Published in final edited form as:
J Med Genet. 2011 March ; 48(3): 197–204. doi:10.1136/jmg.2010.082412.

High incidence of recurrent copy number variants in patients with isolated and syndromic Müllerian aplasia

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Abstract

Background—Congenital malformations involving the Müllerian ducts are observed in around 5% of infertile women. Complete aplasia of the uterus, cervix, and upper vagina, also termed Müllerian aplasia or Mayer–Rokitansky–Kuster–Hauser (MRKH) syndrome, occurs with an incidence of around 1 in 4500 female births, and occurs in both isolated and syndromic forms. Previous reports have suggested that a proportion of cases, especially syndromic cases, are caused by variation in copy number at different genomic loci.

Methods—In order to obtain an overview of the contribution of copy number variation to both isolated and syndromic forms of Müllerian aplasia, copy number assays were performed in a series of 63 cases, of which 25 were syndromic and 38 isolated.

Results—A high incidence (9/63, 14%) of recurrent copy number variants in this cohort is reported here. These comprised four cases of microdeletion at 16p11.2, an autism susceptibility locus not previously associated with Müllerian aplasia, four cases of microdeletion at 17q12, and one case of a distal 22q11.2 microdeletion. Microdeletions at 16p11.2 and 17q12 were found in

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Competing interests None.

Ethics approval This study was conducted with the approval of the Ethics Review Board of Friedrich-Alexander-University, Erlangen-Nuremberg, Germany.

Provenance and peer review Not commissioned; externally peer reviewed.

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4/38 (10.5%) cases with isolated Müllerian aplasia, and at 16p11.2, 17q12 and 22q11.2 (distal) in 5/25 cases (20%) with syndromic Müllerian aplasia.

Conclusion—The finding of microdeletion at 16p11.2 in 2/38 (5%) of isolated and 2/25 (8%) of syndromic cases suggests a significant contribution of this copy number variant alone to the pathogenesis of Müllerian aplasia. Overall, the high incidence of recurrent copy number variants in all forms of Müllerian aplasia has implications for the understanding of the aetiopathogenesis of the condition, and for genetic counselling in families affected by it.

INTRODUCTION

The incidence of congenital malformations involving the Müllerian ducts in the general population is ~5 per 1000, and for infertile women it is more frequent at 35–63 per 1000.[12] Among the most common uterine anomalies are uterine duplications, uterine indentations, and partial uterine aplasias, which occur as a result of incomplete Müllerian and Wollfian duct fusions during development. The Mayer–Rokitanski–Küster–Hauser (MRKH) syndrome (OMIM 277000), occurring in 1 of 4500 live female births, is the most severe type where complete aplasia of the uterus, cervix, and upper vagina is found, leading to failure to menstruate and infertility, despite normal secondary sexual characteristics.[3–5]

The different MRKH subtypes are clinically classified into type I (typical or isolated) patients with normally developed fallopian tubes, ovaries, and urinary tract, and type II (atypical) patients, with Fallopian or ovarian abnormalities, and additional malformations, which typically involve the urinary tract and spine.[67] The acronym MURCS (Müllerian–renal–cervicothoracic somite abnormalities, OMIM 601076) applies to some of these cases. Craniofacial (dysmorphism, microtia) and cardiovascular malformations, and learning difficulties/mental retardation, may also be associated.[8] The reproductive and psychosocial consequences of the disorder are severe; despite this, from the aetiological perspective, it has been relatively poorly studied. Nonetheless, over the last decade, evidence has accumulated to suggest that genetic factors may be important.

Notwithstanding that patients with Müllerian aplasia are usually precluded from reproducing, familial clustering of the disorder has been reported, with apparently autosomal dominant inheritance.[910] For example, in the article by Shokeir,[10] figures 6 and 9 show Müllerian aplasia segregating in apparently autosomal dominant fashion in two or more generations. In a small proportion of cases, a specific genetic aetiology has been identified. Müllerian aplasia with hyper- and rogenism and renal malformations (OMIM 158330) is due to mutations in *WNT4*.[11] Beside single gene involvement, there is evidence implicating copy number variation in the pathogenesis of Müllerian aplasia. Case reports in the literature have linked MURCS to microdeletions at 17q12[12] and 22q11.2[13]; in one small series of 14 cases, both of these loci were identified and two more suggested, a microduplication at 1q21.1 and microdeletion at Xq21.31.[14] Müllerian aplasia has been reported in association with thrombocytopenia–absent radius syndrome, due to microdeletion at chromosome 1q21.1.[15]

