Published OnlineFirst October 15, 2012; DOI: 10.1158/1055-9965.EPI-12-1110

Cancer Epidemiology, Biomarkers & Prevention

Null Results in Brief

Catenin Family Genes Are Not Commonly Mutated in Hereditary Diffuse Gastric Cancer

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Abstract

Background: Approximately one third of the hereditary diffuse gastric cancer (HDGC) families carry germline mutations in the E-cadherin gene (*CDH1*). Risk prediction in members of families with this rare but deadly cancer could be improved by the identification of additional *HDGC* genes in non-*CDH1* families.

Methods: Affected individuals from 22 *CDH1* mutation-negative families were screened for germline mutations in four catenin genes: *CTNNA1*, *CTNNB1*, *JUP*, and *CTNND1*. Catenins interact closely with E-cadherin molecules in cells, and are therefore logical candidate genes for mutation screening in HDGC families.

Results: No nonsynonymous variants were seen in *CTNNA1*, *CTNNB1*, or CTNND1; only *JUP* contained nonsynonymous variants, of which only two rare variants were predicted to be deleterious.

Conclusion: Catenin genes are not commonly mutated in non-*CDH1* HDGC families.

Impact: Germline mutations in *CTNNA1*, *CTNNB1*, *JUP*, or *CTNND1* are unlikely to play a major role in HDGC. *Cancer Epidemiol Biomarkers Prev; 21(12); 2272–4. ©2012 AACR.*

Introduction

Gastric cancer can be divided histopathologically into intestinal and diffuse types. While intestinal type gastric cancer is more common, diffuse type gastric cancer is more likely to have a genetic basis. Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant familial cancer in which 46% of the patients show *CDH1* germline mutations or deletions (1). *CDH1* mutations are thought to account for 1% of all gastric cancer cases overall (2). Because half of the HDGC families are negative for E-cadherin germline mutations, the identification of additional genes underlying this familial cancer is important.

E-cadherin is a transmembrane protein encoded by *CDH1* that functions in the differentiation and polarization of the gastric epithelium through binding to identical molecules on adjacent cell surfaces. Proper function of E-cadherin requires its anchoring to the cytoskeleton by the 4 members of the catenin family [reviewed by Tian and colleagues (3)]. Inactivation of catenins and disruption of cytoplasmic catenin–cadherin binding result in inability of E-cadherin to establish cell-to-cell adhesion even when the extracellular E-cadherin binding domain remains intact. *CTNNA1* encodes α-catenin, which binds actin binding proteins and filaments. The cytoplasmic tail of α-catenin can be bound by either β-catenin (encoded by *CTNNB1*) or γ-catenin (plakoglobin), the product of *JUP*. *CTNND1* encodes p120-catenin, a multi-isoform protein expressed differentially in a variety of cell types. p120 interacts with the juxtamembrane domain of E-cadherin and regulates the lateral clustering and stabilization of the cadherins at the cell membrane. Abnormal expression of both the E-cadherin and catenin genes has previously been shown in diffuse gastric cancer. Given the importance of catenins in regulating E-cadherin function, we hypothesized that screening of these genes in HDGC families without *CDH1* mutations would identify potential new germline mutations responsible for HDGC.

Materials and Methods

Patients and families

Twenty-two families from Canada, the United States, and the United Kingdom were screened. These families, including their characteristics and criteria for ascertainment and testing were part of a larger set described previously (4). Families numbered 3, 5–6, 8, 12, 14, 15, 17, 19, 22–24, 27, 28, 30–35, 37, and 38 were previously confirmed as without *CDH1* mutations (4) and thus included in this study. Constitutional genomic DNA was extracted from blood from at least 1 affected individual of each family, usually the proband. This study was

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Table 1. Gene resequencing results and predicted coding changes

