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Increased heritability of certain types of anorectal malformations

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Abstract

Purpose: Various lines of evidence point to genetic causes for the diverse spectrum of anorectal malformations (ARMs); we therefore studied patterns of heritability in a large case series.

Methods: We searched our ARM database for all patients having family members with congenital anomalies. This group was analyzed to determine the type of ARM and the specific anomalies in affected family members.

Results: Thirty-nine of 1606 patients (2.4%) had a family member with a congenital anomaly. The associated non-ARM anomalies included sacral masses and gynecologic, hematologic, esophageal, duodenal, renal, and spinal anomalies. Of these, 24 patients (1.4%) had 1 or more family members with an ARM. Among females with a positive family history, 73% of patients had either a vestibular or perineal fistula, compared with only 36% in patients without a family history ($P = .0004$). Among males, 35% had perineal fistulas compared with only 10% of those without affected family members ($P = .0051$).

Conclusions: A positive family history in 1.4% is supportive of a strong genetic component to ARM. The risk of having an affected family member is significantly increased in the presence of a vestibular or perineal fistula. These new data allow for more informed counseling of families with an ARM and support the need for further genetic studies.

Anorectal malformations (ARMs) represent a spectrum of abnormalities ranging from mild anal anomalies to complex cloacal malformations. The etiology of such malformations remains unclear and is likely multifactorial. There are however reasons to believe that there is a genetic component. As early as the 1950s, it was recognized that

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Table 1 Patients with more than 1 family member with a congenital anomaly

ARM in patient	Relationship of family member	Anomaly in family member
Vestibular fistula	Aunt	Vaginal atresia
	Uncle	Esophageal atresia
Vaginal fistula	Father	ARM
	Brother	ARM
	Uncle	Sacral tumor
Perineal fistula	Uncle	Currarino's syndrome
	Grandmother	Currarino's syndrome
Urethral fistula	Mother	ARM
	Uncle	ARM
Bladder fistula	Brother	ARM/Fanconi's anemia
	Brother	Aplastic anemia
Urethral fistula	Mother	ARM
	Brother	ARM
	Uncle	ARM

there was an increased risk for a sibling of a patient with ARM to be born with a malformation, as much as 1 in 100, compared with the incidence of about 1 in 5000 in the general population [1]. Since that time, there have been many reports describing families with 2 or more affected members and associations of ARMs with multisystem syndromes [2,3]. In particular, mutations in specific genes encoding transcription factors have been described in patients having Townes-Brocks syndrome [4,5], Currarino's syndrome [6,7], and Pallister-Hall syndrome [8,9], each of which have autosomal dominant modes of inheritance. In addition, it has been found that there is not only an increased incidence of ARM in patients with trisomy 21 (Down's syndrome), but that 95% of patients with trisomy 21 and ARM have imperforate anus without fistula, compared with only 5% of all patients with ARM [10]. Based on this evidence, it is likely that the mutation of a variety of different genes can result in ARM, or that the etiology of ARM is multigenic [2].

Despite apparent genetic associations, the lack of precise data makes counseling parents about the risk of ARM in future children or future generations challenging. Given the known association in trisomy 21 with a specific anomaly, we hypothesized that there would be different familial associations based on the type of ARM. In addition, we hypothesized that there would be an increased association with pelvic or genitourinary non-ARM congenital anomalies in family members secondary to gene abnormalities affecting development.

1. Methods

Our extensive database of patients with ARM was searched to identify patients in whom family members had ARMs or other congenital anomalies identified. Specific information regarding the type of ARM and associated anomalies was evaluated for each identified patient. Review of patient charts was used for all identified patients to supplement data from the database as needed. In addition, information about the anomaly identified and the relationship of the family member was reviewed. Patients were then divided into groups based on the classification of their anorectal anatomy [11] and analysis of associated anomalies performed.

Data analysis was performed using SAS v 9.1 (SAS Institute, Inc, Cary, NC). Comparisons were performed using Fisher's Exact test and relative risks calculated. Results were considered significant with $P < .05$.

This study was reviewed by the international review board and determined to be exempt.

