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Association Between Germline Mutation in *VSIG10L* and Familial Barrett Neoplasia

Ryan E. Fecteau, PhD, **Jianping Kong, PhD**, **Adam Kresak, MS**, **Wendy Brock, RN**, **Yeunjoo Song, PhD**, **Hisashi Fujioka, PhD**, **Robert Elston, PhD**, **Joseph E. Willis, MD**, **John P. Lynch, MD, PhD**, **Sanford D. Markowitz, MD, PhD**, **Kishore Guda, DVM, PhD**, and **Amitabh Chak, MD**

Department of Pathology, Case Medical Center, Case Western Reserve University, Cleveland, Ohio (Fecteau, Kresak, Willis); Division of Gastroenterology, Department of Medicine, University of Pennsylvania, Philadelphia (Kong, Lynch); Division of Gastroenterology and Liver Disease, Department of Medicine, Case Medical Center, Cleveland, Ohio (Brock, Chak); Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio (Song, Elston); Electron Microscopy Facility, Case Western Reserve University, Cleveland, Ohio (Fujioka); Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, Ohio (Elston, Willis, Markowitz, Guda, Chak); Division of Hematology-Oncology, Department of Medicine, Case Western Reserve University, Cleveland, Ohio (Markowitz); Department of Genetics and Genome Sciences, Case Western Reserve University, Cleveland, Ohio (Markowitz); Division of General Medical Sciences-Oncology, Case Western Reserve University, Cleveland, Ohio (Guda)

Abstract

Corresponding Author: Amitabh Chak, MD, Professor of Medicine, Division of Gastroenterology, Case Western Reserve University, 10900 Euclid Ave, Cleveland, OH 44106 (Amitabh.Chak@uhhospitals.org).

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IMPORTANCE—Esophageal adenocarcinoma and its precursor lesion Barrett esophagus have seen a dramatic increase in incidence over the past 4 decades yet marked genetic heterogeneity of this disease has precluded advances in understanding its pathogenesis and improving treatment.

OBJECTIVE—To identify novel disease susceptibility variants in a familial syndrome of esophageal adenocarcinoma and Barrett esophagus, termed familial Barrett esophagus, by using high-throughput sequencing in affected individuals from a large, multigenerational family.

DESIGN, SETTING, AND PARTICIPANTS—We performed whole exome sequencing (WES) from peripheral lymphocyte DNA on 4 distant relatives from our multiplex, multigenerational familial Barrett esophagus family to identify candidate disease susceptibility variants. Gene variants were filtered, verified, and segregation analysis performed to identify a single candidate variant. Gene expression analysis was done with both quantitative real-time polymerase chain reaction and in situ RNA hybridization. A 3-dimensional organotypic cell culture model of esophageal maturation was utilized to determine the phenotypic effects of our gene variant. We used electron microscopy on esophageal mucosa from an affected family member carrying the gene variant to assess ultrastructural changes.

MAIN OUTCOMES AND MEASURES—Identification of a novel, germline disease susceptibility variant in a previously uncharacterized gene.

RESULTS—A multiplex, multigenerational family with 14 members affected (3 members with esophageal adenocarcinoma and 11 with Barrett esophagus) was identified, and whole-exome sequencing identified a germline mutation (S631G) at a highly conserved serine residue in the uncharacterized gene *VSIG10L* that segregated in affected members. Transfection of S631G variant into a 3-dimensional organotypic culture model of normal esophageal squamous cells dramatically inhibited epithelial maturation compared with the wild-type. *VSIG10L* exhibited high expression in normal squamous esophagus with marked loss of expression in Barrett-associated lesions. Electron microscopy of squamous esophageal mucosa harboring the S631G variant revealed dilated intercellular spaces and reduced desmosomes.

CONCLUSIONS AND RELEVANCE—This study presents *VSIG10L* as a candidate familial Barrett esophagus susceptibility gene, with a putative role in maintaining normal esophageal homeostasis. Further research assessing *VSIG10L* function may reveal pathways important for esophageal maturation and the pathogenesis of Barrett esophagus and esophageal adenocarcinoma.

