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Is there a link between ovarian cancer and tooth agenesis?

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Abstract

An epidemiologic study from the year 2008 found a highly significant increase of congenital tooth agenesis in women with ovarian cancer suggesting that a common genetic etiology may predispose women to both conditions. The finding was reminiscent of a previously described family harboring an AXIN2 mutation which could be shown to segregate with both the tooth agenesis and the predisposition to colon cancer transmitted in this family. Since tooth agenesis as a marker for susceptibility to ovarian cancer would be of great relevance to both oncologists and women with inborn missing teeth, the relationship between the two disorders requires a thorough assessment. We examined DNA samples from the ovarian cancer patients who participated in the original study, to look for a possible genetic connection between their ovarian malignancies and tooth agenesis. MSX1, PAX9, AXIN2, EDA, WNT10A, BARX and BRCA1 genes were selected for sequence analysis as they may cause tooth agenesis, are expressed in the female reproductive system, and/or are involved in tumorigenesis in general or specifically in the ovary.

Our study revealed evidence that one half of the dually affected patients had an independent causation of the two conditions, thus reducing the previously estimated ovarian cancer risk for women with congenital tooth agenesis quite significantly.

Keywords

Ovarian cancer; Tooth agenesis; Genetics; MSX1; PAX9; BARX1/2; AXIN2; EDA; WNT10A; BRCA1

1. Introduction

Chalothorn et al. [2008] described an increased prevalence of congenitally missing teeth in women with epithelial ovarian cancer. Twenty percent of these women reported one or two missing teeth, versus three percent in a cancer-free control sample. Surprisingly, ten ovarian

cyst patients (unpublished) displayed an even greater prevalence of hypodontia: forty percent. These observations by Chalothorn et al. suggested that there may be common genetic factors affecting both tooth development and susceptibility to the formation of epithelial tumors or cysts of the ovary, similar to the mutation in the *AXIN2* gene which causes both tooth agenesis and colorectal cancer [Lammi et al., 2004].

Early detection of epithelial ovarian cancer is difficult and as a result, the mortality rate is unacceptably high. If a link were found between tooth agenesis and ovarian cancer, semi-annual screening could become the standard of care for women with tooth agenesis to increase the early detection rate [van Nagell et al., 2007]. This would not only provide a new diagnostic tool but also open up new biological insight into epithelial ovarian cancer which, according to latest findings, may actually originate in the fallopian tube epithelium since gene expression patterns in these two tissues resemble each other closely [Kurman and Shih, 2011].

The lifetime risk for neoplastic ovarian disease is only about a fifth of that for tooth agenesis, which occurs in approximately 3%–9% of the population even when the very common 3rd molar agenesis is excluded [Bergstrom, 1977; Silverman and Ackerman, 1979; Thesleff, 2006]. So far, mutations in *WNT10A*, *MSX1*, *PAX9*, *AXIN2* and *EDA* pathway genes have been shown to cause about 50% of selective tooth agenesis in humans [Bergendal et al., 2011; Bohring et al., 2009; Lammi et al., 2004; Stockton et al., 2000; Tao et al., 2006; Vastardis et al., 1996]. Several of these genes are also expressed in tumor cells of the female reproductive system, suggesting a possible mechanism for the relationship between ovarian disorders and hypodontia.

For example, overexpression of *MSX1* inhibits ovarian carcinoma cell proliferation [Park et al., 2005] while deficiency is common in human ovarian and other malignancies [Park et al., 2001; Sliwinski et al., 2010]. *MSX1* deficiency also causes oligodontia of mostly posterior teeth similar to *PAX9*, a paired box transcription factor which is thought to control mesenchymal BMP4 signaling during odontogenesis [Ogawa et al., 2006; Peters et al., 1998]. *PAX9* expression is also found in epithelial ovarian cancer cell lines [Muratovska et al., 2003]. *BARX1* and 2 are expressed in the developing tooth and are frequently dysregulated in epithelial ovarian cancer [Gould and Walter, 2000; Sellar et al., 2002, 2001]. *AXIN2* is a member of the Wnt signaling pathway, which controls many developmental processes. Individuals with *AXIN2* mutations can have both tooth agenesis and a strong predisposition for colon cancer [Lammi et al., 2004].

