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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.

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 Surprisingly, ten ovarian cyst patients (unpublished) displayed an even greater prevalence of hypodontia: forty percent.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

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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 electro-phoresis 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):

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.

	BRCA1	*EDA*	*WNT10A*	*AXIN2*	Tooth agenesis phenotype (FDI)
Pt#					
2			F228I		
3				S762N	
5	K679stop				
7	K894fs				
10	K527fs				
13	K894fs				
14					**lateral incisor (22), 2nd premolar (25)**
16			**F228I**		**upper 2**nd **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)***