Barrett esophagus (BE) is the only known precursor for esophageal adenocarcinoma (EAC), an aggressive cancer whose incidence has increased dramatically.[1,2] Familial Barrett esophagus (FBE) is a single-trait reflecting inherited susceptibility to both BE and EAC.[3] Positive family history is included in the American College of Gastroenterology recommendations to guide BE screening.[4]

Familial Barrett esophagus accounts for approximately 7% of all BE and EAC cases and shows incompletely penetrant, autosomal-dominant inheritance.[3,5,6] We identified FBE-31, a multiplex, multigenerational family with 14 affected members: 3 with EAC and 11 with BE (Figure 1).[5] Herein, we describe comprehensive genetic and functional characterization of a novel germline variant in the previously uncharacterized gene *VSIG10L* underlying disease susceptibility in this family.

Methods

Proband and Family Recruitment

The probands and families in this study were recruited in an institutional review board–approved study as previously described.[5] Patients granted participation by written, informed consent.

Genetic Studies

Germline DNA was unavailable from deceased family members with EAC, we therefore selected individuals for whole exome capture and sequencing (WES) based on clinical criteria suggestive of a genetic predisposition (ie, young age at BE diagnosis or affected females) (eTable in the Supplement).[7] Variant filtering identified a germline mutation in *VSIG10L* cosegregating in the 4 sequenced individuals (eFigure 1 in the Supplement). Sanger sequencing of *VSIG10L* in family members was performed for segregation analysis, totaling 19 individuals: 12 male and 7 female (eTable, eFigure 2, and eMethods in the Supplement).

Functional Studies of *VSIG10L*

A 3-dimensional organotypic cell culture model of esophageal maturation was used to determine the phenotypic effects of mutant *VSIG10L* on squamous cell maturation (Figure 2). We performed quantitative real-time polymerase chain reaction to determine *VSIG10L* expression in a random set of (n = 238) normal squamous esophagus and stage-specific BE-associated lesions (Figure 3). In situ hybridization of *VSIG10L* transcript of normal esophagus squamous tissue confirmed and localized expression (see eFigure 3 in the Supplement).

Electron Microscopy Studies

Proximal esophagus squamous biopsy specimens from a non-gastroesophageal reflux disease, a BE and/or EAC, and FBE-31 individual III-4 were taken for electron microscopy and examination of intercellular space integrity (eMethods in Supplement).

Statistical Methods

Real-time polymerase chain reaction expression of *VSIG10L* in BE-associated lesions was compared with normal squamous cells using the unpaired *t* test. The logarithm of odds (LOD) score *P* value was obtained using the LODLINK program in the Statistical Analysis for Genetic Epidemiology Program (Case Western Reserve University) (eMethods in the Supplement).

Results

Identification of Germline Mutation in *VSIG10L*

WES revealed 4 candidate germline variants cosegregating in the 4 affected individuals selected for sequencing (eFigure 1 in the Supplement). Of these, a missense variant (S631G) in *VSIG10L* was completely private. Eight of 10 affected individuals (including the 2 EAC

cases) and 3 of 9 unaffected individuals carried the variant (Figure 1; eTable in the Supplement). For this family, the LOD score assuming a dominant 1-locus, 2-allele model with age-specific incomplete penetrance maximized at 0 recombination fraction with $P = .07$. Germline DNA sequencing of *VSIG10L* from probands in another 35 FBE families revealed no additional private variants. Sequencing of 19 EAC biopsy specimens showed 1 somatic missense mutation (G769S) that was predicted to significantly alter protein function (see eMethods in the Supplement).

Structure and Expression

The predicted *VSIG10L* gene product is a membrane-bound protein with 2 immunoglobulin (Ig)-like domains, 2 Ig-like folds, and a small cytoplasmic domain (Figure 4). The S631G variant was found to be highly conserved by multiple sequence alignment (Figure 4) and is predicted to be functionally deleterious (eMethods in the Supplement). *VSIG10L* is highly expressed in normal squamous esophagus and is largely lost in metaplasia, dysplasia, and adenocarcinoma lesions (Figure 3). RNA in-situ hybridization demonstrated VSIG10L expression is confined to the suprabasilar layer of the epithelium (eFigure 3 in the Supplement).

