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Genomic imbalances associated with müllerian aplasia

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ABSTRACT

Background: Aplasia of the müllerian ducts leads to absence of the uterine corpus, uterine cervix, and upper (superior) vagina. Patients with müllerian aplasia (MA) often exhibit additional clinical features such as renal, vertebral and cardiac defects. A number of different syndromes have been associated with MA, and in most cases its aetiology remains poorly understood.

Objective and methods: 14 syndromic patients with MA and 46,XX G-banded karyotype were screened for DNA copy number changes by ~1 Mb whole genome bacterial artificial chromosome (BAC) array based comparative genomic hybridisation (CGH). The detected alterations were validated by an independent method and further mapped by high resolution oligo-arrays.

Results: Submicroscopic genomic imbalances affecting the 1q21.1, 1q12, 22q11.21, and Xq21.31 chromosome regions were detected in four probands. Presence of the alterations in the normal mother of one patient suggests incomplete penetrance and/or variable expressivity.

Conclusion: 4 of the 14 patients (29%) were found to have cryptic genomic alterations. The imbalances on 22q11.21 support recent findings by us and others that alterations in this chromosome region may result in impairment of müllerian duct development. The remaining imbalances indicate involvement of previously unknown chromosome regions in MA, and point specifically to LHX1 and KLHL4 as candidate genes.

Müllerian aplasia (MA) is characterised by absent or rudimentary uterus and upper vagina (müllerian-derived structures) in 46,XX females with normal secondary sexual development.1 In a proportion of women with MA (12–30%), the failure or arrest of development of the müllerian system occurs in conjunction with other developmental defects.2 Renal and skeletal anomalies are the most frequently found but a range of other malformations has been reported. Simpson3 describes at least eight complex disorders which might present MA, and the patients studied by us may overlap with different entities. Most women affected by MA are isolated cases, but descriptions of familial aggregates indicate that this trait may be transmitted as autosomal dominant with incomplete penetrance and variable expressivity.1,3 Chromosomal alterations1 and mutations in the WNT4 gene in females with MA have been sporadically reported. It has been suggested,1,3 however, that the WNT4 mutations associated phenotypes represent a separate genetic category; this proposal conforms to the lack of WNT4 mutations in 25 females with the MRKH anomaly studied by us.4 For the majority of cases, the aetiology of MA remains poorly understood.

Array based comparative genomic hybridisation (array CGH) allows ascertainment of cryptic chromosomal imbalances that escape detection by routine chromosome analysis. It provides a genome-wide screening by hybridising differentially labelled test and reference DNAs to arrays consisting of thousands of genomic clones. This approach has proved useful in determining the aetiology of 15–20% of mental retardation of unknown causes,5 and has led to the identification of novel genes involved in malformation syndromes.6

We report the results of an array CGH screening in 14 women exhibiting MA and additional features, including urinary tract anomalies, cardiac and skeletal defects, hearing impairment, and mental retardation.

METHODS

Patient’s ascertainment

The patients were selected for exhibiting MA, additional clinical signs and a normal G-band karyotype. They were ascertained in public health services from the University of São Paulo (São Paulo, Brazil), namely the Human Genome Research Center and the School of Medicine Hospital.

Screening for genomic imbalances by 1 Mb array CGH

Genomic DNA from patients and normal individuals were hybridised to slides containing triplicates of ~3500 large insert clones evenly spaced at ~1 Mb density over the full genome. The clones were provided by the Wellcome Trust Sanger Institute (UK), and are fully described in the Ensembl Genome Database. Array production, DNA labelling and hybridisation have been previously described.7 Target imbalances were determined based on log2 ratios of the average of the replicates, and sequences were considered as amplified or deleted when outside the ± 0.35 range. Copy number variations (CNVs) that are fully contained in regions previously described as normal variants in the Database of Genomic Variants on Human Genome (DGV) were disregarded.

Validation of the chromosome imbalances detected by 1 Mb array CGH

The microdeletions and microduplications detected by array CGH were validated either by fluorescence in situ hybridisation (FISH) on metaphase spreads from patients’ cultured lymphocytes or by
multiplex ligation-dependent probe amplification (MLPA) (MRC-Holland, Amsterdam, The Netherlands). Fine mapping of the alterations ascertained by 1 Mb array-CGH

More detailed mapping of the microimbalances was performed with the “Syndrome Plus” arrays containing 105K oligonucleotides from Oxford Gene Technology (http://www.ogt.co.uk) with whom we collaborate. The X-chromosome alteration (patient 4) was finely mapped using an X-chromosome tiling path array developed by the Nijmegen University (Nijmegen, The Netherlands). Labelling and hybridisations were performed according to their protocols.

Image acquisition and intensity measurements were made using a Laser Scanner GenePix 4000B and the GenePix software.

