Quantification of the Familial Contribution to Müllerian Anomalies

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OBJECTIVE: To quantify the familial contribution to müllerian anomalies and determine a possible inheritance pattern.

METHODS: Cases of müllerian anomalies, identified by International Classification of Diseases and Current Procedural Terminology codes from January 1994 to March 2006, were collected from the largest hospital systems in the state of Utah. All records were subsequently matched to the Utah Population Database. Controls for this data set were randomly selected and matched based on birth year and gender. Highly specialized software "Kinship Analysis Tools (KAT)" was used for kinship analysis.

RESULTS: A total of 1,397 cases qualified for the final analysis. The kinship analysis tool identified 27 family clusters. The mean familial standardized incidence ratio was 3.43($P<.01$). Using the adjusted "Population Attributable Risk," approximately 10% of cases of müllerian anomalies appear to be attributable to a familial association. The relative risk for müllerian anomalies in each class of kinship was as follows: first-degree relatives 11.6 (95% confidence interval [CI] 5.42–24.82), parents/children 8.78 (95% CI 2.26–34.16), siblings 12.98 (95% CI 5.17–32.62), first cousins 1.44 (95% CI 0.76–2.76), and second cousins 1.30 (95% CI 0.96–1.77).

CONCLUSION: Müllerian anomalies have a strong familial aggregation and follow a polygenic and multifactorial inheritance.

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LEVEL OF EVIDENCE: II

Müllerian anomalies are perhaps the most common of all developmental anomalies. They have been identified in approximately 2–3% of fertile women.[1] Because they are frequently undiagnosed, a precise estimation of their contribution to poor reproductive outcomes is not available. But uterine anomalies are associated with increased risks for spontaneous abortion, infertility, ectopic pregnancies, preterm labor, and fetal malpresentation.[2,3]

The etiology of these developmental disorders is unknown. It has been suggested that genetic factors may contribute to the formation of müllerian anomalies.[4–9] Exposure to environmental xenobiotics during fetal life was also proposed as a potential contributor to the development of müllerian anomalies.[10–15] In consequence, müllerian anomalies would likely be found in families that share similar genetic predisposition and environmental exposures. Evidence for a familial predisposition for müllerian anomalies has been presented in multiple reports examining individuals or small numbers of families.[16–19] However, the inheritance pattern remains poorly defined, and work to date has been limited by the relatively low incidence of the malformations, incomplete diagnosis, and the variability of phenotypic expression. The ability to identify multiple families that have multiple individuals diagnosed with müllerian anomalies is a critical step in understanding the familial character and the mode of inheritance of these anomalies. The University of Utah has a powerful tool, the Utah Population Database, that allows the identification of such families. This database provides access to data concerning approximately six million individuals.

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The central component of the Utah Population Database is an extensive set of Utah family histories, in which family members are linked to demographic and medical information. There are about six million individuals linked into multi-generational families with pedigrees spanning as many as 11 generations.[20] The statistical tools available through the Utah Population Database have facilitated the familial analyses of a number of diseases and conditions, such as preeclampsia and cancers.[21–24] In this study, the Utah Population Database was used to perform a kinship analysis of a large number of individuals affected with müllerian anomalies to describe the familiality of the disease and to determine possible inheritance patterns.

MATERIALS AND METHODS

After respective institutional review board approvals from the University of Utah and Intermountain Health Care, data were collected for all patients with müllerian anomalies who were diagnosed in hospitals and related clinics of the two major health provider systems in the state of Utah (University of Utah and Intermountain Health Care system) over the period extending from January 1994 to March of 2006. The extent of the study was mainly determined by the availability of computerized diagnosis and billing records in the hospitals because most of cases were identified through the International Classification of Diseases, 9th Revision (ICD-9) and Current Procedural Terminology codes. The ICD-9 codes used for the screening for müllerian anomalies were the following: 752.2 (doubling of uterus) and 752.3 (other anomalies of uterus). The Current Procedural Terminology used were 58560 (hysteroscopy with division or resection of uterine septum, 57130 (excision of vaginal septum), and 58540 (hysteroplasty with repair of uterine anomaly (Strassman type). The accuracy of these ICD-9 and Current Procedural Terminology codes in reflecting the correct diagnosis of müllerian anomalies was tested by reviewing the medical record of a random sample of 346 patients. The accuracy of the diagnosis of müllerian anomaly reflected by the ICD-9 and Current Procedural Terminology codes was 91.9% (318 of 346). The distribution of anomalies in the 318 affected patients is given in Table 1. The remaining patients had the following diagnosis: eight uterine polyps, seven leiomyomata, four Asherman syndrome (intrauterine adhesion), three ovarian cysts, one vaginal hysterectomy, one cesarean for breech, one dilation and curettage for miscarriage, one tubal occlusion on hysterosalpingogram, one normal hysterosalpingogram, and one uterine cancer.