Functional Characterization

The phenotypic effects of the S631G variant were assessed in a 3-dimensional organotypic cell culture model.[8] Both nontransfected control keratinocytes and those expressing wild-type *VSIG10L* underwent normal differentiation into a stratified squamous epithelium (Figure 2; eFigure 4 in the Supplement). In contrast, expression of the S631G variant markedly disrupted cellular organization, characterized by epithelial dysmaturation (Figure 2). Similarly, expression of the *VSIG10L* G769S somatic mutation identified in EAC tissue also inhibited squamous maturation (eFigure 5 in the Supplement).

Ultrastructural Studies

Transmission electron microscopy showed that compared with a normal subject and a subject with BE and/or EAC, the proximal esophageal epithelium from individual III-4 who carries the S631G variant demonstrated pronounced dilation of the intercellular space and markedly reduced desmosome formation (Figure 5).

Discussion

Genetic analysis of a large family with FBE uncovered the previously uncharacterized gene *VSIG10L* that is highly expressed in normal esophagus and appears to play a role in epithelial maturation. Moreover, we found loss of VSIG10L expression in BE-associated lesions supporting a functional role for this gene in sporadic disease. Mutant *VSIG10L* impaired squamous cell maturation in an organotypic model and was associated with proton-pump inhibitor refractory dilated intercellular spaces (DIS) and reduced desmosome formation in affected family member III-4, strongly supporting that the inherited defect in *VSIG10L* predisposes to BE and/or EAC in this family.

A *P* value of .07 for a linkage analysis in a single family using reasonable assumptions for BE prevalence and penetrance adds further support that this variant is a FBE susceptibility gene in this family. Incomplete segregation of the *VSIG10L* mutation can be explained by the prevalence of BE, disease bias for males, and the ascertainment bias in identifying families with multiple BE cases and screening individuals with gastroesophageal reflux disease (GERD) symptoms. The prevalence of BE in patients with GERD is 10% to 15%, in accord with the finding of 2 of 10 BE phenocopies in symptomatic males and the model assumptions used in calculating the *P* value for linkage.[9] The 3 instances of unaffected S631G carriers are consistent with an incomplete gender- and age-specific penetrance—2 are young women and 1 is a male with erosive esophagitis (eTable in the Supplement).

Our finding of a private variant in *VSIG10L* in 1 unique family and not in 35 other families suggests marked genetic heterogeneity of FBE. Interestingly, the MSR1 R293X null variant reported in a prior study of FBE did not segregate in this or the 35 other smaller multiplex families with FBE we analyzed but was identified in population controls (unpublished data), illustrating that susceptibility alleles for complex diseases often have limited penetrance, and carriers may not develop disease.[10]

The dramatic findings in the OTC experiments (Figure 2) along with transmission electron microscopy imaging (Figure 5) suggest that mucosal disruption associated with the VSIG10L S631G variant renders the esophagus vulnerable to reflux injury. Dilated intercellular spaces and loss of desmosomes occur early in the pathogenesis of reflux damage.[11,12] Effective treatment with PPIs leads to 100% reversal of DIS.[13] Thus, as noted in individual III-4, we propose that the dominant negative S631G variant predisposes to reflux injury and the subsequent development of BE and EAC in this family. Genome-wide association studies also suggest that variants in genes involved in esophageal maturation predispose to the development of BE and EAC.[14,15]

The discovery of this novel gene opens avenues of translational research for elucidating the function of *VSIG10L* in BE pathogenesis, prevention, and treatment. Immediate clinical implications for carriers of the S631G variant in this family include early screening and close monitoring. Future studies of *VSIG10L* variants and associated genes in the population may allow risk stratification of susceptibility to BE and EAC.

Conclusions

VSIG10L is a susceptibility gene for familial BE and/or EAC. Future investigations aimed at elucidating function and downstream effects of mutations in *VSIG10L* should reveal as of yet uncharacterized pathways operating in esophageal maturation and susceptibility to BE and EAC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

Question

Do germline DNA mutations underlie susceptibility to familial Barrett esophagus, familial aggregation of Barrett esophagus, and esophageal adenocarcinoma?