Table 1 Description of the clinical features of the patients, with their chromosome imbalances and mode of inheritance.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical description of patients</th>
<th>Type and extension of the imbalance</th>
<th>Parental origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3-year-old female child presenting complete uterine and vaginal agenesis, fused external labia and ovaries undetected by ultrasound examination</td>
<td>dup 2.7 Mb at 1q21.1</td>
<td>Both inherited from normal mother</td>
</tr>
<tr>
<td></td>
<td></td>
<td>dup 0.6 Mb at 22q11.22</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>del 2.6 Mb at 22q11.21</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>18-year-old female with mild to moderate learning disabilities, vaginal agenesis and a very rudimentary uterus (bilateral muscular buds), long face, prominent nose, short philtrum, high palate, slender hands, slight dorso-lumbar scoliosis, unilateral kidney agenesis at right, slight increase of the aortic arch and hypothyroidism.</td>
<td>del 1.2 Mb at 17q12</td>
<td>de novo</td>
</tr>
<tr>
<td>3</td>
<td>45-year-old female with mental impairment, complete absence of the uterus and vagina, seizures since the age of 1 year, mild facial asymmetry, slight deviation of the nasal root and maxilla, high palate, long and slender arms and legs, long and thin hands and feet, horizontal nystagmus and onychodystrophy</td>
<td>del 1 Mb at Xp21.31</td>
<td>Unknown—parents unavailable</td>
</tr>
<tr>
<td>4</td>
<td>32-year-old female with absence of uterus and upper third of the vagina, Sprengel deformity of the shoulder, short neck with limited motion, low posterior hairline, high palate, moderate to severe hearing impairment at right, and discrete brachidactyly in upper limbs</td>
<td>del 1 Mb at Xp21.31</td>
<td>Unknown—parents unavailable</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Five submicroscopic copy number alterations were identified in four out of 14 (29%) patients with MA tested. These alterations were all independently ascertained and do not overlap. Table 1 presents the clinical features of the affected probands carrying chromosome imbalances, as well as the size, position and mode of inheritance of the chromosome alterations. Figure 1 illustrates the process of detecting chromosome alteration by 1 Mb array screening, its confirmation by FISH and its fine mapping by oligo-arrays. Figure 2 displays the physical map of the four chromosome regions exhibiting genomic imbalances.

Patient 1

This patient carries two duplications, one at 1q21.1 and the other at 22q11.21. Both imbalances were inherited from her phenotypically normal mother. The 1q21.1 duplication (fig 2A) region spans ~3.5 Mb, and contains small segments scattered in the area which have been previously described as normal variation (DGV). However, polymorphic variation has not been reported in the distal area of the duplication, which encompasses both the recurrent microdeletion/microduplication (Ensembl Genome Database) and the thrombocytopenia-absent radius syndrome (TAR, OMIM 274000) susceptibility segments. A recent study\(^5\) showed that 50 investigated TAR syndrome patients had variable deletions within this 1q21.1 region, including a common 200 Kb segment that is duplicated in our patient. The majority of these TAR patients (75%) inherited the chromosome alteration from a phenotypically normal parent. Interestingly, some cases of TAR syndrome have been reported in conjunction with MA.\(^2\) These data suggest that genetic factors for TAR and MA phenotypes map to this 1q21.1 area, and that incomplete penetrance and variable expressivity probably occur.

As a complicating factor, patient 1 exhibits an additional ~1 Mb duplication that partially overlaps the distal segment of the 22q11.21 microdeletion/microduplication syndromes region (fig 2C). This area is partially covered by CNVs, except for a ~200 Kb segment containing several genes. The 22q11 duplication syndrome presents variable phenotype, often very mild to normal, but the absence of müllerian derivatives was never recorded.\(^13\)

Patient 2

This patient has been previously reported by us\(^6\) for presenting uterus agenesis associated with a de novo 22q11.21 deletion in the DiGeorge/velocardiofacial syndrome region (DG/VCFs, OMIM 158400/192450). Deletions in this region had only been previously associated with MA in female fetuses,\(^14,15\) but recently two other reports of females with absent uterus and deletions in this region strengthen our results.\(^16\) We have now further mapped the rearrangement in our patient, which revealed that the deletion spans 2.5 Mb but is in fact interrupted by a small chromosome segment with apparently normal copy number (fig 1B, fig 2C). The non-deleted segment contains the \(TBX1\) gene, the main gene responsible for five major clinical features associated with the del 22q11.21 syndrome, namely: conotruncal anomaly face, cardiac defects, thymic hypoplasia, velocardiofacial insufficiency with cleft palate, and parathyroid dysfunction with hypocalcaemia. Accordingly, our patient does not present features of \(TBX1\) deletion. Further, she displays features often present in DG/VCFs but not linked to alterations of the \(TBX1\) gene, namely: learning disabilities, a facial type suggestive of 22q deletion, scoliosis, and kidney agenesis.