2. Results

A total of 1606 patients with ARM were identified in our database. Of this group, 39 (2.4%) had at least 1 family member with a congenital anomaly. Six (15%) of these 39 patients had more than 1 affected family member (Table 1). Associated non-ARM anomalies within the index patients and family members were primarily genitourinary or pelvic anomalies (28 [72%] of 39 anomalies) (Table 2). The genitourinary or pelvic anomalies ranged from simple

Table 2 Associated non-ARM anomalies

Index patient (n = 39)	Family members (n = 46)
Cardiac (6)	Cardiac (0)
Ventricular septal defect (2)	
Atrial septal defect (4)	
Sacral mass (4)	Sacral mass (4)
	Currarino's syndrome (3)
Gastrointestinal (9)	Gastrointestinal (2)
Duodenal atresia	Duodenal atresia
Esophageal atresia	Esophageal atresia
Omphalocele (2)	
Malrotation (2)	
Duplicated appendix	
Ileal atresia	
Colonic atresia	
Genitourinary (16)	Genitourinary (4)
Duplicated müllerian structures (6)	Duplicated Müllerian structures
Bifid scrotum (3)	Bicornate uterus
Hypospadias (2)	Vaginal atresia
Single kidney (2)	Single kidney
UPJ obstruction	
Undescended testis	
Hematologic (0)	Hematologic (2)
	Fanconi's anemia
	Aplastic anemia
	Other (2)
	Meningocele
	Down's syndrome

UPJ, ureteral pelvic junction.

Table 3 Classification of ARM in those with affected family members compared with the entire series

Classification of ARM	% of those with affected family member	% of the entire series
Females		
Vestibular/perineal fistula	74*	37
Cloaca	18*	47
Atresia	5	0.5
Vaginal fistula	5	1
Males		
Bulbar fistula	35	27
Perineal fistula	35*	11
Bladder fistula	12	12
Prostatic fistula	6*	31
Anal stenosis	6	0.3

* $P < .05$ compared with the percentage of the entire series.

bicornate uterus and sacral lipoma to vaginal atresia and presacral teratomas.

A total of 24 (1.4%) of the 1606 patients had at least 1 family member with an ARM. The male to female ratio in this group was 1:2.4, compared with a ratio of 1:1.1 for the entire series. There were 14 siblings with ARM, of which 3 were twins.

The types of ARMs seen in those with affected family members differed from those observed in the series as a whole (Table 3). In males with a perineal fistula, there was a 7% chance (6 of 82 patients) of having an affected family member (relative risk, 3.45; 95% confidence interval, 1.75-6.79). Among females with a perineal or vestibular fistula, there was a 5% chance (16 of 312 patients) of having an affected family member (relative risk, 2.02; 95% confidence interval, 1.54-2.66).

Of the patients with a perineal or vestibular fistula, 3.0% (12/394) had a family member with an ARM and 5.6% (22 of 394) had a family member with some congenital anomaly. Given the estimated incidence of ARM in 1 in 5000 live births, these numbers place a relative of a child with a perineal or vestibular fistula at nearly 150 times increased chance of being affected.

In contrast, there was a reduction in relative risk of having an affected family member for patients with a cloaca (0.38, $P < .05$) or prostatic (0.18, $P < .05$) fistula; these anomalies were less common in patients with affected family members.

3. Discussion

We studied the familial incidence of ARM in the largest reported case series. Among all of the patients in the series, we found a 1.4% incidence of a positive family history for ARM, supporting the previous estimate of approximately 1% [1]. To date, no associations between specific types of ARMs and positive family history have previously been reported. Our analysis of this large case series, however, has revealed an increased association of specific types of ARM, namely, perineal or vestibular fistulas, with affected family members. Thus, patients with these types of ARMs have 2 to 3 times higher chance of having a family member with an ARM. Both the 1.4% overall incidence and 3% incidence in patients with perineal or vestibular fistula are significantly higher than would be predicted based on an overall incidence of ARM of 1:5000 (0.02%). These results strongly support a genetic component to the etiology of ARM. In addition, these findings are supported by previous findings of the EUROCAT working group, which reported epidemiologic differences among the various types of anal anomalies suggesting different embryological or genetic origins [12].

Before this study, parents of a child with an ARM or a family member with an ARM received counseling only regarding the approximately 1% chance of having another child with a malformation based on literature from the 1950s that included little detail [1]. Our study provides a first step in giving physicians information on risk based on specific classifications of ARM. Thus, based on our findings, parents of children with perineal or vestibular fistulas can now be told that there is a 3% chance of another family member being affected. In addition, parents of boys born with a perineal fistula or girls born with a perineal/vestibular fistula can now be counseled that there is a 7% or 5% chance, respectively, of having a family member with a congenital anomaly. Furthermore, in our series, there was less family transmission among patients with either cloacas or prostatic fistulas.

