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Research Letter

Report of a del22q11 in a Patient With Mayer-Rokitansky-Küster-Hauser (MRKH) Anomaly and Exclusion of *WNT-4*, *RAR-gamma*, and *RXR-alpha* as Major Genes Determining MRKH Anomaly in a Study of 25 Affected Women

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To the Editor:

Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, comprising utero-vaginal atresia in otherwise phenotypically normal women with a normal karyotype (46,XX), has an incidence of about 1/5,000 among newborn girls. Anomalies of the genital tract range from upper vaginal atresia to total Müllerian agenesis (congenital absence of the Fallopian tubes, uterus, and upper vagina). Since the development of the urinary tract takes place concomitantly with the Müllerian ducts, affected women frequently also have renal anomalies. Skeletal and auditory defects are also frequently found, while other associations are rare.

Mendelsohn et al. [1994] and Kastner et al. [1997] described MRKH-like defects in mice with mutations in the genes controlling the synthesis of retinoic acid receptors (*RXR* and *RAR*). In humans, the gene for the *RAR-gamma* receptor is located in 12q; Kucheria et al. [1988] described two unrelated girls with Müllerian duct agenesis who carried an autosomal translocation (12;14)(q14;q31) involving the same region. Recently, Biason-Lauber et al. [2004] described a mutation in the *WNT-4* gene in a woman with Müllerian agenesis and a mild degree of virilization, a finding not associated with the MRKH phenotype.

We studied 25 women with the MRKH defect. The affected women also had vertebral (7/25) and other skeletal defects (6/25) including Klippel-Feil and Sprengel anomalies, clinodactyly, brachydactyly, and syndactyly of digits and congenital hip dislocation. Kidney defects (10/25), cardiac anomalies (2/25), and hearing impairment (2/25) were also found. All patients had a normal 46,XX karyotype and were normal as to the *RAR-gamma*, *RXR-alpha*, and *WNT-4* genes. The study included clinical and ultrasonographic examination of the urogenital system and radiographs of the vertebral column. Single-strand conformation polymorphism (SSCP) was performed

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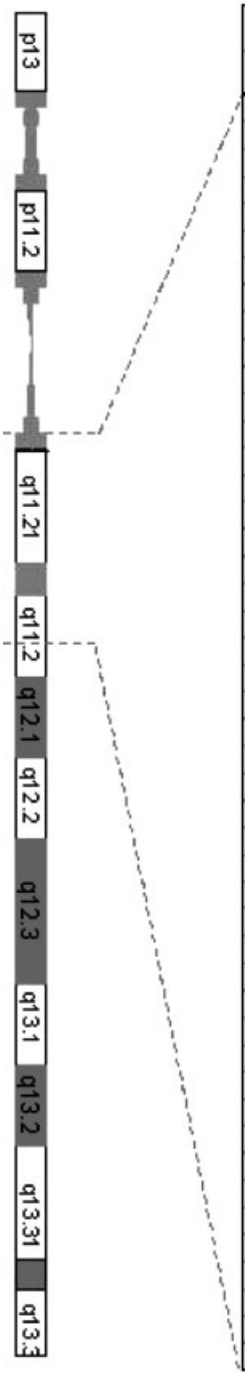
as the mutation screening method in PCR products of the *RAR-gamma*, *RXR-alpha*, and *WNT-4* genes. Amplicons that showed altered bands by SSCP analysis were sequenced (MegaBace™), allowing the identification of the following single nucleotide polymorphisms (SNP) previously described: in the *RAR-G* gene a C > T substitution at 7–8 intron zone (IVS 7 + 21C > T) in two patients and one relative, and a C > T substitution in exon 8 in six patients (c.1280C > T). For the *WNT-4* gene, base substitutions were also detected in two patients in exon 5 (c.1026C > T). No obviously pathogenic mutation was detected in any of the three genes investigated. The recent report of Clément-Ziza et al. [2005],