Table 1. Distribution of Müllerian Anomalies in the Sample Patients

Diagnosis	No. of Patients	Frequency (%)
Septate incomplete	87	27.4
Bicornuate	76	23.9
Septate complete	41	12.9
Didelphis	29	9.1
Arcuate	27	8.5
Unicornuate	27	8.5
Müllerian aplasia	15	4.7
Transverse septum	5	1.6
Longitudinal septum	4	1.3
Possible septum	4	1.3
Septate vs bicornuate	3	0.9
Total	318	100.0

The majority of hospitals and related clinics (90%), including the major health systems in the state, provided the requested records allowing the identification of most cases of müllerian anomalies diagnosed in the state of Utah over the last 12 years. The geographic distribution of these hospitals covered all the populated areas of the state of Utah (Fig. 1). After collection of cases, patients' identifiers were used to link patients to matching data present in the Utah Population Database. Controls were individuals who did not have uterine anomalies (not part of the case data set) and were randomly selected from the Utah Population Database by matching based on birth year and female gender. Five controls were selected for each case, and sampling was done without replacement so as not to use the controls multiple times.

The kinship analysis was conducted by working with software developed and managed by the Utah Population Database. Highly specialized software, Kinship Analysis Tools (KAT; University of Utah, Salt Lake City, UT), was used to estimate the magnitude of familial risk.[25] These programs are highly efficient and specifically written to take advantage of the particular resources of Utah Population Database. There are two sets of programs that the statistical team at the Utah Population Database uses, termed Dynaped and Kinclass. The Dynaped allows calculation of familial disease incidence, familial average phenotypes, identification of founders and estimation of individual family members' relative risks via pedigree-structured Poisson regression, and extension of the above methods to alternative inheritance models. The Dynaped kinship analysis tool was used to find families with excess müllerian anomalies. Statistics computed for each family were the number of descendants, observed number of affected, expected number of affected, P value, familial standardized incidence

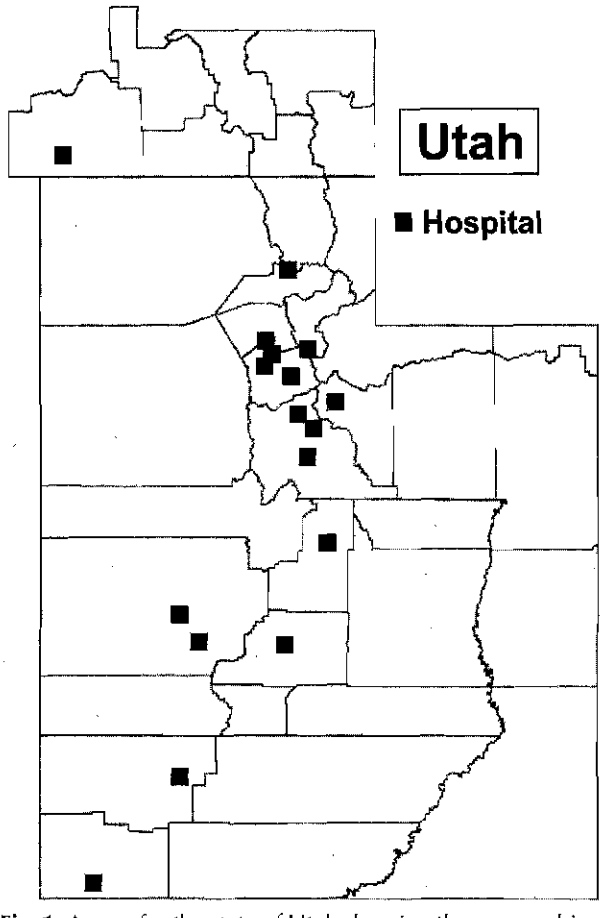

Fig. 1. A map for the state of Utah showing the geographical distribution of the hospitals that participated in the study. These hospitals serve most of the populated areas of the state.