WNT10A is the most commonly mutated gene in tooth agenesis [Bohring et al., 2009] with a large number of missing teeth in homozygotes, but only a few missing teeth in about 50% of heterozygotes. *EDA* gene mutations are associated with mild, selective tooth agenesis in carrier females of this X-linked disorder. Both *WNT10A* and *EDA* mutations would signify coincidental tooth agenesis if identified in ovarian cancer patients with tooth agenesis. Mutations in *BRCA1* are the best-known causes of breast and ovarian cancer but are not implicated in tooth agenesis.

The primary goal of our study was to investigate if the five well-established tooth agenesis genes *WNT10A*, *EDA*, *PAX9*, *MSX1*, and *AXIN2* as well as the *BARX1*, 2 and the *BRCA1* genes show any evidence of involvement in the tooth agenesis/ovarian cancer association by doing a thorough sequence analysis of these candidate genes in the original patient sample from the study by Chalothorn.

2. Materials and methods

2.1. Subject recruitment

The original protocol was IRB approved by the University of Kentucky. Fifty subjects with ovarian cancer and another ten with ovarian cystic disease, each with or without tooth agenesis, were recruited by Chalothorn from the University of Kentucky Ovarian Screening Clinic. Inclusion criteria were peri- or post-menopausal, ages 45 or older and no obvious signs of a syndrome. Additional unrelated patients with tooth agenesis and no personal or family history of ovarian disease were recruited by us under a separate IRB approval from Baylor College of Dentistry. All participants were Caucasians except one who was of Japanese ancestry.

The final patient cohorts consisted of four groups: 1) 10 patients with agenesis of 1–2 teeth and ovarian cancer, 2) 40 patients with only ovarian cancer, 3) 35 patients with agenesis of 1–8 teeth without ovarian disorders, and 4) the 10 patients with ovarian cysts of whom 4 had mild tooth agenesis.

The wild-type sequence and allele frequencies of common variants in control populations were obtained from the NCBI SNP database and the NHLBI Exome sequencing project (ESP).

Informed consent was obtained and a thorough patient history and dental exam was performed. If a patient was unsure of history, the patient's dentist was consulted to confirm etiology of any missing teeth. DNA samples were obtained using BuccalAmp swabs and sent to Texas A&M University Baylor College of Dentistry for analysis.

2.2. DNA Extraction from buccal swabs

Since buccal swabs may not have yielded sufficient material for the analysis of eight to ten genes, the samples were amplified by Whole Genome Amplification (WGA) with the GenomiPhi WGA system (GE Healthcare). Successful genome amplification was verified by gel electrophoresis of amplified samples together with a quantitation marker.

2.3. Polymerase chain reaction and sequencing of products

The exons of each gene were PCR amplified with GoTaq reagents (Promega) using a 96-well plate format for the 95 samples and one negative control. Several of the amplicons were very GC-rich and required PCR optimization and the use of 5% DMSO. Quality and quantity of PCR products was confirmed by gel-electrophoresis, followed by treatment with ExoSapIt (USB) and addition of sequencing primers. Automated dideoxy chain terminator sequencing was done by Seqwright, TX and GenScript, NJ.

2.4. Analysis of sequencing results

All sequences were visually inspected for heterozygous base changes and compared to the corresponding wild type sequences using the NCBI BLAST program. Once a nucleotide change was found, the SNP (single nucleotide polymorphism) database was consulted to determine if the SNP is a common polymorphism. For common SNPs, the allele frequencies were compared between the different experimental groups and between experimental groups and Caucasian population controls reported in NCBI and NHLBI databases. For *MSX1* polymorphisms, the Caucasian control allele frequencies from a study by Jezewski et al. (2003, supplement) were also employed. Chi-square statistics was used for the determination of statistical significance of allele frequency differences.