Findings

In this genetic study of a multigenerational family with 14 affected individuals, a rare variant in the uncharacterized gene *VSIG10L* was associated with susceptibility to Barrett esophagus and esophageal adenocarcinoma. Functional studies revealed this germline mutation in *VSIG10L* disrupts esophageal epithelial maturation.

Meaning

This discovery of the first susceptibility variant in familial Barrett esophagus reveals novel biology in disease pathogenesis and indicates early screening and close clinical monitoring for individuals harboring the germline variant.

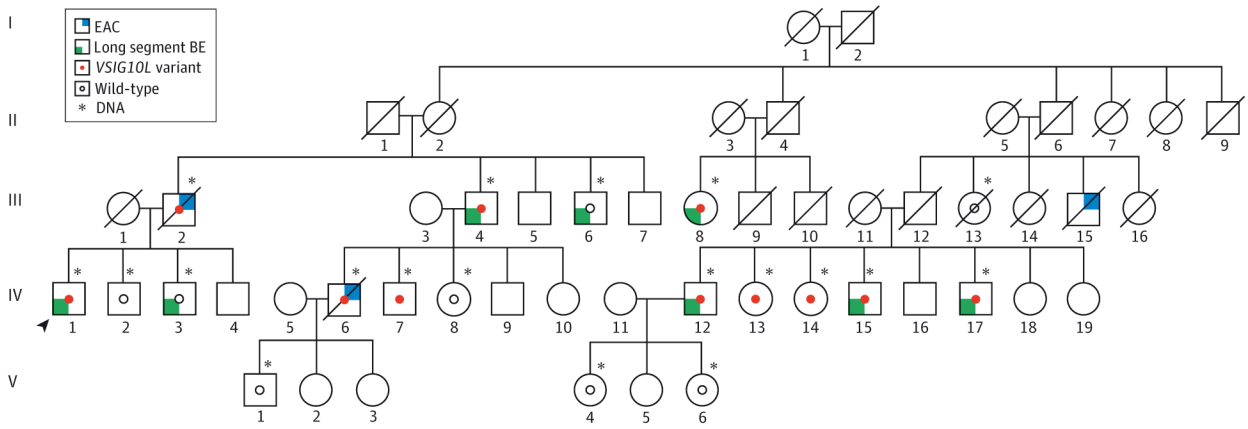

Figure 1. Pedigree of Familial Barrett Esophagus Family 31

Generations are indicated by roman numerals at left and individuals within a generation are numbered. Asterisks denote individuals from whom blood samples or formalin-fixed, parafin-embedded DNA was obtained. The proband is indicated by a black arrowhead (individual IV-1). Clinical characteristics and affectation status are specified in eTable in the Supplement. Exome sequencing and variant filtering work flow is outlined in eFigure 1 in the Supplement. BE indicates Barrett esophagus; EAC, esophageal adenocarcinoma.