Typically, syndromes due to recurrent copy number variants exhibit wide phenotypic variability, a fact which has been recognised since the early descriptions of the 22q11.2 microdeletion syndrome[16] and which continues to be recognised in the new syndromes which have been described since the advent of high resolution array based studies (reviewed in Mefford and Eichler[17]). A further example of this variability is given by the 16p11.2 microdeletion syndrome. This was initially described in cohorts of patients with autism.[1819] Later, the recognition was made that patients with 16p11.2 microdeletions have a more complex phenotype than those patients with presumed multifactorial forms of autism spectrum disorder, including dysmorphism, congenital anomalies, growth disturbance,

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motor delay, and epilepsy.[2021] Most recently, a strong association between 16p11.2 microdeletions and obesity was reported, especially where cognitive disability was also present.[22] A detailed explanation of how such pronounced phenotypic variability can arise from copy number variation at a single locus is lacking; environmental factors, epigenetic changes, and 'second hits'[23] may all play a part. For the present, it is likely that the full range of phenotypic variability of these disorders has yet to be explored, and that more associations may be identified through the study of different patient cohorts.

We report copy number analysis of DNA samples from a cohort of 63 individuals with Müllerian aplasia, of which 38 were classified as isolated or typical Müllerian aplasia (60 3%) and 25 were classified as syndromic or atypical Müllerian aplasia (39 7%), from which 11 had a diagnosis of MURCS association.

METHODS

Patient samples

Patient samples and phenotypic data were collected by the Department of Obstetrics and Gynecology, Erlangen, Germany. The project was approved by the Ethics Review Board of Friedrich-Alexander-University, Erlangen-Nuremberg, Germany. Informed consent for genetic studies was obtained in each case.

Copy number assay

Copy number analysis was performed on one of two platforms: Agilent 244K oligonucleotide array, as described,[24] and the Affymetrix SNP 6.0 genotyping platform, as described.[25] An initial pilot study was performed using the Agilent 244K array; subsequently, the Affymetrix SNP 6.0 array was used in order to facilitate comparison with a cohort of 7366 population controls, which had been assayed using the same platform. These control individuals were of European ancestry and were recruited from the WTCCC2 and GAIN (Genetic Association Information Network) consortia as described.[25]

Affymetrix SNP 6.0 data were analysed using Affymetrix powertools and Birdsuite software. Copy number variant calls made using Agilent software were converted to genotyping calls in order to enable comparison with the control cohort. We used a cut-off for calling copy number variants of 200 Kb.

Validation of results

Quantitative PCR (qPCR) on patient DNA was used to confirm deletions or amplifications at specific genomic loci. Results were normalised to *ACTB* DNA levels, which served as a control. Primer and probe sequences are shown in table 1. Experiments were performed as described earlier[26] on the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, California, USA). Parental samples were not available for analysis; thus, information about whether a given copy number variant arose de novo was not available.

RESULTS

Our study demonstrated a strikingly high incidence of recurrent copy number variants, with nine (14%) of the 63 samples studied having a copy number variant of this type (table 2 and figures 1 and 2). These copy number variants were confirmed by qPCR (figure 3). For isolated Müllerian aplasia, this applied to 4/38 (10 5%) patients, and for syndromic Müllerian aplasia, to 5/25 (20%) patients. The results are summarised in tables 2–4. In isolated Müllerian aplasia, we identified two microdeletions at 17q12, and two at 16p11.2.

Of the five syndromic cases, two had microdeletions at 17q12, two had microdeletions at 16p11.2, and one had a 'distal' 22q11.2 microdeletion. A sixth case had a microduplication at 2q11.2, a locus recently reported to represent a novel recurrent copy number variant, though as yet phenotypic data for this new disorder have not been provided.[27] Interpretation in this case is made additionally difficult by the presence of a large (4.6 Mb) deletion at 2p24.3. Either, or conceivably both, of these imbalances may have contributed to the phenotype in this case. There were two instances of microdeletion at 16p11.2 in the control cohort; no instances of microdeletion at 17q12 and 22q11.2 (distal) were identified.

We observed that 4/63 (6%) of cases in our series had microdeletions at 16p11.2, the first time that this locus has been associated with Müllerian aplasia. Recent reports have identified microdeletions at this locus in cohorts of patients with autism spectrum disorder[19] and obesity.[22] These patients often have syndromic features such as dysmorphic facial features and some congenital malformations including vertebral anomalies,[2028] and in one case, micropenis,[20] but not female reproductive tract malformations. The enrichment of this locus in patients with Müllerian aplasia compared with controls (4/63 patients compared with 2/7366 controls) is highly statistically significant (p=6.96e-8, Fisher's exact test) and suggests a hitherto unappreciated role for genes within the deletion interval in the development of the Müllerian derivatives.