One potential difficulty with our study is that, despite its size, it is not population based, so there is the potential for bias based on our referral pattern and a disproportionate number of complex ARMs in our series. It is possible, given that most of the patients in this series were referred from other centers, that we actually see a slightly higher rate of those with affected family members. These families may in fact seek evaluation at our center because of their prior knowledge of ARM and treatment options. Despite these potential limitations, it is unlikely that these referral patterns have a significant enough impact to diminish the patterns observed. In the future, it would be desirable to gather similar data by performing a multiinstitutional or population-based review of patients with ARM. Such a review would likely be affected by difficulties in ascertaining the type of ARM and obtaining details of family history, information that has been actively sought and recorded in our database since its inception.

Adding support to the likelihood that ARMs represent genetic "inborn errors of development" is our new finding that 15 (0.9%) of 1606 patients with ARM have a family member with a non-ARM congenital anomaly. Of these, we found that more than 50% were genitourinary or pelvic. Based on these numbers, it would seem prudent to have a higher degree of suspicion for such anomalies in families in which a member has an ARM. However, given that the number and severity of such anomalies in our series were

fairly small, it is hard to justify routine screening of all family members. It is important however to realize that, in 4 patients, the anomalies identified were sacral masses having potential malignant or neurologic complications that would likely be able to be identified by a simple rectal examination or suggested by a plain x-ray of the pelvis.

Animal studies also point to genetic causes of ARM. For example, lines of mice [13,14] and pigs [15] with inherited ARMs have been described, and there is recent evidence of increased incidence of ARMs in certain breeds of dogs [16]. Although, to date, the specific mutations have not been studied in the mouse lines, a recent report has identified several regions of the pig genome that are linked to the ARM phenotype [17]. Furthermore, gene targeting in mice has demonstrated the importance of a number of genes, singly or in combination, for normal hindgut development. In this way, it is likely that ARM is similar to the prototypical congenital anomaly of the digestive system, Hirschsprung's disease (congenital colonic aganglionosis), which also affects about 1 in 5000 live births [18-20].

Prior descriptions of affected families, multisystem syndromes including ARM, studies of knockout mice, and this report all point to the role of genetic factors and even specific genes in development and malformation of the distal hindgut. To date, these findings have not been translated into studies of humans with ARM. In the future, it will be essential to identify specific genes associated with human ARM. To accomplish this task, it will be necessary to develop a patient registry and genomic DNA repository for patients with ARM. It will then be necessary to test candidate genes based on previous studies with knockout mice against genes of families with multiple affected members. In addition, linkage analysis will need to be performed to link familial ARM to specific loci on the human chromosome. A need to advance research in this area has been put forward by the World Congress of Pediatric Gastroenterology, Hepatology and Nutrition [21]. Such studies in the past have been limited by the rarity of the malformation with the care of such patients not localized to individual centers. The continued use of large case series and multicenter registries will be essential to conducting further studies to better understand the genetics of these malformations.

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References

[1] Anderson RC, Reed SC. The likelihood of recurrence of congenital malformations. J Lancet 1954;74(5):175-6.

[2] Boocock GR, Donnai D. Anorectal malformation: familial aspects and associated anomalies. Arch Dis Child 1987;62(6):576-9.

[3] Kubiak R, Upadhyay V. Isolated imperforate anus in monozygotic twins: case report and implications. J Pediatr Surg 2005;40(3):E1-E4.

[4] Kohlhase J, Wischermann A, Reichenbach H, et al. Mutations in the *SALL1* putative transcription factor gene cause Townes-Brocks syndrome. Nat Genet 1998;18(1):81-3.

[5] Townes PL, Brocks ER. Hereditary syndrome of imperforate anus with hand, foot, and ear anomalies. J Pediatr 1972;81(2):321-6.

[6] Currarino G, Coln D, Votteler T. Triad of anorectal, sacral, and presacral anomalies. AJR Am J Roentgenol 1981;137(2):395-8.

[7] Ross AJ, Ruiz-Perez V, Wang Y, et al. A homeobox gene, *HLXB9*, is the major locus for dominantly inherited sacral agenesis. Nat Genet 1998;20(4):358-61.

[8] Böse J, Grotewold L, Ruther U. Pallister-Hall syndrome phenotype in mice mutant for *Gli3*. Hum Mol Genet 2002;11(9):1129-35.

[9] Hall JG, Pallister PD, Clarren SK, et al. Congenital hypothalamic hamartoblastoma, hypopituitarism, imperforate anus and postaxial polydactyly—a new syndrome? Part I: Clinical, causal, and pathogenetic considerations. Am J Med Genet 1980;7(1):47-74.