Patient 3

This patient has a de novo ~1.3 Mb deletion on 17q12, which includes the region of the renal cyst and diabetes syndrome (RCAD; OMIM 137920) (fig 1A, fig 2B). This syndrome is caused by mutations or deletions of one copy of the gene encoding hepatocyte nuclear factor-1-\(\beta\) (TCF2), and is characterised by renal anomalies, with or without diabetes.\(^17\) In spite of her TCF2 haploinsufficiency, patient 3 has neither diabetes nor renal malformations. Further, she presents severe learning disabilities, seizures, absent uterus, and other clinical features that are not part of the RCAD syndrome. Abnormalities of the genital tract, including rudimentary or bicornuate uterus, have sporadically been described in patients with TCF2 mutations, and the deletion of TCF2 in our patient has possibly added to her MA. However, other genes map within her 17q12 deletion and likely contributed to her phenotype, particularly the \(LHX1\) gene. No phenotype has ever been associated with alterations of the \(LHX1\) gene in humans,\(^18\) but studies in mice\(^19\) show that \(lim1\), which is 99.5% homologous to the human \(LHX1\) gene (GeneCards), is expressed in the epithelium of the developing müllerian duct during its formation. Female \(lim1\)-null mice present normal ovaries but completely lack all derivatives of the müllerian ducts (oviducts, uterus, cervix and the upper region of the vagina); similarly, our patient has complete absence of uterus and vagina (she refused oviducts investigation) but displays normal ovaries and secondary sexual characteristics. On the other hand, it is important to observe that the \(lim1\) heterozygous mice, which would correspond to our patient’s \(LHX1\) haploinsufficiency, do not show any müllerian phenotype. Conversely, \(lim1\)-null mice usually die before birth, and escapers, in addition to MA, also lack anterior head and kidney formation; our patient has no kidney abnormalities and, although she exhibits some syndromic features, her phenotype is far milder than the one manifested by the \(lim1\)-null mice. If we postulate that \(LHX1\) is causally connected to MA, then humans must have a different dosage threshold response than mice to its product, manifesting MA even when the gene is in haploinsufficiency.

Patient 4

The patient carried a ~1 Mb deletion on Xq21.31, which was finely mapped using a tiling path array for chromosome X.\(^11\) The lost segment encompasses a single gene, \(KLHL4\), which therefore appears as candidate for the phenotype (fig 2D). This gene is widely expressed in fetal tissues, and has also been detected in the female reproductive system and kidney, but its function is still unknown. Alternatively, the adjacent genes \(DACH2\) and \(POFB1\), related to organogenesis and premature ovarian failure (POF), respectively, may have altered expression due to their close proximity of the rearrangement. Association between POF and müllerian developmental abnormalities has been previously reported.\(^20\) One could argue that preferential inactivation of the \(X\) carrying the deletion may lead to the manifestation of any recessive gene present in its normal homologue, but the X
Figure 2  Physical map of the chromosome regions found duplicated or deleted in four patients. The chromosome bands, corresponding phenotypes and candidate genes are represented.
inactivation pattern of this patient did not indicate a major skew, at least in blood (data not shown).

CONCLUSIONS

Two patients presented de novo chromosome alterations, namely patients 2 and 3. The parental origin of the deletion on chromosome Xq21.31 in patient 4 could not be determined because the patient refused her parents to be investigated. Our data implicate the altered regions in MA, and point in particular to LHX1 and KLHL4 as candidate genes.

The chromosome alterations in patient 1, which were inherited from her phenotypically normal mother, have unclear significance and represent a problem for a genetic counselling. In the last 2 years, a number of papers have documented the unexpected large scale CNV occurring in normal individuals.5 It is possible that some of these variants represent increased risk factors for particular phenotypic anomalies whose clinical manifestation may be dependent on other genetic and environmental factors. The MA in this patient has been associated to LHX1 and KLHL4 as candidate genes.

Multifactorial/polygenic inheritance of MA has long been postulated,1 1 and our study supports the multiplicity of genetic factors probably involved in the genesis of müllerian defects. Our data point to new chromosome regions possibly harbouring genes related to MA, although the genotype–phenotype correlation becomes more difficult to establish as we move further away from simple monogenic dominant patterns of inheritance. In this regard, initiatives such as DECIPHER, which compile copy number alterations in specific phenotypes, are more helpful and allow us to understand the phenotypic sequelae of such variations.

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Competing interests: None declared.

Patient consent: Informed consent was obtained for publication of the patients’ details in this report

REFERENCES