In keeping with previously published studies[1214] we found microdeletions at 17q12 in patients with both syndromic and apparently isolated Müllerian aplasia. This finding is in line with previous reports in the literature linking this locus to syndromic Müllerian aplasia[14] and, in a single case, to apparently isolated Müllerian aplasia.[12]

Several case reports have demonstrated an association between the velocardiofacial syndrome (VCFS) associated microdeletions at 22q11.2 and Müllerian aplasia.[131429] Recently, a novel genomic disorder was reported due to microdeletions at an adjacent, telomeric, locus, and this disorder was given the name '22q11.2 distal deletion'.[30] The complex genomic architecture at this locus gives rise to 'nested' microdeletions within the critical interval. In the original report of this syndrome,[30] attention was drawn to two cases with distal nested microdeletions, one of which had an isolated congenital heart defect. We now describe a case with syndromic Müllerian aplasia/MURCS and the same, distal nested microdeletion.

We identified a microduplication at 2q11.2 in a patient with features of MURCS association. Copy number variation at this locus has recently been reported and the suggestion has been made that this constitutes a novel genomic disorder.[27] However, no phenotypic data concerning this duplication are available, and the interpretation in our case is further confounded by the co-existence of a previously undescribed 4.6 Mb deletion on chromosome 2p. Clarification of the possible contribution of these two individual copy number variants to abnormalities of Müllerian development must await further examples.

The intervals delineated by these copy number variants harbour in some cases interesting candidate genes. The approximately 0.55 Mb interval at 16p11.2 contains *TBX6*, a gene previously implicated in the development of paraxial mesoderm[31] but with no known role in formation of the Müllerian ducts. There are no other compelling developmental candidate genes in the critical interval. The 1.4 Mb interval at 17q12 harbours two genes with known roles in reproductive tract development: *HNF1B*, in which mutations have been described in patients with renal cysts and diabetes[32]; these patients also have genital tract malformations such as bicornuate uterus and uterus didelphys, but an absence of uterus and fallopian tubes has been reported.[32] Second, this region harbours *LHX1*. Mutations in this gene have not been described in humans, but mice with targeted knockout of *Lhx1* have an absence of

uterus and oviducts.[33] Sequencing of *LHX1* in patients with Müllerian aplasia has to date not revealed any mutations (Bernardini *et al*,[12] and our unpublished data). The 'distal 22q11.2[9] locus harbours just four genes, *RTDR1*, *RAB36*, *GNAZ*, and *BCR*. Germline mutations in none of these four genes have been described in humans, and developmental malformations have not been reported in BCR-null[34] or GNAZ-null mice,[35] for which mouse data are available.

Eleven non-genomic disorder type copy number variants were identified in the case cohort which were absent in controls, but none of these occurred in more than one patient, and the absence of parental samples is an additional factor limiting our interpretation of their significance. Table 4 lists copy number variants occurring in a single patient in the case cohort and not in the control cohort. Three of these, at 2p24.1–24.3, 15q21.1, and 18q23, were selected for validation by qPCR; all three were confirmed (data not shown). The deletion of 4.6 Mb at 2p24.1–24.3 occurred in a patient with a double segment imbalance, the other copy number variant being a microduplication at 2q11.2, discussed above. One imbalance harbouring a potentially interesting candidate gene was noted, a 200 Kb duplication at 18q23 encompassing SALL3, a member of the SALL gene family, of as yet unknown function. Replication of this study on larger cohorts will be needed in order to determine whether these single occurrence copy number variants are significant.

DISCUSSION

Previous reports have identified copy number variants in Müllerian aplasia,[1214] but these have been case reports and small series, and studies of medium or large scale series have to date not been carried out. Here, we give results of copy number analysis of a series of 63 patients, identifying a strikingly high incidence of 9/63 (14%) of previously characterised microdeletions. Microdeletions at 16p11.2 and 17q12 in particular are highly enriched in the case population in comparison to the control population. Both of these microdeletions, and the 22q11.2 distal microdeletion, have previously been associated with congenital malformations: of the spine in the case of 16p11.2,[28] the genitourinary tract in the case of 17q12,[36] and cardiovascular system in the case of the distal 22q11.2 deletion.[30] Thus, although data on inheritance for these cases are lacking, there can be little doubt that these microdeletions are contributing to the phenotypes of isolated and syndromic Müllerian aplasia.

The apparent contribution of genomic disorder type copy number variants to congenital malformations occurring in isolation is of particular interest. A recent study of non-syndromic tetralogy of Fallot, a complex congenital heart malformation, gives weight to the idea that genomic disorders may be associated with isolated congenital malformations.[37] Copy number variants corresponding to known genomic disorders were identified at 22q11.2 (two, both loss), and at 1q21.1 (four gain, one loss) in 7/512 (1.4%) of individuals. In our study, copy number variants associated with genomic disorders were identified in 4/43 (9%) of isolated Müllerian aplasia cases, a significantly higher figure than for isolated tetralogy of Fallot.