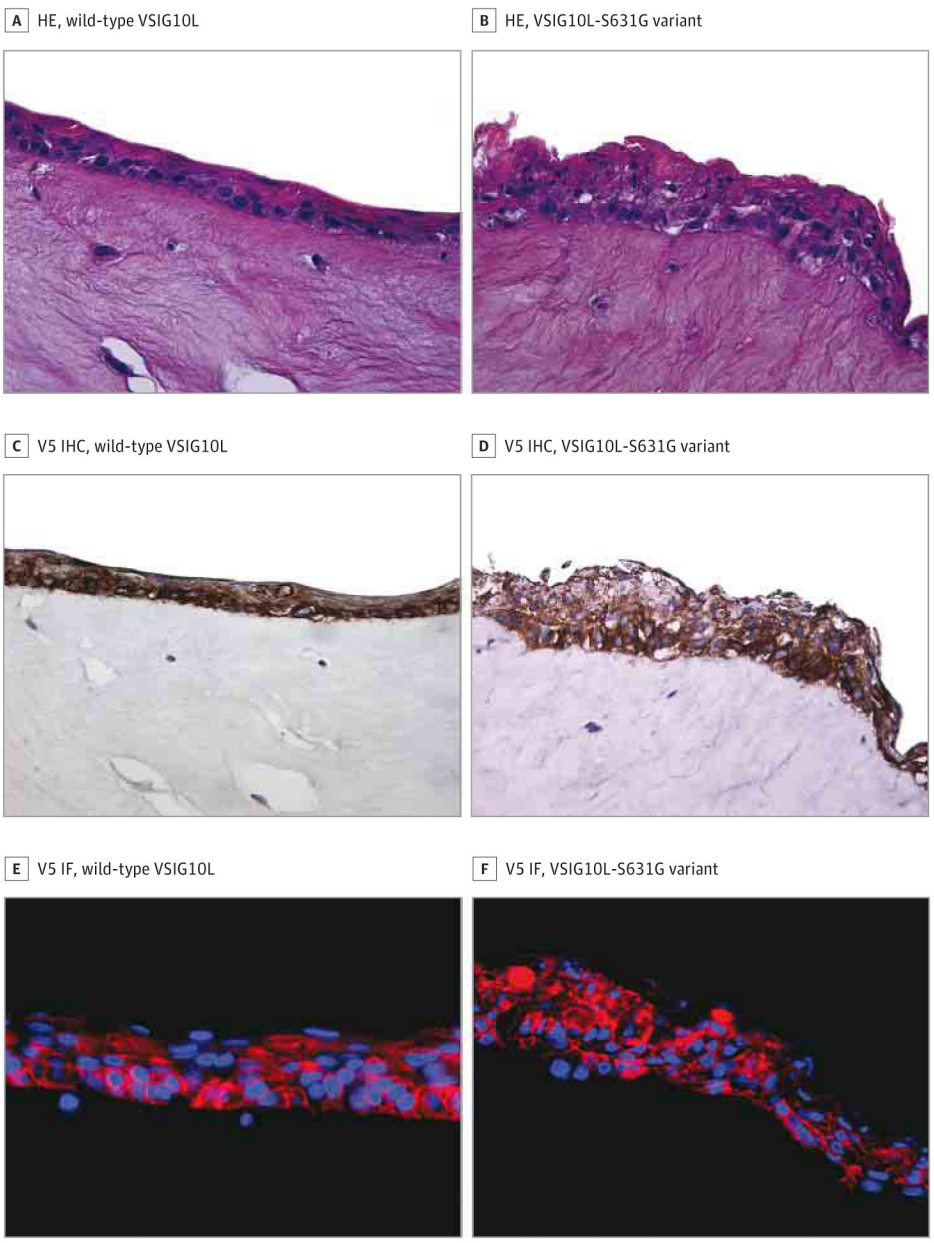

Figure 2. Photomicrographs of Organotypic Cell Culture Model Expressing Wild-Type VSIG10L and VSIG10L S631G Variant

A and B, Hematoxylin-eosin (H&E) analysis (original magnification ×40 for all panels) of wild-type VSIG10L expressed in esophageal keratinocytes shows a normal stratified squamous morphology having spherical nuclei in the basal cells that become more elongated with a parallel orientation to the surface as they mature apically. Expression of the mutant S631G VSIG10L variant induces a marked change in morphology characterized by epithelial dysmaturation having irregular shaped nuclei extending to the surface with loss of polarity and hyperkeratosis. C and D, Immunohistochemical analysis for the V5-tagged epitope shows diffuse expression of V5-tagged VSIG10L protein. E and F, Immunofluorescence targeted against the V5 epitope shows a membranous pattern of

expression in the wild-type expressing cells that becomes more disorderly on introduction of the S631G variant. IF indicates immunofluorescence; IHC, immunohistochemistry.

