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

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Abstract

Background: Congenital malformations involving the Mullerian ducts are observed in around 5% of infertile women. Complete aplasia of the uterus, cervix, and upper vagina, also termed Mullerian aplasia or Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome occurs with an incidence of around 1 in 4,500 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 Mullerian aplasia, we performed copy number assays in a series of 63 cases, of which 25 were syndromic and 38 isolated.

Results: We report a high incidence (9/63, 14%) of recurrent copy number variants in this cohort. These comprised four cases of microdeletion at 16p11.2, an autism susceptibility locus not previously associated with Mullerian 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 4/38 (10.5%) cases with isolated Mullerian aplasia, and at 16p11.2, 17q12 and 22q11.2 (distal) in 5/25 cases (20%) with syndromic Mullerian aplasia.

Conclusion: Our 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 Mullerian aplasia. Overall, the high incidence of recurrent copy number variants in all forms of Mullerian aplasia has implications for our understanding of the aetiopathogenesis of the condition, and for genetic counseling in families affected by it.

Introduction

The incidence of congenital malformations involving the Mullerian ducts in the general population is ~5 per 1000, and of infertile women more frequent at 35-63 per 1000 [1,2].

Among the most common uterine anomalies are uterine duplications, uterine indentations, and partial uterine aplasias, which occur as a result of incomplete Müllerian and Wolffian duct fusions during development. The Mayer-Rokitanski-Küster-Hausler (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 [6,7]. 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 Mullerian aplasia are usually precluded from reproducing, familial clustering of the disorder has been reported, with apparently autosomal dominant inheritance [9,10]. For example, figures 6 and 9 (reference [10]) show Mullerian 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. Mullerian aplasia with hyperandrogenism 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 Mullerian 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]. Mullerian 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 recognized since the early descriptions of the 22q11.2 microdeletion syndrome [16] and which continues to be recognized in the new syndromes which have been described since the advent of high-resolution array-based studies (reviewed in ref [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 [18,19]. 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, motor delay, and epilepsy [20,21]. 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 marked 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. Here, we report copy number analysis of DNA samples from a cohort of 63 individuals with Mullerian aplasia, of which 38 were classified as isolated or typical Mullerian aplasia (60.3%) and 25 were classified as syndromic or atypical Mullerian aplasia (39.7%), from which 11 had a diagnosis of MURCS association.

Results

Our study demonstrated a strikingly high incidence of recurrent copy number variants, with 9 (14%) of the 63 samples studied having a copy number variant of this type (Table 1 and Figures 1 and 2). These CNVs were confirmed by quantitative PCR (Figure 3). For isolated Mullerian aplasia, this applied to four of 38 (10.5%) of patients, and, for syndromic Mullerian aplasia, to five of 25 (20%). The results are summarized in Tables 1-3. In isolated Mullerian 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 [24]. 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 (6.3%) of cases in our series had microdeletions at 16p11.2, the first time that this locus has been associated with Mullerian 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, [20,25] and in one case, micropenis [20], but not female reproductive tract malformations. The enrichment of this locus in patients with Mullerian aplasia compared with controls (4/63 patients compared with

2/7,366 controls) is highly statistically significant ($p = 6.96 \times 10^{-8}$, Fisher's exact test) and suggests a hitherto unappreciated role for genes within the deletion interval in the development of the Mullerian derivatives.

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

Several case reports have demonstrated an association between the velocardiofacial syndrome (VCFS) associated microdeletions at 22q11.2 and Mullerian aplasia [14,26,27]. 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' [28]. The complex genomic architecture at this locus gives rise to 'nested' microdeletions within the critical interval. In the original report of this syndrome [28], 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 Mullerian 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 made that this constitutes a novel genomic disorder [24]. 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 CNVs to abnormalities of Mullerian 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 development of paraxial mesoderm [29] but with no known role in formation of the Mullerian 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 [30]; these patients also have genital tract malformations such as bicornuate uterus and uterus didelphys, but absence of uterus and fallopian tubes has been reported [30]. Secondly, this region harbours *LHX1*. Mutations in this gene have not been described in humans, but mice with targeted knockout of *Lhx1* have absence of uterus and oviducts [31]. Sequencing of *LHX1* in patients with Mullerian aplasia has to date not revealed any mutations (ref [12], and our unpublished data). The 'distal 22q11.2' 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 [32] or *GNAZ*-null mice [33], for which mouse data are available.

11 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 3 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 (Figure 3). 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 CNVs are significant.

Discussion

Previous reports have identified copy number variants in Mullerian aplasia [12,14], 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 characterized 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 [25], the genito-urinary tract in the case of 17q12 [34] and cardiovascular system in the case of the distal 22q11.2 deletion [28]. Thus, although data on inheritance for these cases is lacking, there can be little doubt that these microdeletions are contributing to the phenotypes of isolated and syndromic Mullerian 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 [35]. 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 Mullerian 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 [20,21]; and obesity [22]. Both isolated and syndromic forms of Mullerian 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 hypothesized that this microdeletion, and others like it, provides 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 counseling in Mullerian aplasia. At present, few women with the isolated form of this malformation are referred to clinical geneticists. Mullerian aplasia results in infertility, and so it might be argued that the need for genetic counseling 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 Mullerian aplasia, notwithstanding that our poor understanding of the penetrance and variable expressivity of these disorders makes genetic counseling 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 Mullerian 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.