Our study extends the phenotypic variability associated with microdeletions at 16p11.2. The previously known phenotypic consequences of microdeletions at this locus are: autism spectrum disorder[18]; epilepsy[21]; developmental delay/learning disability and dysmorphism/congenital anomalies[2021]; and obesity.[22] Both isolated and syndromic forms of Müllerian aplasia can now be added to this list. Increasingly, these findings raise the question of which factor or factors are responsible for determining the phenotypic outcome in patients with 16p11.2 microdeletions in particular and genomic disorders in general. It can be hypothesised that this microdeletion, and others like it, provide an early and general

developmental perturbation, the ultimate and precise consequence of which is independent of the perturbation itself, but depends on other factors. Future studies may begin to address this question, through sequencing, identification of 'second hits',[23] and other modalities.

Our results have implications for genetic counselling in Müllerian aplasia. At present, few women with the isolated form of this malformation are referred to clinical geneticists. Müllerian aplasia results in infertility, and so it might be argued that the need for genetic counselling is less (although it might be important in cases where egg donation is being considered). More critically, though, the diagnosis of a microdeletion at 16p11.2, 17q12 or 22q11.2 (distal) has potential implications for other family members. Copy number variants associated with genomic disorders may be inherited from a phenotypically normal parent and transmitted to other family members. We anticipate that families would wish to be made aware of the associations with autism (16p11.2) or young onset diabetes and renal cysts (17q12) and cardiac malformations (22q11.2 (distal)) as well as with Müllerian aplasia, notwithstanding that our poor understanding of the penetrance and variable expressivity of these disorders makes genetic counselling difficult. In conclusion, our data support the contention that detailed copy number assays should be carried out in the assessment of both isolated and syndromic forms of Müllerian aplasia. We feel that the high incidence of recurrent copy number variants in these patients makes it reasonable to recommend that they should be referred to a clinical geneticist for assessment, and, where appropriate, for genetic testing and family counselling.

Acknowledgments

The authors thank all of the patients who participated in this study and Departments of Obstetrics and Gynaecology in Tübingen (Professor D Wallwiener), Heidelberg (Professor T Strowitzky) and Wuppertal (Professor Hucke), Germany for help in recruitment of patients. CS-S, MS, VM, TF, MH, NH, and NPC are funded by the Wellcome Trust. CS-S is the recipient of an Intermediate Clinical Fellowship from the Wellcome Trust. RS was funded by the Deutsche Forschungsgemeinschaft (DFG-STR923/2-2). The authors wish to thank Cordelia Langford and Sarah Widaa of the Microarray facility, and Sarah Edkins and Emma Gray of the DNA collections group at the Sanger Institute for assistance with sample processing and genotyping.

Funding Other funders: Wellcome Trust; Deutsche Forschungsgemeinschaft.

