Does the Y chromosome have a role in Müllerian aplasia?

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Objective: To investigate whether Y chromosomal genetic material has a role in the development of Müllerian aplasia in Finland. We have studied the *TSPY1* gene and 38 additional male-specific fragments covering areas of both the long and short arms of the Y chromosome in Finnish patients with Müllerian aplasia.
Design: A retrospective study.
Setting: University hospital and genetic laboratory.
Patient(s): A sample set of 110 Finnish patients with well-diagnosed Müllerian aplasia and 20 healthy relatives (13 mothers, 4 fathers, and 3 sisters from different families) were included in the study. One hundred healthy female controls with a background of at least one normal pregnancy with delivery were used as controls.
Intervention(s): Blood samples for DNA extraction.
Main Outcome Measure(s): Detection of Y chromosomal fragments by polymerase chain reaction in female patients with Müllerian aplasia.
Result(s): None of the female patients showed presence of the earlier reported *TSPY1* gene or 38 additional Y chromosomal markers.
Conclusion(s): Our results indicate that the studied Y-specific fragments, namely *TSPY1* and 38 Y chromosomal markers, are not responsible for the syndrome in these Finnish patients with Müllerian aplasia. (Fertil Steril® 2010;94:120–5. ©2010 by American Society for Reproductive Medicine.)

Key Words: Müllerian aplasia, TSPY1, Y chromosome

Müllerian aplasia features primarily congenital absence of the vagina and functional uterus. Most patients with Müllerian aplasia have the normal female chromosome constitution (46,XX), hormonally active functioning ovaries, and normal secondary sexual characteristics. In Finland a population-based study showed that the minimum incidence of Müllerian aplasia is one in 5,000 newborn girls (1). The majority of these patients have Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome, the most common form of Müllerian aplasia, which is characterized by vaginal aplasia, rudimentary uterine horns, and normal or hypoplastic fallopian tubes (2). A less common form is total Müllerian aplasia, where all Müllerian derivatives (uterus, upper vagina, and fallopian tubes) are absent. Renal and skeletal anomalies occur in 10% to 40% of all patients with Müllerian aplasia (3).

Received November 17, 2008; revised February 2, 2009; accepted February 3, 2009; published online March 26, 2009.
M.S. has nothing to disclose. J.P. has nothing to disclose. M.P. has nothing to disclose. M.H. has nothing to disclose. H.L. has nothing to disclose. K.A. has nothing to disclose.
Supported by Victoria Foundation, Medicinska Understödsföreningen Liv och Hälsa Foundation, Päivikki and Sakari Sohlberg Foundation in Finland.
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The etiology of Müllerian aplasia is complex. Familial clustering has been noted but is rare, and the most favored hypothesis is multifactorial inheritance (4). This is strengthened by the fact that Müllerian anomalies were not found in female children born to patients with Müllerian aplasia through surrogacy (5). Several candidate genes, such as the anti-Müllerian hormone gene (*AMH*) and its receptor (*AMHR*) (6–8), as well as members of the *WNT* and *HOXA* gene families (9–11), have been investigated for mutations in patients with Müllerian aplasia. Given that only three patients have mutations in *WNT4*, the cause of the syndrome for the majority of the patients remains unknown.

During early embryonic development in males the anti-Müllerian hormone (AMH) causes the regression of the Müllerian ducts shortly after initiation of SRY (sex-determining region Y) expression. In Müllerian aplasia the partial regression of the Müllerian duct derivatives resembles this phenomenon, which in males is regulated by a number of genes, including *SRY* residing on the Y chromosome.

Recently, Plevraki et al. (12) reported the presence of fragments of a Y chromosomal gene, testis-specific protein 1-Y-linked (*TSPY1*), in two out of six females in whom MRKH syndrome was diagnosed. The biologic function of *TSPY1* is unclear, but it is a candidate oncogene for gonadoblastoma and is supposed to function as a proliferation factor during spermatogenesis (13). Although it may not be *TSPY1* per se

0015-0282/$36.00
doi:10.1016/j.fertnstert.2009.02.004

that causes the Müllerian aplasia syndrome, this finding suggests that the presence of Y chromosomal fragments could play a role in the etiology of Müllerian aplasia.

The aim of this study was to investigate whether male-specific Y chromosome fragments were present in Finnish patients with Müllerian aplasia, and therefore have a role in development of Müllerian aplasia. In addition to attempting to amplify fragments of the *TSPY1* gene, we also included 38 additional loci to cover most areas of the male-specific Y chromosome. The sample set we investigated includes 110 patients with Müllerian aplasia and 20 relatives, which comprises, to our knowledge, a significantly larger sample series than in any published studies concerning this syndrome to date.