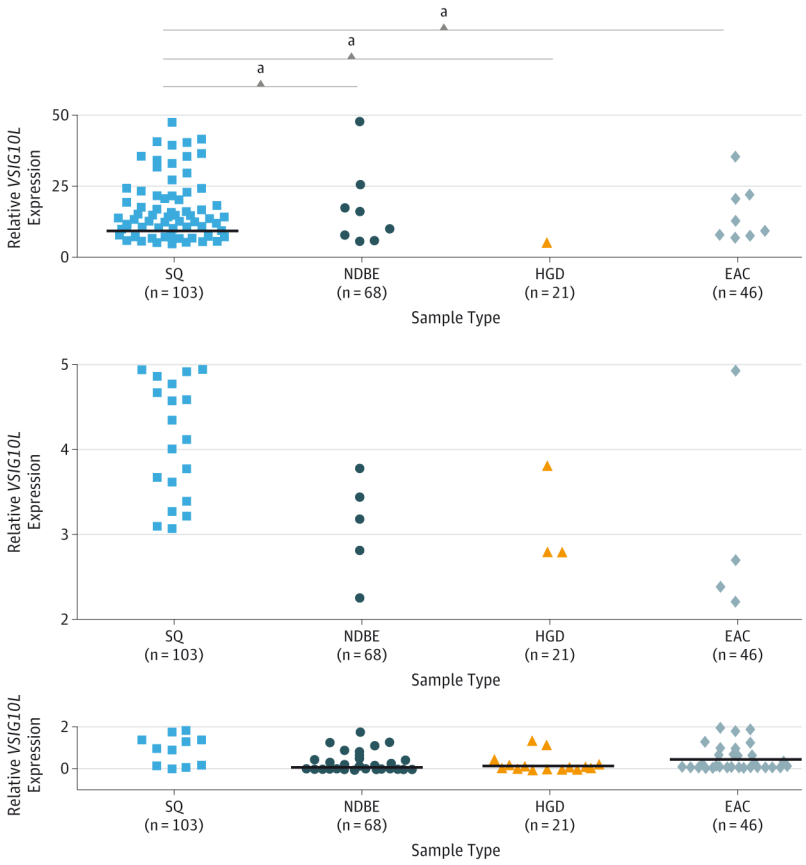

Figure 3. *VSIG10L* Transcript Expression in Normal Esophagus and Barrett-Associated Lesions

Quantitative real-time polymerase chain reaction of *VSIG10L* transcript from independent set of tissues including normal esophageal squamous (SQ), nondysplastic Barrett esophagus (NDBE), high-grade dysplasia (HGD), and esophageal adenocarcinoma (EAC). Expression level of *VSIG10L* in each of the SQ, NDBE, HGD, and EAC are shown as linear fold-change relative to mean *VSIG10L* expression of the entire cohort (N = 238).

[a] $P < .001$.