Materials and 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 [36] and the Affymetrix SNP 6.0 genotyping platform, as described [37]. 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 7,366 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 [37].

Affymetrix SNP 6.0 data were analyzed using Affymetrix *powerTools* and *Birdsuite* software. CNV 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 CNVs of 200 Kb.

Validation of results

qPCR on patient DNA was used to confirm deletions or amplifications at specific genomic loci. Results were normalized to *ACTB* DNA levels, which served as a control. Primer and probe sequences are shown in Table 4. Experiments were performed as described earlier [38] on the

ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). Parental samples were not available for analysis; thus, information about whether a given copy number variant arose *de novo* was not available.

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Conflicting Interests Statement

The authors declare no conflict of interest.

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Figure legends

Figure 1

Copy number variants associated with genomic disorders in patients with isolated and syndromic Mullerian 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; arrayCGH 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 gray, 90-98% similar; light to dark yellow, 98-99% similar; light to dark orange, >99% similar; are indicated.

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 4-6 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 normalized to results obtained at the *ACTB* locus and are presented as relative values.

Tables

Table 1

Genomic disorder type copy number variants in isolated and syndromic Mullerian 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

Table 2

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

Case	Locus of Deletion	Age (yrs)	Classification	Findings	Isolated/ syndromic	Renal	Cervical/vertebral	Cranio- facial	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 (computer tomography 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 (computer tomography and laparotomy)	Normal	No clinical indications	Normal psychomotor development	Ht (146 cm) Wt (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	Ht (161 cm) Wt (74 kg)	
5	17q12	24	V5b C2b U4b A0 M0 Type I	blind-ending vagina, rudimentary uterus, tubes and ovaries normal	Isolated	Normal (computer tomography and laparotomy)	Normal	No clinical indications	Normal psychomotor development	Ht (171 cm) Wt (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	Ht (173 cm) Wt (70 kg)	Diabetes type 2, cortico- 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	Ht (159 cm) Wt (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 (computer tomography and laparotomy)	Mild scoliosis	No clinical indications	Normal psychomotor development	Ht (154 cm) Wt (58 kg)	
9	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 L tube, tube R agenesis, ovaries normal	Syndromic	Agenesis of right kidney	Normal	No clinical indications	Normal psychomotor development	Ht (165 cm) Wt (51 kg)	Bilateral inguinal hernias
10	22q11.2	16	V5b C2b U4b A2a MRSC, Type II/ MURCS	1 cm blind-ending vagina. Rudimentary uterus. Normal R ovary, streak L 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	Ht 153 cm (3 rd -10 th); Wt 48.5 kg (10 th -25 th)	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

Please refer to ⁸ for explanation of VCUAM classification of Mullerian 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).

Table 3. Non-genomic disorder type copy number variants in isolated and syndromic Mullerian 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

Table 4. 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	GCACCCCACTTGAAGCA
	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