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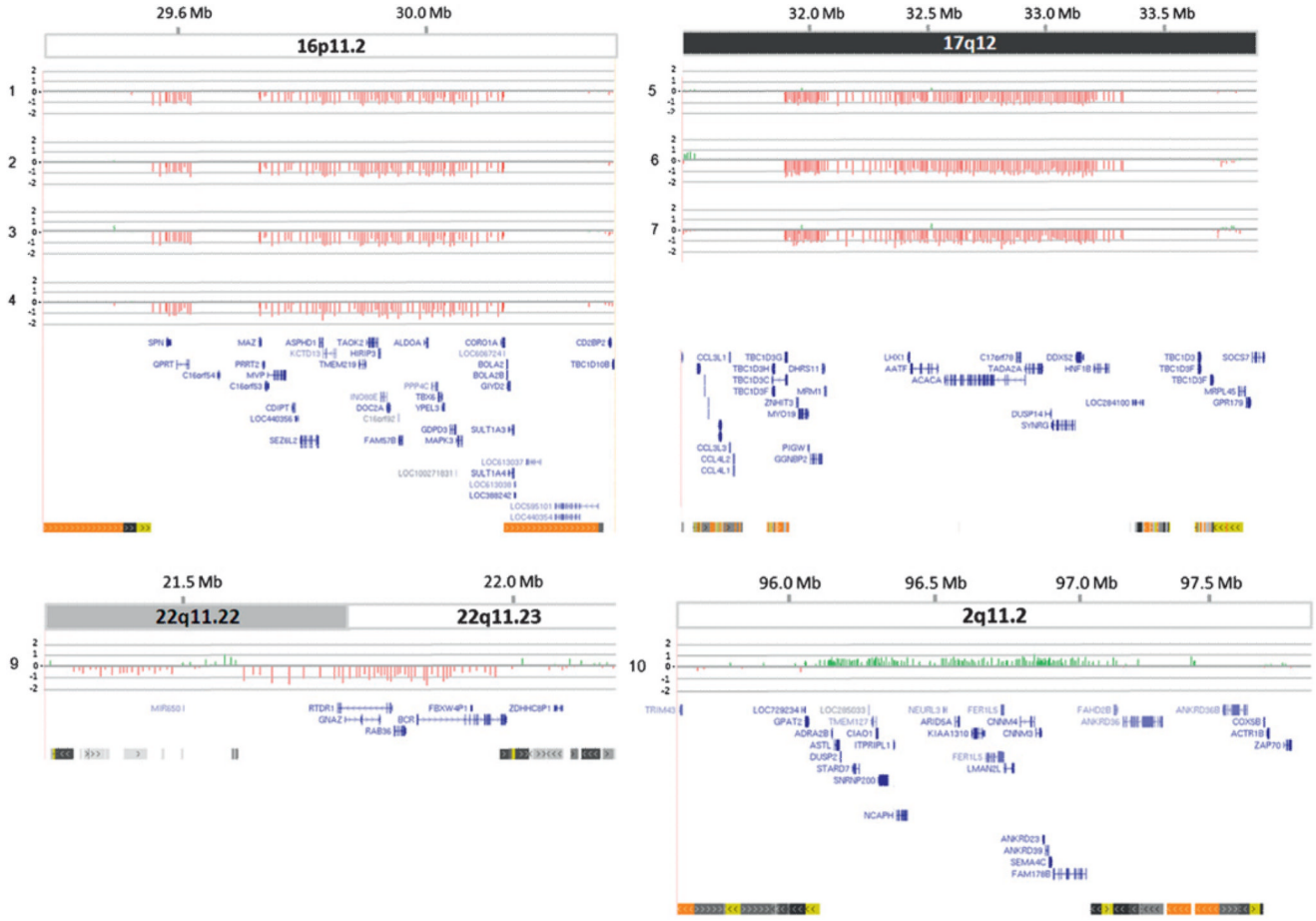

Figure 1.

Copy number variants associated with genomic disorders in patients with isolated and syndromic Müllerian aplasia. Each figure presents microarray data for one of four genomic loci, at 16p11.2, 17q12, 22q11.2 (distal) and 2q11.2. Presented from top down are: scale showing distance from tip of 'p' arm in megabases (UCSC genome browser March 2006 Hg18/NCBI Build 36); chromosome ideogram showing chromosome band; array comparative genomic hybridisation (CGH) result showing value for each probe (log2 ratio), where zero corresponds to diploid copy number, −1 to a heterozygous deletion and +0.58 to a heterozygous duplication. Patient ID is shown to the left of each graphic. DNA from case 8 was assayed on the Affymetrix 6.0 platform only; data for this case are given in figure 2. Gene content of the region is shown below. For simplicity of presentation, alternately spliced isoforms are not shown. Finally, segmental duplications of >1 Kb of non-repeat masked sequence are shown. Light to dark grey, 90–98% similar; light to dark yellow, 98–99% similar; light to dark orange, >99% similar; are indicated.

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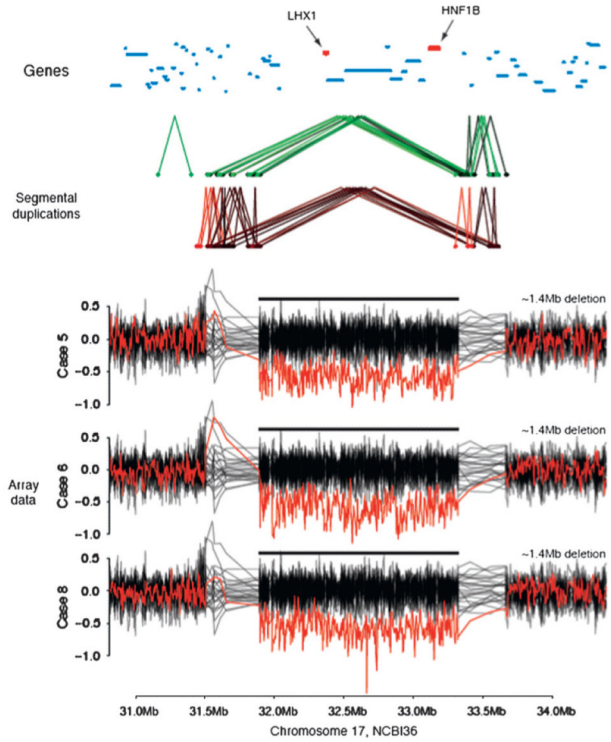

Figure 2.