Gene	Variant	Frequency[a]	Amino acid change	SIFT	Polyphen	dbSNP ID (build 137)	Flanking sequence
CTNNA1	IVS4 (+45) A/G	1/44 (0.02)	N/A	N/A	N/A	rs28363396	ttaaagttgttcattttact[A/G]tctagagggaaaaactcatt
	IVS7 (+8) C/G	16/44 (0.36)	N/A	N/A	N/A	rs288028	CTTGCGTAGACAGgtaatct[C/G]gatgaaagtgctgattgttt
	x15 (2220) G/A	16/44 (0.36)	Silent	N/A	N/A	rs1059110	GGACCACTCAAAATACATC[G/A]GATGTCATCAGTGCTGCCAA
	x15 (2226) C/G	1/44 (0.02)	Silent	N/A	N/A	rs11552052	CTCAAAATACATCGGATGT[C/G]ATCAGTGCTGCCAAGAAAT
CTNNB1	IVS14 (-17) T/G	1/44 (0.02)	N/A	N/A	N/A		ctttctattctccttgctt[T/G]tgcatgtttatctagACTG
	x16 (2452) C/T	1/44 (0.02)	Silent	N/A	N/A	rs4135386	CTCCAGGTGACAGCAATCAG[C/T]TGGCCTGGTTTGATACTGAC
JUP	x3 (213) T/C	36/44 (0.82)	Silent	N/A	N/A	rs7405731	GTGCCCCCCAGCCAAGGTGAT[T/C]CTGGAGTACCAGATGTCCAC
	x3 (431) G/A	1/44 (0.02)	Arg144His	Tolerated	Possibly damaging		CGATGCCGAGCTGGCCACTC[G/A]CGCCCTGCCCGAGCTCACCA
	x4 (532) C/T	1/44 (0.02)	Arg178Trp	Not tolerated	Probably damaging		TGTCGAAGAAGGAGGCGTCG[C/T]GGCGGGCCCTGATGGGCTCG
	IVS5 (+17) T/C	7/44 (0.16)	N/A	N/A	N/A	rs12942034	CAAGgtgggccctccccaac[T/C]ctcccgaggcctgaagccca
	x6 (964) G/A	1/44 (0.02)	Val322Met	Tolerated	Benign		ATGGTGGGCCCCAGGCCCTC[G/A]TGCAGATCATGCGTAACTAC
	IVS10 (−34) C/A	30/44 (0.68)	N/A	N/A	N/A		gcctgcctcaccccttccca[C/A]ctctccctgcttcccacgt
	IVS12 (+22) A/G	30/44 (0.68)	N/A	N/A	N/A	rs7216034	tgagtatcctaggttggacc[A/G]cagtagttggttgtgcaagt
	x14 (2108) A/T	30/44 (0.68)	Silent	N/A	N/A		ATGAGCCCTATGGAGATGAC[A/T]TGGATGCCACCTACCGCCCC
CTNND1	x3 (24) G/C	1/44 (0.02)	Silent	N/A	N/A		GACGACTCAGAGGTGGAGTC[G/C]ACCGCCAGCATCTTGGCCTC
	x6 (483) C/T	1/44 (0.02)	Silent	N/A	N/A	rs10896644	CCAGTCGCTATGGGACCAGA[C/T]GGGTTGCCTGTGGATGCTTC
	IVS20 (−45) A/G	2/44 (0.05)	N/A	N/A	N/A		agctctggcacacactcatg[A/G]ggttcctgtctactcata

[a]Number of times variant was observed/number of chromosomes examined (frequency).

approved by the joint British Columbia Cancer Agency/University of British Columbia (BCCA/UBC) Clinical Research Ethics Board. Informed consent was obtained for all participants.

Resequencing

The sequencing methodology used was described previously (4). Amplicons were designed to allow assessment of each entire coding exon including intron–exon junctions. Seventeen exons of *CTNNA1*, 15 exons of *CTNNB1*, 13 exons of *JUP*, and 19 exons of *CTNND1* were screened. Primers and PCR conditions are given in Supplementary Online Material. Bidirectional sequence data were obtained on ABI-3700 sequencers then assembled and viewed in Polyphred/Consed. The programs SIFT ("Sorting Intolerant from Tolerant") (5) and Polyphen-2 (6) were used to assess the potential functional impact of variants.

Results

The 22 families were screened for mutations in the 4 catenin genes. Variants observed are listed in Table 1. Four synonymous variants were found in *CTNNA1*, 2 in *CTNNB1*, 8 in *JUP*, and 3 in *CTNND1*. Only *JUP* contained nonsynonymous variants: a conservative substitution (Val322Met) and 2 that were predicted to be "possibly damaging" by Polyphen, Arg144His, and Arg178Trp, with the latter indicated as "not tolerated" by SIFT.

Discussion

The only mutations with possible functional significance were found in *JUP*. Both of these were heterozygous and rare (observed in only 1/50 chromosomes). Because plakoglobin and β-catenin have overlapping roles in binding α-catenin, and no mutations were found in *CTNNB1*, it is unlikely that the rare mutations found in the *JUP* gene would have severe functional consequences. Catenin genes have been screened in gastric cancer tumor tissue previously with little evidence for a strong role of catenin mutations in gastric tumorigenesis. To our knowledge, our study is the first to screen these genes in HDGC families.

Because the kinase-driven pathways of the cadherin–catenin complex are critical to its structural integrity (7) and α-actin may act in the regulation of the cytoskeletal organization (8), it is possible that changes in the regulatory pathways of the cadherin–catenin complex could play a larger role than mutations within the catenin genes. It seems likely that genetic variation in catenin genes themselves has little if any effect on HDGC risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: D. Huntsman, A.R. Brooks-Wilson
Development of methodology: S. Leach, A.R. Brooks-Wilson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.M. Schuetz, S. Leach, P. Kaurah, J. Jeyes, D. Huntsman, A.R. Brooks-Wilson

Published OnlineFirst October 15, 2012; DOI: 10.1158/1055-9965.EPI-12-1110

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.M. Schuetz, Y. Butterfield, A.R. Brooks-Wilson
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Grant Support

This study was funded by Genome BC and Genome Canada.

Received September 28, 2012; accepted October 1, 2012; published OnlineFirst October 15, 2012.

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Cancer Epidemiol Biomarkers Prev 2012;21:2272-2274. Published OnlineFirst October 15, 2012.