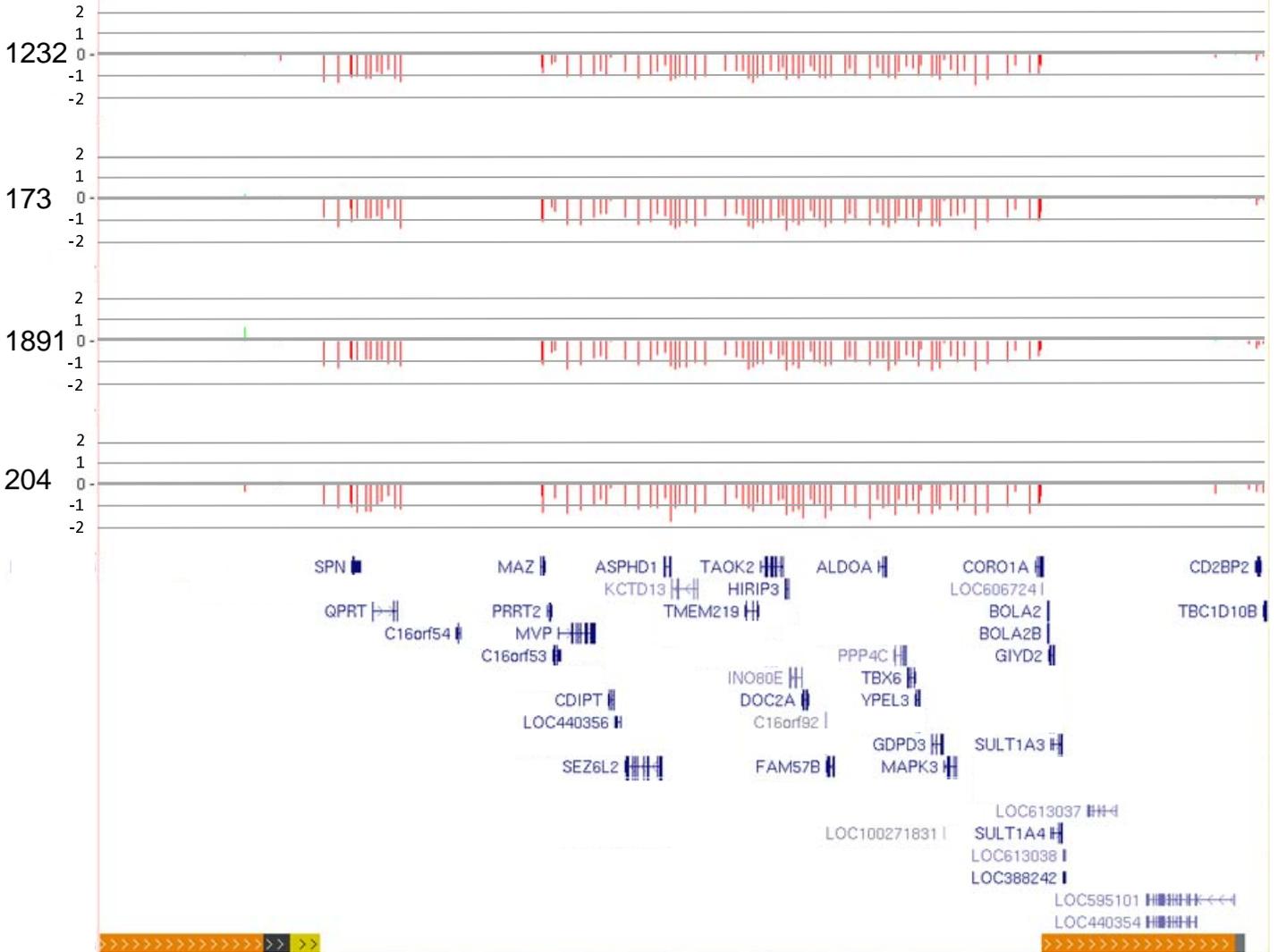
	Gnaz probe	ACAGCTGAGCCCCTGACCGGC
2q11.2	BCR fw	GATCCTGCACCCGAACAAA
	BCR rev	CCAATTCCATTCCAAACACTAACA
	BCR probe	CCATCCCCTCCTCCTTCTGAATGC
2p24.124.3	Vsn1 fw	CTCAGAGAGAAGTCACCCATCAAC
	Vsn1 rev	AATGAGAGGGTGTGCAAGTGAA
	Vsn1 probe	CCCTGCCTGGGAAGCTGGCC
2p24.124.3	GDF7 fw	GATGGGACTTTTGGCTTGCTAA
	GDF7 rev	CAGAGCAGCGGACGTCTTC
	GDF7 probe	CCAAAGCTCGGTTCGGATATCCCG
15q21.1	15q21 fw	GCTGATTATAAACGGAGCCATATTC
	h15q21 rev	CCTGGCTGCTTTTGACATCAT
	h15q21 probe	TTGAGACCAGGCCTTCACTTTCTCGGAA
18q23	hSall3 fw	GGCTTGGGCAAGTGAAGGA
	hSall3 rev	TGGCCACGCAGAGAATGTT
	hSall3-probe	AGACCCGGACCCTTCGAGCTCCA
control	Beta-actin fw	AGGTGCACAGTAGGTCTGAACAGA
	Beta-Actin rev	AAGTGCAAAGAACACGGCTAAGT
	Beta-Actin probe	TCCCCATCCCAAGACCCAGC

*Probes were dual-labelled with FAM (5') and TAMRA (3')

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30.0 Mb

16p11.2

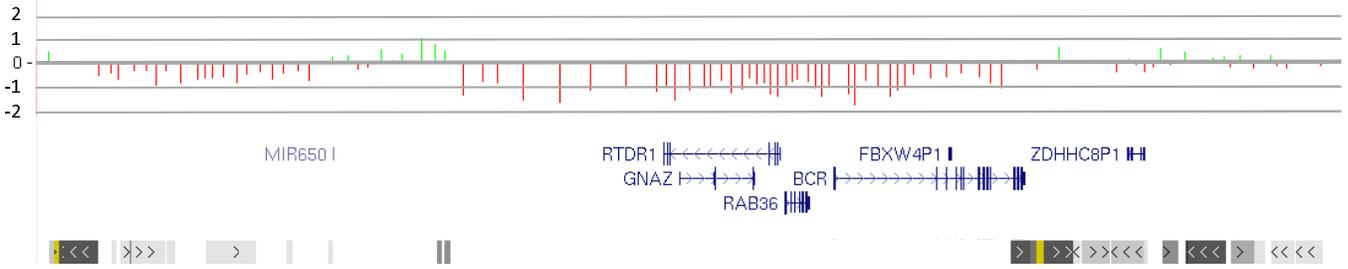


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22q11.22

22q11.23



32.0 Mb

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33.0 Mb

33.5 Mb

Scale
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17q12

214

3379

2194