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ratio, and relative risks. A familial standardized incidence ratio is a kinship-weighted average of the ratio of observed to expected incidence of disease among family members.[25] The results were filtered to detect families that had at least five affected descendants and a familial standardized incidence ratio statistically different from 1 ($P \leq .01$) to identify families with a clustering of anomalies. In these families, the observed number of anomalies exceeded the expected number. Expected numbers were estimated by multiplying the overall population prevalence by the number of descendants in a family who could have been observed to have the disease. Also using the case control analysis, Dynaped allowed the calculation of the Population Attributable Risk, which is the proportion of müllerian anomalies in our data set that can be attributed to familiality. The second program, Kinclass, determines kinship relationships for a set of individuals according to a desired set of criteria, such as first- and second-degree relatives who are still alive. The Kinclass program was used to compute the logistic regression for müllerian anomalies using the patients and the same set of controls and subsequently calculate the relative risk of having müllerian anomalies in each kinship class.

RESULTS

We identified 1,985 cases of suspected müllerian anomalies based on the general ICD-9 and Current Procedural Terminology codes used for screening. Three hundred thirty cases in the data set could not be linked to Utah Population Database. Among the 1,655 cases found in the Utah Population Database, eight cases were listed twice in the data set, with two different project identification numbers, and 250 cases did not have parents or children recorded in the database, so they were dropped from the analysis. The final number of cases available for the final the analysis that follows was 1,397 (Fig. 2).

Using the kinship analysis function, we identified 29 founders with families that had from five to 14

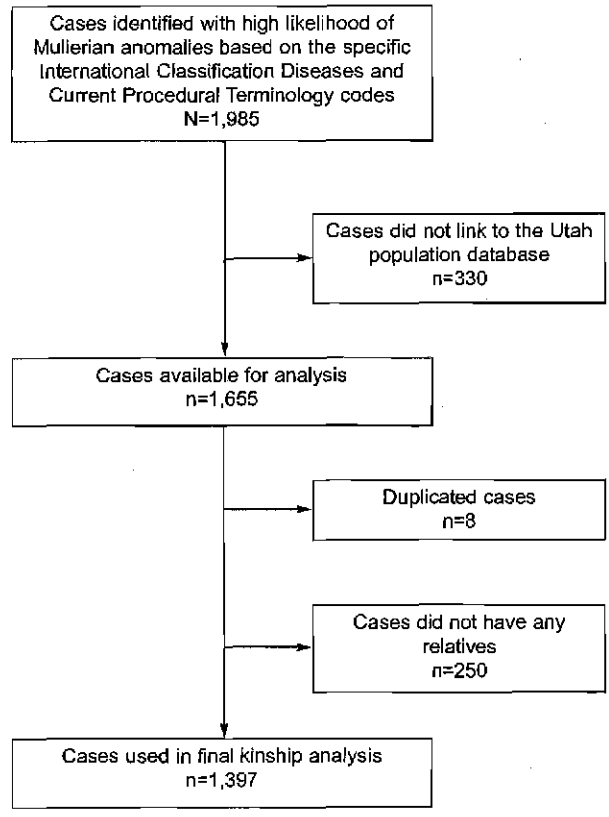

Fig. 2. A diagram of the study population.

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Table 2. Family Clusters

Founder	No. of Descendants	No. of Affected Family Members	Familial Standardized Incidence Ratio	95% Confidence Interval	Clusters
1	6,126	8	3.2	1.4–6.3	1
2	5,146	7	3.3	1.3–6.8	1
3	6,632	8	3.4	1.5–6.7	2a
4	3,767	7	4.6	1.8–9.5	2b
5	4,128	6	3.6	1.3–7.8	2c
6	14,425	9	2.26	1.0–4.2	2d
7	4,422	7	3.9	1.6–8.0	3
8	2,016	5	6.1	2.0–14.2	4
9	2,702	5	4.5	1.5–10.5	5a
10	2,294	6	6.4	2.3–13.9	5b
11	4,525	6	3.4	1.2–7.4	6
12	2,951	5	4.2	1.4–9.8	7
13	6,074	7	3.2	1.3–6.6	8
14	9,676	7	2.9	1.2–6.0	9
15	793	5	16.3	5.3–38.0	10
16	1,079	5	11.7	3.8–27.3	10
17	3,557	6	4.2	1.5–9.1	11
18	4,719	8	4.1	1.8–8.1	12
19	5,482	8	4.0	1.7–7.9	13a
20	10,264	10	2.5	1.2–4.6	13b
21	1,376	5	8.7	2.8–20.3	14
22	4,071	6	3.5	1.3–7.6	15
23	16,601	13	2.1	1.1–3.6	16
24	4,445	6	4.1	1.5–8.9	17a
25	5,463	7	3.7	1.5–7.6	17b
26	2,502	5	5.2	1.7–12.1	18
27	7,001	8	2.8	1.2–5.5	19
28	12,986	12	2.3	1.2–4.0	20
29	4,217	6	3.7	1.4–8.1	21

affected descendants and a pedigree size ranging from 793 to 16,602 descendants (Table 2). Founders are individuals for whom there are no ancestral genealogical relationships in the Utah Population Database. Thus, they are the earliest generation in the database. Further analysis identified the 256 affected descendants of these 29 founders. Careful analysis of these affected individuals showed that they constituted 27 family clusters, with some clusters having more than one founder. Also, some of the clusters shared some,