Affymetrix 6.0 data for three patients with deletions at 17q12 (case 7 was not run on this platform). Log2 ratios for the three samples are highlighted in dark red, with the other samples from the same genotyping plate shown in black. The segmental duplication structure, taken from the UCSC genome browser, is shown. Protein coding genes are indicated by dark blue lines, with *LHX1* and *HNF1B* highlighted in red.

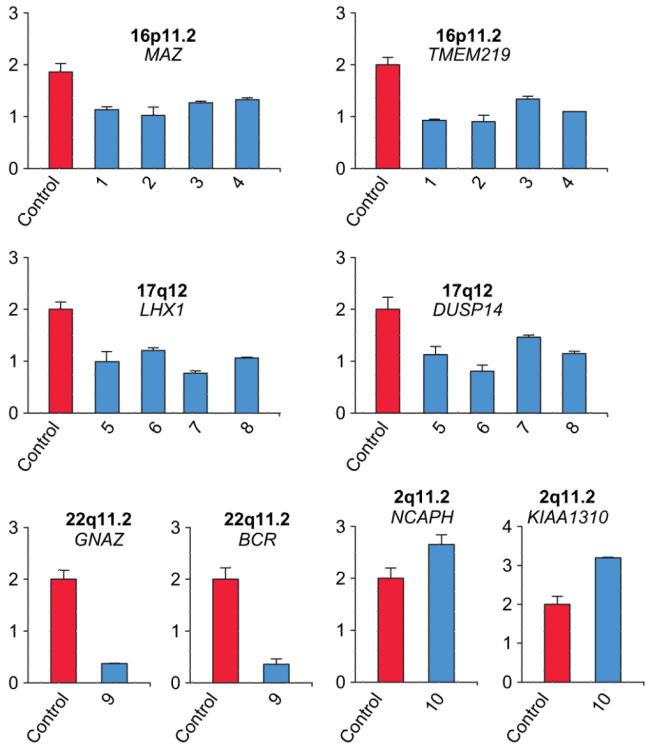

Figure 3.
Quantitative assessment of deletions and amplifications at indicated genomic loci. Quantification was performed on DNA of 4e6 control persons (red bars) and affected individuals (blue bars). Primer and probe sequences were chosen to amplify intronic regions of the indicated genes. Data were normalised to results obtained at the *ACTB* locus and are presented as relative values.

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Table 1

Primer and probe sequences used for quantitative PCR *

Region	Primer/probe	Sequence
1q21.1	Pex11b fw	CACGCTGATGTGCTTGTGATG
	Pex11b rev	TTTGACAATGATGAGGCCTGAA
	Pex11b probe	TGCGGCCTGCACTGGCCC
1q21.1	CD160 fw	TTGAACCCAGGAGTCCACAAG
	CD160 rev	ACAGACGGCGGGAAACTCTT
	CD160 probe	CCAGGACCGCCTCCGAAGGTG
2q11.2	NCAPH fw	GGGCGGCCTCCTCCTT
	NCAPH rev	TTCCAGGAAAACCACCATTTTAA
	NCAPH probe	CTCCTAAAGCGTGCTCGGTGTCTCTCC
2q11.2	Kiaa1310 fw	GGTGCTGGCCAAGCAAGT
	Kiaa1310 rev	GCTCACCGACCCCAAGGT
	Kiaa1310 probe	TACTGTCTGTCCACGCGAGGTCTTTCTG
16p11.2	MAZ fw	GGCTCAAAGGGCCCAATAG
	MAZ rev	CCTCCCTGTGCCCAGAAGT
	MAZ probe	AGGGATGCCCATGTACCACTCAGGC
16p11.2	Tmem219 fw	GCACCCCACTTGGAAGCA
	Tmem219 rev	TGAGGCTCGCGGACTTTAA
	Tmem219 probe	TCAGATCTTGGCCCTACCCCTCCTGT
17q12	LHX1 fw	CATGTGCCTGGGAAGAAAGG
	LHX1 rev	TGCCCTGTCTCTTCCAAGCT
	LHX1 probe	AGCCTGACTCGGCCCAGAAGCC
17q12	Dusp14 fw	TCTGGTGCATGGATAGAAGCAA
	Dusp14 rev	CCACCGCAGAGAAAGACTCAA
	Dusp14 probe	TGACTTTCAGCGATGCCAAGTGTCCA
2q11.2	Gnaz fw	GCCTGGTAGAGAGGTCTGTCTTG
	Gnaz rev	GGGAAATCACTTGGGCAGAA
	Gnaz probe	ACAGCTGAGCCCCTGACCGGC
2q11.2	BCR fw	GATCCTGCACCCGAACAAA
	BCR rev	CCAATTCCATTCCAAACACTAACA
	BCR probe	CCATCCCCTCCTCCTTCCTGAATGC
2p24.124.3	Vsnl1 fw	CTCAGAGAGAAGTCACCCATCAAC
	Vsnl1 rev	AATGAGAGGGTGTGCAAGTGAA
	Vsnl1 probe	CCCTGCCTGGGAAGCTGGCC
2p24.124.3	GDF7 fw	GATGGGACTTTTGGCTTGCTAA
	GDF7 rev	CAGAGCAGCGGACGTCTTC
	GDF7 probe	CCAAAGCTCGGTTCGGATATCCCG
15q21.1	15q21 fw	GCTGATTATAAACGGAGCCATATTC
	h15q21 rev	CCTGGCTGCTTTTGACATCAT
	h15q21 probe	TTGAGACCAGGCCTTCACTTTCTCGGAA