MATERIALS AND METHODS

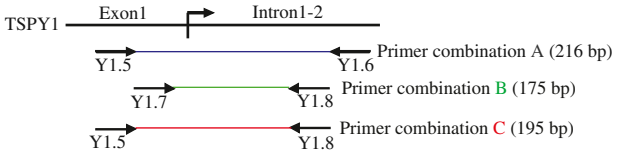
Blood samples were collected from 110 Finnish patients with Müllerian aplasia and 20 relatives (13 mothers, 4 fathers, and 3 sisters from different families) through the Departments of Obstetrics and Gynecology of the five University Hospitals (Helsinki, Kuopio, Oulu, Tampere, and Turku) in Finland. Genomic DNA was extracted with use of the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN) according to the manufacturer's recommendation. One hundred female control samples were collected from the Department of Obstetrics and Gynecology of the Helsinki University Hospital, and women who provided these samples had at least one normal pregnancy with delivery. Healthy male controls (four for the *TSPY1* studies and one for the additional Y chromosomal fragment studies) were obtained from the Finnish Red Cross for validation of the polymerase chain reaction (PCR) method and for verification of sequencing products. The study protocol has been approved by the Finnish Ministry of Social Affairs and Health and by the Ethics Committee of the Department of Obstetrics and Gynecology, Helsinki University Hospital, Finland.

TSPY1

The presence of the *TSPY1* gene fragment (NT_011878, NCBI) was initially analyzed by PCR with two primer pairs (Fig. 1, combinations A and B) according to Plevraki et al. (12). In brief, the first PCR was performed with use of primers Y1.5 (5′ CTA GAC CGC AGA GGC GCC AT 3′) and Y1.6 (5′ TAG TAC CCA CGC CTG CTC CGG 3′). A second reaction (nested PCR) using the above PCR product as a template then was performed with flanking primers Y1.7 (5′ CAT CCA GAG CGT CCC TGG CTT 3′) and Y1.8 (5′ CTT TCC ACA GCC ACA TTT GTC 3′) (Fig. 1, combination B). In contrast to Plevraki et al., we obtained fragments in all female samples for both of these reactions but of a different size than in the male samples. Therefore, we attempted to optimize the specificity of the PCR fragments by using the same primers, Y1.5, Y1.6, Y1.7, and Y1.8 primers, in two other combinations. Only primer combination Y1.5 and Y1.8 (Fig. 1, combination C) gave a *TSPY1*-specific product confirmed by sequencing. All samples were analyzed subsequently with this primer combination.

FIGURE 1

Polymerase chain reaction primers used for amplification of the *TSPY1* gene (NT_011878, NCBI) according to Plevraki et al. (12). Base positions of the primers (5′–3′) are Y1.5 (9996136–9996155), Y1.6 (9996332–9996352), Y1.7 (9996156–9996176), and Y1.8 (9996311–9996331). The primers were used in three different combinations (*A, B,* and *C*). We obtained a *TSPY1*-specific PCR product using primer combination *C*.



Sandbacka. Y chromosome in Müllerian aplasia. Fertil Steril 2010.

Each PCR was performed in a 15-μL reaction mix containing 133 nmol of each deoxynucleoside-triphosphate (dNTP) (Finnzymes, Espoo, Finland), 667 nmol of forward and reverse primers, 1× PCR buffer including $MgCl_2$, 0.5 units AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA), and 100 ng of patient, relative, and control DNA or 50 ng of male control DNA. Amplification conditions were as follows: 10 minutes of initial denaturation at 95°C, followed by 35 cycles of 30 seconds at 95°C, 64°C, and 72°C, respectively, followed by final extension at 72°C for 10 minutes. Amplification products were verified by electrophoresis through 1.5% agarose gels and visualized by staining with ethidium bromide. Product sizes were determined with use of a size marker (Quick-Load 100 base pair [bp] DNA Ladder; New England BioLabs, Ipswich, MA). Negative and positive controls were run concurrently. To verify whether the amplified fragments were from the *TSPY1* gene, fragments from four male controls were sequenced with BigDye version (Applied Biosystems) and compared with the UCSC database with BLAT searches (http://genome.ucsc.edu). Amplification products of the *TSPY1* gene in females were carefully sequenced and compared with the UCSC database.

Additional Y Chromosomal Markers

In addition to *TSPY1*, 38 loci covering both the long and short arms of the Y chromosome (Fig. 2) were analyzed for all samples. The analyzed loci included a set of 33 Y chromosomal markers (sY84, sY134, sY117, sY102, sY151, sY94, sY88, sY283, sY157, sY158, sY81, sY182, sY147, sY86, sY105, sY82, Y6PHc54pr, sY97, sY14, sY254, sY95, sY127, sY149, Fr15-lipr, Y6HP52pr, Y6D14pr, sY160, sY144, sY255, sY159, sY277, Y6HP35pr, and sY145), which we refer to as the Y panel. To further improve the coverage of the Y chromosome, more loci were found for the short arm by searching the NCBI UniSTS database