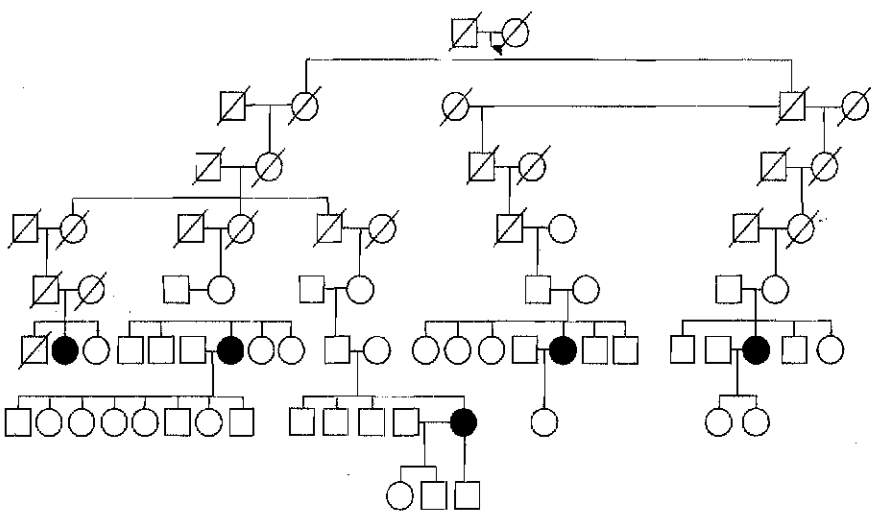

Fig. 3. A pedigree showing a family with an increased risk of müllerian anomalies by the study criteria (at least five affected family members and a familial standardized incidence ratio significantly different from 1). *Circle,* female; *square,* male; *crossed,* deceased; *black,* anomaly.

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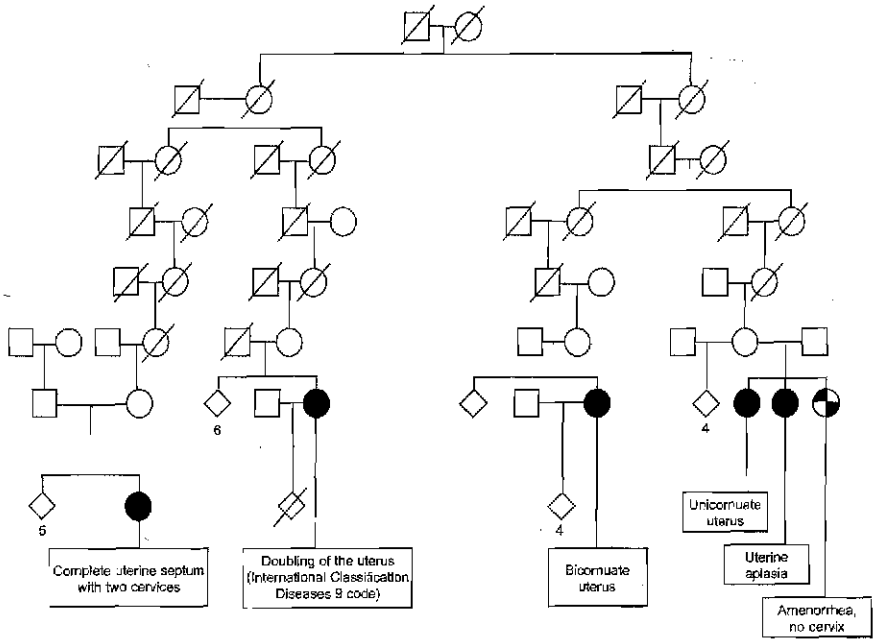

Fig. 4. A pedigree that has three affected sisters. One of the sisters presented with primary amenorrhea and the absence of a cervix on physical examination. *Circle,* female; *square,* male; *diamond,* sex undetermined; *crossed,* deceased; *black,* anomaly.

