the pseudoautosomal region of the X chromosome (Xp22.3) was found in patient 30. The region contains a single, highly conserved gene,

TABLE 3

Description of CNVs identified using array CGH and the associated müllerian abnormality.

Patient	Diagnosis	Chromosome position	Size (Mb)	Duplication (dup) or deletion (del)	Inheritance
1	Unicornuate uterus (MURCS)	1q44	0.32	Del	De novo
2	MRKH I	1q21.1	4.6	Del	De novo
17	Partial septate uterus, TVS, absent lower third of vagina	4q35.2	1.1	Del	Maternally inherited
19	MRKH I	7q31.2	1.8	Del	NK
21	Vaginal atresia	13q21	0.41	Del	Paternally inherited
23	MRKH I	15q26.3	0.54	Del	Paternally inherited
30	Uterine didelphus, double cervix	Xp22.3	0.33	Dup	De novo[a]
31	MRKH II	17q12	1.9	Del	De novo[a]
33	Uterus didelphus, double cervix and vagina	1q21.1	0.257	Dup	Paternally inherited

Note: The size in megabase (Mb) pairs of the microdeletion/microduplication is provided with the chromosome location, list of gene(s) within the CNV region, and inheritance details. NK = not known; TVS = trans-vaginal sonography.

[a] We were able to demonstrate only that CNV not inherited from one parent.

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DHRSX, which encodes an enzyme of the short-chain dehydrogenase/reductase family that is expressed in renal tissue and may play a role in the development of the genitourinary system.

Congenital anomalies associated with MRKH type II are variably reported and may be present in one-to two-thirds of affected women. It is uncertain how common renal, skeletal, or other congenital abnormalities are in other non-MRKH müllerian disorders. We found that these were present frequently, with renal abnormalities identified in 41.7% (10/24) and spinal abnormalities in 50% (12/24) of our non-MRKH cohort (Table 1). Furthermore, the presence of these associated anomalies did not correlate with the frequency of detection of a microdeletion or microduplication. Indeed, of the three women in whom we identified CNVthat was likely to be pathogenic, only one had both spinal and renal anomalies.

Clinical and biochemical features of hyperandrogenism have been reported in some women with müllerian duct abnormality. In four cases, *WNT4* mutations have been identified and the gonadal phenotypes were very similar to that of the *wnt4* knockout mice (19–21). In mice, Wnt4 plays a key role in müllerian development. Wnt4-deficient mice lack müllerian ducts and demonstrate partial male-female sex reversal (22). The gonads have degenerated follicles and display increased expression of steroidogenic enzymes necessary for T biosynthesis. To our knowledge, no large studies have investigated the presence of biochemical abnormalites in women with müllerian disorders. In this study, we found clinical features of hyperandrogenism in a proportion of women, with acne present in 9/35 (30%) and hirsutism in 3/35 (9%) of cases. However, no biochemical evidence of androgen excess was demonstrated with normal serum hormone levels (androstenedione, DHEAS, T, LH, FSH, E_2) and normal urinary excretion of steroid metabolites as assessed by GC-MS, suggesting that androgen excess is not common in our cohort of women with müllerian disorders.

Recurrent genomic microduplications have been described in other congenital disorders, such as split-hand/-foot malformation (45). As with the 1q21.1 TAR locus, the duplications exhibit variable expressivity and reduced penetrance, with affected and unaffected family members harboring a similar microduplication. Similarly, in this study, four cases of genomic imbalance were found to have been inherited from a clinically unaffected parent. It is likely, therefore, that müllerian disorders represent further examples of congenital malformations in which variable penetrance may be related to multiple genetic and nongenetic etiological factors. The presence of a CNV in one of several chromosomal regions may constitute a susceptibility factor, conferring a higher risk to offspring, and affected women should be counseled appropriately. The reproductive option of surrogacy for women with MRKH does not prevent the potential inheritance of a CNV by the offspring, and, if present, the option of preimplantation genetic diagnosis should be explored.

In addition, a significant proportion of women in this cohort have associated congenital abnormalities, although there was no biochemical evidence of hyperandrogenism. Further study should, in particular, help to determine whether those women who are found to have abnormal array CGH results are those most likely to have associated abnormalities and also whether CNV is less prevalent in those women who have the least severe müllerian abnormalities. Given the range of problems encountered by these women, there is a need for more extensive and detailed further study of the genetic basis of these conditions as well as a need to study long-term clinical outcome. Our findings, together with those recently described, suggest that recurrent microdeletion or microduplication may frequently be associated with a spectrum of müllerian disorders, including MRKH. Given the high rate of CNV in this study, array CGH should be considered in the clinical investigation of women with müllerian disorders, just as is now the current practice in the investigation of learning disabilities.

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SUPPLEMENTAL APPENDIX

List of urinary steroid metabolites assayed by GC-MS.

Metabolite	Unit
24-h androgen metabolites	
Androsterone	μg/24 h
Aetiocholanolone	μg/24 h
DHA	μg/24 h
16-OH-DHA	μg/24 h
Androstenetriol	μg/24 h
24 H cortisol metabolites	
11-oxo-aetiocholanolone	μg/24 h
11B-OH-androsterone	μg/24 h
11B-OH-aetiocholanolone	μg/24 h
Tetrahydrocortisone	μg/24 h
Tetrahydrocortisol	μg/24 h
Allo-tetrahydrocortisol	μg/24 h
A-cortolone	μg/24 h
B-cortolone	μg/24 h
A-cortol	μg/24 h
B-cortol	μg/24 h
24-Hour intermediates	
17-hydroxypregnanolone	μg/24 h
Pregnanediol	μg/24 h
Pregnanetriol	μg/24 h
11-oxo-pregnanetriol	μg/24 h
THS	μg/24 h
24-h mineralocorticoid metabolites	
THDOC	μg/24 h
RHA	μg/24 h
THB	μg/24 h
Allo-THB	μg/24 h
Urine cortisol	
Urine volume	mL
Urine creatinine	mmol/L
Urine cortisol	nmol/L
Urine cortisol/creatinine ratio	μmol/mol creatinine
24-Hour urine cortisol	nmol/24 h

Note: THB = Tetrahydrobiopterin; RHA = RNA helicase A; THDOC = Tetrahydrodeoxycorticosterone.

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