3. Results

Sequence analysis of the *EDA*, *WNT10A* and *BRCA1* genes yielded several interpretable results (Table 1):

1. One of the ten patients with combined ovarian cancer/tooth agenesis was a carrier of the known Arg69Leu mutation in *EDA* (rs132630309), explaining the tooth agenesis, and she also had a frameshift mutation in *BRCA1* (rs80357686) most probably responsible for the ovarian cancer.
2. Another tooth agenesis/ovarian cancer patient had the *BRCA1* frameshift mutation rs80357082, which was also present in two other ovarian cancer patients without tooth agenesis, indicating that this patient's ovarian cancer and her tooth agenesis have different roots and also that the investigated population shared common ancestors since the frequency of this mutation seems to be quite high, but no population allele frequencies are available (presumably due to gene patent issues).
3. Three of the remaining eight tooth agenesis/ovarian cancer patients had the *WNT10A* Phe228Ile mutation (rs121908120), which in heterozygous form is probably the most common mutation encountered in mild tooth agenesis while causing severe tooth agenesis when homozygously inherited. There were also three patients with the *WNT10A* Phe228Ile mutation in the "ovarian cancer only" group, however about 50% of people with this mutation do not present with tooth agenesis.

These results taken together suggest that at least half of the samples from the combined ovarian cancer/tooth agenesis patients identified in the epidemiological study by Chalothorn show evidence for independent causation of the two conditions. In the ten ovarian cyst patients we found mutations neither in *BRCA1* nor in the tooth agenesis genes *WNT10A* or *EDA*; but one of these women had the previously reported C1995insG mutation in *AXIN2* (see below).

Sequence analysis results from the other investigated genes are presented in Tables 2 and 3 and are briefly presented below. These results were calculated for the whole group, including ovarian cancer and ovarian cyst groups.

In *BARX1*, the missense mutation Ala48Thr was detected in 17 samples, but no significant allele frequency differences between the groups were found. The other five polymorphisms in this gene were also insignificant.

In *BARX2*, we found four heterozygous Ser64Pro mutations; three of them in individuals with tooth agenesis, with and without ovarian cancer. Ser64Pro is fairly conserved among species suggesting its importance in protein function; however since the control allele frequency reported in the SNP database is similar to the one found in this study, it may not play a role in TA. On the other hand, we do not know if the control population used for SNP data was screened for missing teeth. The synonymous Pro203Pro polymorphism in exon 2 occurred in the ovarian cancer cohort in 50% of those with tooth agenesis versus 33% without tooth agenesis however the difference was not statistically significant. The remaining polymorphisms found for *BARX2* did not demonstrate allele patterns consistent with an association with a disease phenotype.

A substantial number of polymorphisms were found in the *MSX1* gene including Ala40Gly (formerly Ala34Gly). The minor allele frequency of this polymorphism was significantly higher in all groups with tooth agenesis (regardless of ovarian disease status) compared to the NHLBI ESP Caucasian control group ($n > 2000$). However using the allele frequency values for 154 Caucasian controls which were reported by Jezewsky et al., 2003) in their study about *MSX1* mutations in cleft lip/palate patients, the differences were not statistically significant. The allele frequencies of the *MSX1* polymorphisms rs8670 and rs 186861426 were also significantly higher in most groups with tooth agenesis using the NHLBI ESP (Table 3), but not with the Jezewski control frequencies (we also noticed that these SNPs appear to be syntenic with Ala40Gly).

In *AXIN2* we found the missense mutation Ser762Asn in a single ovarian cancer patient without tooth agenesis. This mutation may thus contribute to cancer susceptibility but not to tooth agenesis. We also found the previously reported C1995insG mutation in one patient with combined ovarian cysts and tooth agenesis however we suspect that it is a PCR artifact since its appearance was dependent on PCR conditions and it occurs after a run of seven G nucleotides.