[10] Torres R, Levitt MA, Tovilla JM, et al. Anorectal malformations and Down's syndrome. J Pediatr Surg 1998;33(2):194-7.

[11] Peña A. Anorectal malformations. Semin Pediatr Surg 1995;4(1):35-47.

[12] Cuschieri A. Descriptive epidemiology of isolated anal anomalies: a survey of 4.6 million births in Europe. Am J Med Genet 2001;103(3):207-15.

[13] Searle AG. The genetics and morphology of two 'luxoid' mutants in the house mouse. Genet Res 1964;5:171-97.

[14] Kluth D, Lambrecht W, Reich P, et al. SD-mice—an animal model for complex anorectal malformations. Eur J Pediatr Surg 1991;1(3):183-8.

[15] van der Putte SCJ, Neeteson FA. The pathogenesis of hereditary congenital malformations of the anorectum in the pig. Acta Morphol Neerl Scand 1984;22(1):17-40.

[16] Vianna ML, Tobias KM. Atresia ani in the dog: a retrospective study. J Am Anim Hosp Assoc 2005;41(5):317-22.

[17] Cassini P, Montironi A, Botti S, et al. Genetic analysis of anal atresia in pigs: evidence for segregation at two main loci. Mamm Genome 2005;16(3):164-70.

[18] Bates MD. Development of the enteric nervous system. Clin Perinatol 2002;29(1):97-114.

[19] Kapur RP. Hirschsprung disease and other enteric dysganglionoses. Crit Rev Clin Lab Sci 1999;36(3):225-73.

[20] Tam PKH, Garcia-Barcelo M. Molecular genetics of Hirschsprung's disease. Semin Pediatr Surg 2004;13(4):236-48.

[21] Martín MG, Silveira TR, Grand R, et al. Genetics of gastrointestinal and hepatobiliary disorders: Working Group Report of the First World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. J Pediatr Gastroenterol Nutr 2002;35(Suppl 2):S118-27.

Discussion

John Gosche, MD (Jackson, MS): Have you looked at the associated anomalies that we see with imperforate anus, and is there an effect at having multiple anomalies?

Michael Bates, MD, PhD (response): We have not look at that in any great detail, but that is an important question. There is mouse and human data that would suggest particular genes that may be involved in VACTERL-type associations, and so that would be of great interest to us so that we can more confidently go after those genes.

Jacob Langer, MD (Toronto, Ontario, Canada): Most of the ones with family histories that I have seen have had Currarino's triad. How many of yours have that problem?

Michael Bates, MD, PhD (response): That's a good question. I don't recall the exact numbers off the top of my head, but it's not all of the patients, definitely not all the patients in the series.

Albert Dibbins, MD (Portland, ME): I saw a family a number of years ago that we could trace through 3 generations—a man with a perineal fistula who had 2 wives. There were 3 daughters. Each of the 3 daughters had perineal or rectovestibular fistulae, and then 2 of the daughters had children. Both the boys had high imperforate anuses and the girl had a rectovestibular fistula, and by that time, all 3 of these children had renal and ear and radial anomalies, and it seemed as if this was obviously X chromosome connected. As you traced it through generations, it was becoming more severe. Did you see progression like that in your multigeneration families that you had a chance to look at?

Michael Bates, MD, PhD (response): No, we have not. That's an interesting question as to whether there is increased survivability because of improvements in care of patients with multiple anomalies or whether there is something, for example, in an environment that is resulting in a more severe phenotype. That's a very interesting observation.

Alberto Pena, MD (Cincinnati, Ohio): I want to invite all of you, my colleagues, to be more proactive in detecting these familial types because we have seen patients with perineal fistula, and then, when we specifically ask the mother, she says I think have the same defect. Many ladies are walking around with the same defect, except that nobody discussed it. And then the grandmother says, oh, my gynecologist also told me that I have something like that. There is another group of patients, the so-called Currarino, where we expect a big presacral mass, but sometimes in every baby that we have—we have families where the baby had a perineal fistula and then we order AP x-ray films of the sacrum and find little defects that represent a small presacral mass and sometimes you find the entire family with that small presacral mass. We suspect that this is much more common than we suspected, but we have to look for those associations. Thank you very much.

Michael Bates, MD, PhD (response): Thank you for that comment. One aspect of the paper that I didn't present in the interest of time that is in the abstract and is in the manuscript is that there are patients who have anorectal malformations who have family members with a variety of caudal lesions, including presacral masses that didn't have an anorectal malformation per se, and there appears to be an increased incidence of that as well, so there may be a variety of phenotypes that eventually we may be able to ascribe to particular gene lesions.