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but not all, affected descendants (Table 2). An example of these family clusters is given in Figure 3. Members of these families had a threefold higher risk of müllerian anomalies than controls (the mean familial standardized incidence ratio was 3.43, with $P<.01$, compared with all families combined). Apart from the previous family sets, we searched for families that had two or more affected first- or second-degree family members (mothers-daughters, sisters, and aunts-nieces). We successfully identified multiple families, including three sets of aunt-nieces, four sets of mother-daughters, 10 sets of two sisters, and two sets of three sisters. An example of such families is given in Figure 4.

The case control analysis was used to calculate the Population Attributable Risks. Using the adjusted Population Attributable Risks, the risk for müllerian anomalies attributable to familial affiliation approximates 10% (95% confidence interval 7–13%).

The relative risks for each kinship class were computed using conditional logistic regression. The Kinclass program uses the same control set that Dynaped used above, with five controls per case matched based on birth year and gender. The relative risk per kinship class is given in Table 3. There appears to be a nearly 12-fold increase in risk for müllerian anomaly for first-degree relatives of affected individuals. The relative risk for first cousins is approximately 1.4, and for second cousins it is 1.3. The relatively large drop in risk from siblings to cousins suggests a polygenetic/multifactorial mode of inheritance.

Table 3. Relative Risk per Kinship Class

Relationship	Relative Risk	95% Confidence Interval	*P*
First-degree relatives	11.6	5.42–24.82	<.001
Parents/children	8.78	2.26–34.16	.001
Siblings	12.98	5.17–32.62	<.001
First cousins	1.44	0.76–2.76	.29
Second cousins	1.30	0.96–1.77	.11

DISCUSSION

In this study, we found strong evidence for familiality contributing to müllerian anomalies. Differentiating between genetic and environmental contributions is particularly difficult when studying families because family members most often share not only genetic predisposition but also environmental exposures. The relative risk of having a müllerian anomaly in a first-degree relative of an affected individual is more than 12 times higher than controls. Increased risk remains detectable in distant relatives as far as second-degree cousins, denoting a strong genetic element. However, in addition to genetic predisposition, socioeconomic and geographic factors (such as environmental exposures and access to health care) may also contribute to the development of and/or detection of müllerian anomalies. This is suggested by the magnitude of the elevated risk in immediate family members (parents and siblings) in comparison with the modest increase in second cousins. Indeed, the pattern of familial clustering of cases of uterine malformations is consistent with polygenetic/multifacto-

rial disorders. In these conditions, genetic predisposition and local or environmental factors contribute to the likelihood of diagnosis of müllerian anomalies for close family members, whereas more distant relatives are only affected by genetic predisposition.

A potential source of bias in this study may be due to the possibility of case clustering among close relatives owing to heightened awareness of these conditions and common access to diagnosis. Alternatively, müllerian anomalies can be associated with normal reproductive function and can remain undiagnosed in affected individuals. The undiagnosed cases will contribute to an underestimation of the prevalence of müllerian anomalies and to an underestimation of their familial character. We believe that targeted screening of families of cases and families of controls using three-dimensional ultrasonography or magnetic resonance imaging will yield a stronger evidence of familiality.

This analysis included different types of müllerian anomalies that may be caused by different genetic alterations. This may account for the varying phenotypic expressions observed in this study. A kinship analysis of specific types of anomalies might yield a monogenetic phenotype. However, the observation that members of the same family had different phenotypic expression of müllerian anomalies (Fig. 4) does not support a specific genetic etiology for each type of anomaly.

In terms of the ability to generalize these finding, this study represents U.S. families with ancestry from Northern and Western Europe. Extensive investigation of the families in the Utah Population Database reveals that it is a noninbred population and is representative of the white population of the United States.[26,27] The representative nature of the population can be explained by several factors, such as the large founding size, the high rates of gene flow, and ancestors with diverse countries of origin. Using this resource, discoveries such as the *BRCA1* and *BRCA2* breast cancer mutation[28,29] and the *APC* gene mutation in colon cancer were made.[30]

Our study provides insight into the familial distribution of müllerian anomalies based on the analysis of a large number of affected individuals by using population analysis techniques unique to the Utah Population Database. This analysis should be extended by familial kinship studies based on different types of anomalies and by studies employing sensitive diagnostic techniques for uterine morphologic characterization within kindreds of interest. Comprehensive identification and characterization of müllerian anomalies within kinships would allow genetic linkage analysis to help identify potentially important genes underlying these common developmental anomalies. Also, a geographic localization of cases with correlation to known toxic exposures during gestation may provide evidence of an environmental contribution to the development of müllerian anomalies.

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