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Region	Primer/probe	Sequence
18q23	hSall3 fw	GGCTTGGGCAAGTGAAGGA
	hSall3 rev	TGGCCACGCAGAGAATGTT
	hSall3-probe	AGACCCGGACCCTTCGAGCTCCA
control	Beta-actin fw	AGGTGCACAGTAGGTCTGAACAGA
	Beta-Actin rev	AAGTGCAAAGAACACGGCTAAGT
	Beta-Actin probe	TCCCCATCCCAAGACCCCAGC

* Probes were dual labelled with FAM (5′) and TAMRA (3′).

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Table 2

Genomic disorder type copy number variants in isolated and syndromic Müllerian aplasia

Case	Locus	Size	Copy number	Co-ordinates (Hg18)	Phenotype	Platform/confirmation
1	16p11.2	0.55 Mb	Del	16:29487535–30085308	MURCS	Agilent, Affymetrix 6.0, qPCR
2	16p11.2	0.60 Mb	Del	16:29561000–3010700	MA	Agilent, Affymetrix 6.0, qPCR
3	16p11.2	0.55 Mb	Del	16:29560500–30106808	MURCS	Agilent, qPCR
4	16p11.2	0.55 Mb	Del	16:29487535–30085308	MA	Agilent, qPCR
5	17q12	1.4 Mb	Del	17:31889000–33322000	MA	Agilent, Affymetrix 6.0, qPCR
6	17q12	1.4 Mb	Del	17:31889000–33322000	MURCS	Agilent, Affymetrix 6.0, qPCR
7	17q12	1.4 Mb	Del	17:31889297–33322972	MURCS	Agilent, qPCR
8	17q12	1.4	Mb	Del 17:31889000–33322000	MURCS	Affymetrix 6.0, qPCR
9	22q11.2	0.39 Mb	Del	22:21588000–21973000	MURCS	Agilent, Affymetrix 6.0, qPCR
10	2q11.2	1.30 Mb	Dup	2:96052862–97390919	MURCS	Agilent, qPCR

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Table 3

Summary of the clinical findings in patients with copy number variants of genomic disorder type and isolated or syndromic Müllerian aplasia