Position on chr.22 (Mb)	Gene name	MLPA probe	DGS/VCFS deletion	Deletion in our patient
15.94	IL17R		+	+
15.97	*CECR6*		+	+
15.99	*CECR5*		+	+
16.03	*CECR1*		+	+
16.33	*CECR2*		+	+
16.48	BCL2L13		+	+
16.60		BID	+	+
16.81	Q8N9H1_HUMAN		-	?
17.69	HIRA		-	?
16.64	O94909_HUMAN		-	?
17.27	*DGCR6*		-	?
17.39	*DGCR2*		-	?
17.49	TSSK2_HUMAN		-	?
17.49	*DGCR14*		-	?
17.54	CLTCL1		-	?
17.70		HIRA	-	-
17.79	MRPL40		-	-
17.84	CDC45L		-	-
17.88		CLDN5	-	-
18.07	SEPT5		-	-
18.11	TBX1		-	-
18.20		KIAA1652	-	-
18.44	*DGCR8*		-	-
18.48	RANBP1		-	-
18.49	ZDHHC8		-	-
18.57	Q6ICL0_HUMAN		-	-
18.68	*DGCR6L*		-	-
18.88		FLJ14360	-	-
19.04	USP41		-	-
19.10	SCARF2		-	-
19.19		PCQAP	-	-
19.39	PIK4CA		-	-
19.45	SERPIND1		-	-
19.54		SNAP29	-	-
19.6	CRKL		-	-
19.66		LZTR1	-	-
20.25	UBE2L3		-	?
20.44	MAPK1		-	?
20.60	PPM1F		-	?
20.63	TOP3B		-	?
20.70	IGLV4-69		-	?
20.87	IGLV6-57		+	?
20.92	VPREB1		+	?
21.81	RAB36		+	?
22.45	SMARCB1		+	?
22.52	SLC2A11		+	?
22.56		MIF	+	+

FIG. 1. Deletion found in our MRKH patient, as compared to the maximum region size of the 22q11 deletion thus far reported in the DGS/VCFS showing the mapping of the MLPA probes we used and the location of other genes known to be in the region. The deleted (−), present (+) and unknown (?) regions found in our patient are indicated at the table's last column. The critical regions for DeGeorge syndrome (DGCR) and cat eye syndrome (CECR) are also shown.

excluding the *WNT-4* gene as a major gene in determining the MRKH anomaly, is thus fully confirmed by the results here presented.

Five of our patients, with more complex phenotypes (genital, renal, cardiac, and skeletal defects), were selected for comparative genomic hybridization microarrays (array-CGH). Slides containing triplicates of ~3,500 large insert clones spaced at ~1 Mb density over the full genome were produced in the Leiden University Medical Center. The large insert clones set used to produce these arrays was provided by the Wellcome Trust Sanger Institute (UK); information on the contents of the full set is available at the Wellcome Trust Sanger Institute mapping database site, Ensembl (http://www.ensembl.org/). In one of these five patients (Fig. 2), microarrays detected a large deletion (~4 Mb in size) at 22q11, which was confirmed and further mapped by Multiplex Ligation-dependent Probe Amplification (MLPA) using the SALSA P023 DiGeorge MLPA kit (Fig. 1), kindly donated by MRC-Holland (Amsterdam, Netherlands), and was used according to the manufacturer's instructions [Schouten et al., 2002]. All probes included in the SALSA P023 DiGeorge kit are described at www.mrc-holland.com. Using five DNA polymorphic markers (D22S1638, D22S1648, D22S944, D22S1623, and D22S1709), we showed that the patient's mother does not carry the deletion. The father was not available for investigation, but the remaining normal five alleles in the patient were not shared by her mother, showing that the de novo deletion was maternally derived. She had a rudimentary uterus and vaginal agenesis with normal secondary sexual characteristics, and (17y4mo at examination) mild to moderate learning disabilities, and signs suggestive of 22q11 phenotype including minor craniofacial anomalies (long face, prominent nose, short philtrum, and high palate), slender hands, slight dorsolombar scoliosis, and a slight increase of the aortic arch. She was under treatment for hypothyroidism due to Hashimoto's thyroiditis, also present in her otherwise normal older sister. At examination, the patient's height was 161 cm, weight 54 kg, and OFC was 54 cm. A cerebral MRI examination showed minor lesions of white matter, secondary perhaps to ischemic lesions (the patient was born with severe Rh incompatibility problems that required complete blood exchanges 5 days after birth).

Deletions in chromosome 22q11 cause different congenital defects [Ryan et al., 1997]. DiGeorge syndrome (DGS) and velocardiofacial syndrome (VCFS) are the most common congenital anomalies associated with del22q11. Since both conditions are caused by the same deletion, they are often referred to as the DGS/VCFS syndrome. The most common deletion is 3 Mb in size, found in about 90% of the patients. Among patients with this deletion, 7% present a 1.5 Mb deletion; just a few have smaller deletions, unbalanced translocations or no detectable abnormalities in the region 22q11 [Edelmann et al., 1999]. Although the corresponding phenotype is highly variable, there exists no apparent correlation between the deletion size and the phenotype manifestations.

FIG. 2. Facial features of the patient with the 22q11 deletion.

The deletion found in our patient includes loci responsible for DGS/VCFS. Uterus anomalies are known to occur only in the 'cat-eye' syndrome, located at ~0.6 Mb distance from the deletion zone. To our knowledge, this is the first report of a patient with MRKH anomaly and a deletion in the DGS/VCFS region, if we do not consider as such one case of uterus anomalies previously reported in this region [Devriendt et al., 1997]: a 19-week female fetus with Potter sequence, MRKH anomaly and a 22q11 deletion inherited from the father, with VCFS, but without urological anomalies.

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