Sequencing of *PAX9* revealed five known and one new polymorphism (Table 2). One of these, a C to T transition adjacent to the A240P polymorphism, leads to a synonymous histidine codon which was significantly more frequent in samples from tooth agenesis patients (Table 3).

4. Discussion

A genetic connection between development and cancer is intuitively understandable in that both processes are characterized by rapid cell growth which often involves the same signaling pathways. However it is less intuitively understandable that a developmental deficit like tooth agenesis should be associated with the uncontrolled expansion of cell growth found in cancer. The finding that a nonsense mutation in *AXIN2*, which should lead to increased WNT signaling, can cause both severe tooth agenesis and a predisposition for colon cancer in humans [Lammi et al. 2004] is therefore somewhat surprising because

increased WNT signaling leads to supernumerary teeth in experimental animal models [Järvinen et al., 2006]. *Axin2* can also promote oncogenicity in colon cancer through the upregulation of *Snail1* [Wu et al., 2012] and a gain-of function mutation, not a nonsense mutation, in *Axin2* would be more compatible with its double role as suppressor of tooth development and promoter of colon cancer. A double role is easier to fathom in the case of mutations in *MSX1*, a protein that is able to interact with both DNA and other proteins and can act as a transcriptional suppressor or as an activator depending on cellular context.

Since a causal linkage between tooth agenesis and ovarian cancer would have major ramifications for the large number of women with minor tooth agenesis, the issue requires quite urgent clarification. Therefore we undertook this investigation and approached the question of common genetic factors in tooth agenesis and ovarian cancer by screening known tooth agenesis causing genes for mutations as suggested in the original study by Chalothorn. We added the *BRCA1* gene and the newly attributed tooth agenesis gene *WNT10A* to their list of candidate genes.

We did not find a single candidate gene or mutation that could explain the increased co-occurrence of tooth agenesis with ovarian cancer. The controversial *AXIN2* C1995insG mutation was found in an ovarian cyst-not cancer-patient with tooth agenesis. But we did find *BRCA1* mutations in two of the ten cases of ovarian cancer/ tooth agenesis patients, explaining the ovarian cancer. Additionally, one of these two also had an *EDA* mutation, clearly demonstrating separate origins of ovarian cancer and tooth agenesis. Furthermore we found a well known tooth agenesis-causing *WNT10A* mutation in three members of the ovarian cancer/tooth agenesis group arguing for independent causation of the tooth agenesis in these patients. Our findings do not prove that the two conditions arose independently from each other but they make it seem quite likely for at least half of the cases described in the original study.

The observation by Chalothorn et al. that their 10 ovarian cyst patients had an even higher prevalence of hypodontia than the ovarian cancer cohort reinforces the notion of independent origin since so far no genetic link has been detected between epithelial ovarian cancer and ovarian cysts making it unlikely that a single gene could be responsible for all 3 disorders.

Additional evidence for independent causation comes from a recent study [Küchler et al., 2013] which did find increased rates of (self-reported) cancer in patients with tooth agenesis. However, ovarian cancer in contrast to prostate, breast, and nervous system malignancies, was not among the significant results. We also have been enrolling a few hundred subjects in our own “Missing Tooth Study” over the past 10 years and have not noted any case with ovarian cancer or cysts in her/his family’s medical history; however the reason for this could be the higher prevalence of tooth agenesis and incomplete recall of family health issues.

On the other hand, we know that the investigated genes represent only a minority of all the possible candidates that may be involved in hypodontia and it would certainly be worthwhile to investigate additional genes which are commonly associated with ovarian cancer such as

BRCA2, BRCA-interacting protein, *ErbB2* and *p53*, for example, which are also quite strongly expressed in the tooth bud of developing mouse embryos [Diez-Roux et al., 2011].