Case	Locus of deletion	Age (years)	Classification	Findings	Isolated/ syndromic	Renal	Cervical/vertebral	Craniofacial	Cognitive	Growth	Other
1	16p11.2	30	V5b C2b U4b A0 MSN Type II/ MURCS	Blind ending vagina, rudimentary uterus, tubes and ovaries normal	Syndromic	Normal (CT and laparotomy)	Hypoplasia of the wrist	No clinical indications	Moderate disturbed psychomotor development	Normal (no exact measures)	Epilepsy, moderate bilateral hearing loss
2	16p11.2	20	V5b C2b U4b A0 M0 Type I	Blind ending vagina, rudimentary uterus, tubes and ovaries normal	Isolated	Normal (CT and laparotomy)	Normal	No clinical indications	Normal psychomotor development	Height 146 cm Weight 47 kg	
3	16p11.2	32	V5b C2b U4b A0 MR Type II	Blind ending vagina, rudimentary uterus, long uterus horns, tubes and ovaries normal	Syndromic	Left atrophic kidney	Scoliosis	No clinical indications	Normal psychomotor development	Normal (no exact measures)	
4	16p11.2	18	V5b C2b U4b A0 M0 Type I	Blind ending vagina, rudimentary uterus, tubes and ovaries normal	Isolated	Normal (laparoscopy and laparotomy)	Normal	No clinical indications	Normal psychomotor development	Height 161 cm Weight 74 kg	
5	17q12	24	V5b C2b U4b A0 M0 Type I	Blind ending vagina, rudimentary uterus, tubes and ovaries normal	Isolated	Normal (CT and laparotomy)	Normal	No clinical indications	Normal psychomotor development	Height 171 cm Weight 56 kg	
6	17q12	37	V5b C2b U4b A0 MRSC Type II/ MURCS	Blind ending vagina, rudimentary uterus, tubes and ovaries normal	Syndromic	Left kidney agenesis with absent ureter	Pelvic misalignment resulting in different leg length	No clinical indications	Normal psychomotor development	Height 173 cm Weight 70 kg	Diabetes type 2, aortic-pulmonary septal defect
7	17q12	31	V5b C2b U4b A0 MR Type II	Rudimentary uterus, tubes normal, ovaries normal	Syndromic	Absent right kidney, left pelvic kidney	Right convex kyphoscoliosis	No clinical indications	Normal psychomotor development	Height 159 cm Weight 40 kg	Left kidney transplantation
8	17q12	25	V5b C2b U4b A0 M0 Type I	Blind ending vagina, rudimentary uterus, tubes and ovaries normal	Isolated	Normal (CT and laparotomy)	Mild scoliosis	No clinical indications	Normal psychomotor development	Height 154 cm Weight 58 kg	
9	22q11.2	16	V5b C2b U4b A2a MRSC Type II/ MURCS	1 cm blind ending vagina. Rudimentary uterus. Normal right ovary, streak left ovary.	Syndromic	Fused pelvic kidney	Short neck; hemivertebrae C1 and C3, C5, C6, ventral and dorsal fusion C2 and C3; cleft vertebral arch C4, C5, C6; scoliosis thoracic vertebral column; hypoplastic first ribs, flattened sacrum	Dysplastic auricles, right sided cleft lip, cleft palate	Normal psychomotor development	Height 153 cm (3rd–10th) Weight 48.5 kg (10th–25th)	Atrial septal defect, persistent left superior vena cava, unroofed coronary sinus, patent ductus arteriosus; multiple nevi; left hypoplastic thumb, hypoplastic middle phalanx, absent distal phalanx second digit, absent middle and distal phalanges, fifth digit
10	2q11.2 and 2p24.24.3	28	V5b C2b U4b A1b MR Type II/ MURCS	Blind ending vagina, no uterus, no uterus horns, very short left tube, tube right agenesis, ovaries normal	Syndromic	Agenesis of right kidney	Normal	No clinical indications	Normal psychomotor development	Height 165 cm Weight 51 kg	Bilateral inguinal hernias

Please refer to Opelt *et al*[8] for explanation of VCUAM classification of Müllerian abnormalities.

Briefly, V5b, complete atresia of the vagina; C2b, bilateral aplasia of the cervix; U4b, aplasia of the uterus; A, adnex malformations with A1b, bilateral tubal malformation but normal ovaries; A2a, unilateral tubal hypoplasia; M, associated malformation, R, renal system; S, skeleton; C, cardiac; N, neurological; 0, normal.

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Table 4

Non-genomic disorder type copy number variants in isolated and syndromic Müllerian aplasia

Case	Locus	Size	Copy number	Co-ordinates (Hg18)	Phenotype	Overlap	Gene content	Platform/confirmation
9	14q32.33	0.46 Mb	Del	14:104840347–105295569	MURCS	0.53	Multiple	Agilent, Affymetrix 6.0
10	2p24.124.3	4.6 Mb	Del	2:16875751–21460570	MURCS	0.12		Agilent, qPCR
11	1q31.1	0.40 Mb	Dup	1:187058991–187457103	MA	0.46		Affymetrix 6.0
12	2p23.1	0.21 Mb	Dup	2:31493349–31706481	MA	0	SRD5A2	Affymetrix 6.0
13	5p11	0.4 Mb	Del	5:45989457–46401198	MURCS	0.28		Affymetrix 6.0
13	11p11.12	0.76 Mb	Del	11:50334299–51095288	MURCS	0.29		Affymetrix 6.0
14	5q14.3	0.4 Mb	Del	5:91827411–92261197	MA	0		Agilent, Affymetrix 6.0
15	6q11.1	0.41 Mb	Dup	6:62969555–63392084	MA	0.41	KHDRBS2	Affymetrix 6.0
16	15q21.1	0.28 Mb	Del	15:45521438–45801152	MURCS	0	SEMA6D	Agilent, qPCR
17	16q11.2	0.2 Mb	Dup	16:45210000–45414000	MA	0		Agilent
18	18q23	0.2 Mb	Dup	18:74729993–74935915	MA	0	SALL3	Agilent, qPCR