In conclusion we challenge the currently held notion of a predisposition for developing ovarian cancer in women with tooth agenesis and propose that a larger epidemiological study should be conducted to confirm any link between ovarian cancer and tooth agenesis. Ideally, a large family with both ovarian cancer and tooth agenesis may be recruited for linkage analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.ejmg.2014.02.013>

Table 1

A list of the Ovarian Cancer patients with/without tooth agenesis who were found to have mutations in the breast/ovarian cancer gene *BRCA1*, the cancer/tooth agenesis gene *AXIN2* and the tooth agenesis genes *EDA* and *WNT10A*. Bold entries represent patients with combined ovarian cancer/tooth agenesis; the others have ovarian cancer without tooth agenesis. Patient numbers 1–50 are ovarian cancer patients; numbers above 50 are ovarian cyst patients. The phenotype has been reported by Chalothorn et al. and does not show any remarkable features. Upper lateral incisors and lower second premolars are the most commonly missing teeth in the general population [Shimizu and Maeda, 2009]. Tooth agenesis caused by monoallelic *WNT10A* mutations is known to have a 50% penetrance and involve maxillary premolars and lateral incisors.

Pt#	<i>BRCA1</i>	<i>EDA</i>	<i>WNT10A</i>	<i>AXIN2</i>	Tooth agenesis phenotype (FDI)
2			F228I		
3				S762N	
5	K679stop				
7	K894fs				
10	K527fs				
13	K894fs				
14					lateral incisor (22), 2nd premolar (25)
16			F228I		upper 2nd premolar (25)
19	M1652I				
20			F228I		
27					lower 2nd premolar (35)
29					upper lateral incisors (12, 22)
30			F228I		upper lateral incisors (12, 22)
32			F228I		upper 2nd premolar (15)
33	Q1096fs	R69L			incisor (22), molar (27)
34			F228I		
35	K894fs				upper lateral incisors (12, 22)
36					micro-molars (16, 17)
43					upper lateral incisor (12)
56				C1995insG	2nd premolars (35, 45)

Table 2

List of all polymorphisms and their allele frequencies in selected study groups (OV $\pm$ TA: All patients with ovarian disease (cancer and cysts) with or without tooth agenesis. TA $\pm$ OV: All patients with tooth agenesis regardless of ovarian disease status. OV + TA: All patients with both ovarian disease and tooth agenesis).

Mutation	Gene	rs#	Site	Allele frequency (minor allele)				Remarks
				Total (n = 95)	OV $\pm$ TA (n = 60)	TA $\pm$ OV (n = 49)	OV + TA (n = 14)	
PAX9	rs12881240	H239H		0.30'	0.24	0.31'	0.18	Significant NHLBI
	rs4904210	A240P		0.31	0.34	0.26	0.29	0.36
	rs61734510	K172K		0.011	0.008	0.01		0.008
	rs17104965	d ₂₅₉ C > T		0.032	0.033	0.031	0.036	0.02
	rs17766200	d ₄₂₉ T > C		0.126	0.075	0.163	0.036	0.093
AXIN2		d ₂₀₉ G > T		0.005		0.01		
	CI002733	C1995insG		0.005	0.008	0.01	0.036	PCR artifact?
	rs35415678	L688L		0.016	0.008	0.03	0.036	0.04
	rs117688560	S762N		0.005	0.008			0.0014
	rs9906513	e7+39 T > A			N/D	0.03	0.036	0.04
MSX1		22nt5' gene		0.01	0.008	0.01		49 TA samples
	rs 186861426	18nt5' ATG		0.12''	0.075'	0.15''	0.036	Significant NHLBI
		7nt5' ATG		0.005		0.01		
	rs36059701	A40G		0.22''	0.217'	0.255''	0.32''	Significant NHLBI
	rs34165410	G116G		0.026	0.0167	0.04	0.036	0.04
		T39T		0.005		0.01		
	rs8670	d ₆ C > T		0.29''	0.24	0.34''	0.286	Significant NHLBI
	rs1095	d ₆₈ C > T		0.005	0.008		0.036	0.117 ^a
	rs2229262	d ₇₀ C > T		0.005		0.01		0.019 ^a
	rs12532	d ₂₇₆ A > G		0.3	0.31	0.28	0.286	0.24
BARX1	rs33929633	e2-15		0.1				See below ^c
	rs 191789925	A48T		0.096	0.087	0.095	0.036	0.066
	rs12684081	e3+29 C > T		0.295	0.306	0.274	0.25	0.27
	rs149299341	e3+49 C > G		0.006	0.01			0.008

Mutation		Allele frequency (minor allele)					Remarks
Gene	rs#	Site	Total (n = 95)	OV ± TA (n = 60)	TA ± OV (n = 49)	OV + TA (n = 14)	Control
BARX2	rs11793856	P240P	0.26	0.25	0.276		0.267
	rs2297835	d ₁₇ G > T	0.005	0.008			0.01
	rs59397123	d ₁₀₈ G > A	0.06	0.05	0.06		0.092
	rs117979658	e ₁ +46 G > T		0.017			Low cover
	rs142822705	S64P	0.02	0.017	0.02		OV ± TA only
	rs149011396	Ile93Ile	0.005		0.01		0.0014
EDA	rs10791010	e4-5 C > T		0.18		0.25	0.2
	rs10791011	P203P		0.18		0.25	b OV ± TA only
	rs60183112	d ₁₃₀ insC		0.23	0.286		b OV ± TA only
		d ₃₉ A > G		0.008			
	rs132630309	R69L			0.036		0.011
	rs2274469	e3+27 T > A			0.11		OV + TA only
	rs11426919	e4-25indelA			0.5		Artifact
	rs10579679	e4+11delCT			0.36		OV + TA only
	rs140058036	e5+47 G > C			0.07	0.06	OV + TA only
	rs62604271	e5+125 G > T			0.07		OV + TA only
	rs2296765	e6-11 C > T			0.25	0.37	OV + TA only
		d ₃₅ C > G			0.036		OV + TA only

^a Ethnically unmatched control.

^b rs10791010, rs10791011 and rs60183112 appear to be syntenic, the latter also as a separate polymorphism.

^c Homozygotes are not recognizable in the sequencing reaction. ' = significant, " = highly significant difference compared to NHLBI Caucasian control population but not in comparison with the (smaller) control group described in (ref. 29, supplement).

^d ₂₅₉ C > T denotes SNP location 259 nucleotides 3' of stop codon.

Table 3

Detailed analysis of significant allele frequency differences in all study groups (OV_{no}TA: All patients with ovarian disease only. OV ± TA: All patients with ovarian disease with or without tooth agenesis. TA ± OV: All patients with tooth agenesis regardless of ovarian disease status. TAnoOV: All patients with tooth agenesis, no ovarian disease. OV + TA: All patients with both ovarian disease and tooth agenesis).

Mutation		Allele frequency (minor allele)							Remarks	
Gene	rs#	Site	Total (n = 95)	OVnoTA (n = 46)	OV ± TA (n = 60)	TA ± OV (n = 49)	TAnoOV (n = 35)	OV + TA (n = 14)		Control
PAX9	rs12881240	H239H	0.30′	0.25	0.24	0.31′	0.36″	0.18	0.20	Significant
MSX1	rs186861426	18nt5′/ATG	0.12″	0.087′	0.075′	0.15″	0.2″	0.036	0.025	Significant
	rs36059701	A40G	0.22″	0.185	0.217′	0.255″	0.23′	0.32″	0.143	Significant
	rs8670	*6C > T	0.29″	0.23	0.24	0.34″	0.37″	0.286	0.2	Significant

*significant, " highly significant when compared to the NHLBI Caucasian control population but not in comparison with the (smaller) control group described in (Jezewski et al., supplement). Genotype frequency in the NHLBI Caucasian control population displayed Hardy–Weinberg Equilibrium for all Mx1 SNPs. The Pax9 SNP CAC > CAT deviated modestly from HWE probably due to its dependence on the adjacent SNP GCG > CCG, creating a potential CpG dinucleotide, which has a tendency to either mutate the third position C of the CAC codon to a T or the first position G of the adjacent GCG to a C. rs36059701 and rs8670 are syntenic.