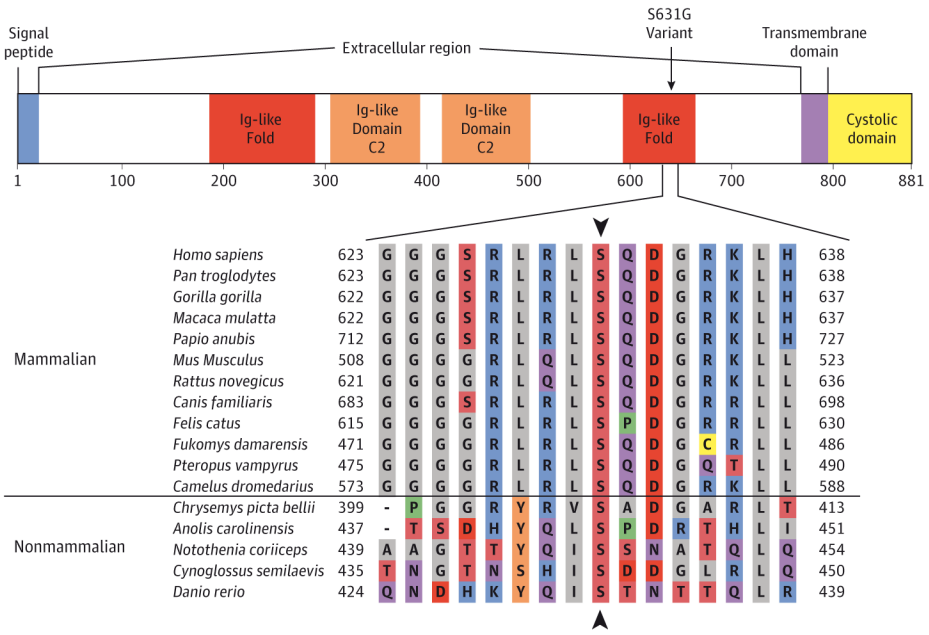

Figure 4. VSIG10L Protein Structure and Variant Conservation

Map of VSIG10L predicted protein domains (top) and multiple sequence alignment of VSIG10L orthologs (bottom). The S631G variant discovered in familial Barrett esophagus family 31 occurs within 1 of the predicted Ig-like folds as indicated by the arrow on the map. The multiple sequence alignment displays a stretch of amino acids within this Ig-like fold that encompasses the mutated serine residue (arrowheads), which is highly conserved in mammalian and nonmammalian species. Ig indicates immunoglobulin.

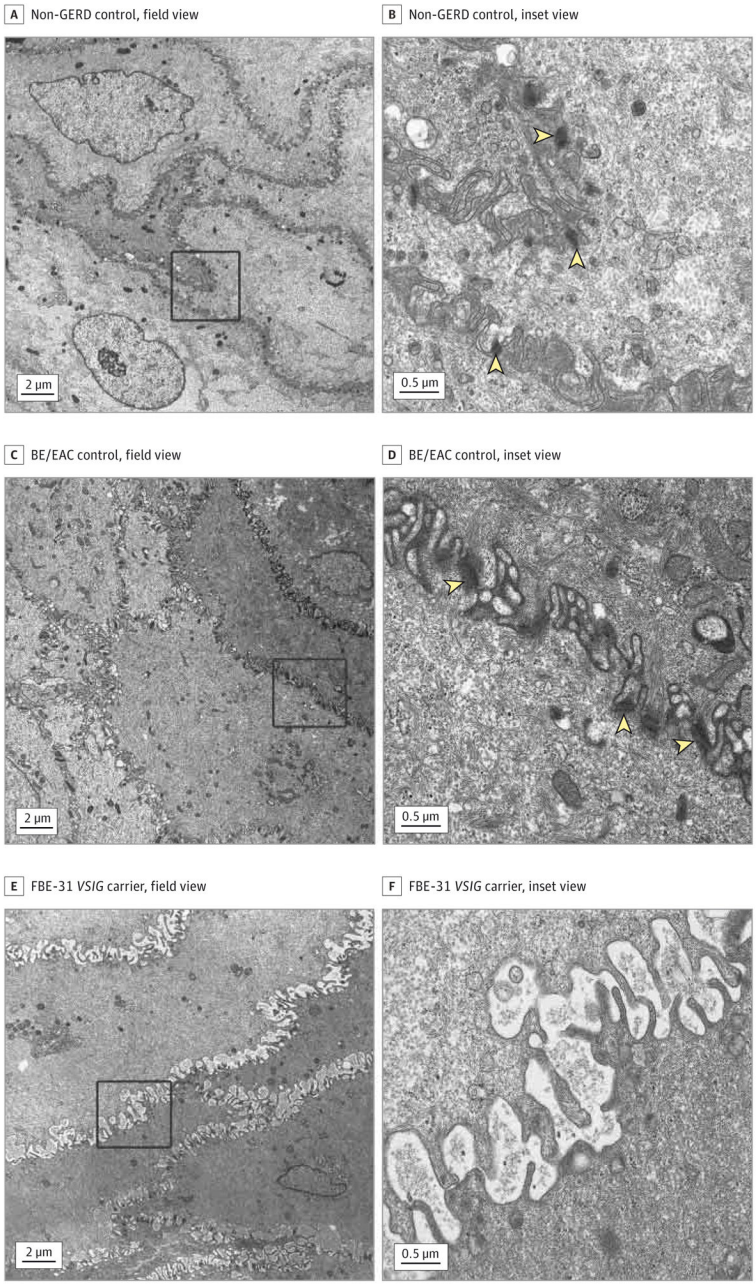

Figure 5. Photomicrographs of Electron Microscopy Studies in Esophageal Mucosa

Proximal esophagus biopsy samples from a non-GERD control patient, a patient with both BE and EAC, and individual III-4 from FBE family 31 harboring the *VSIG10L* S631G variant. Zoom of boxed area in each figure shown at right. A and B, The suprabasilar layer of the esophageal epithelium in both the non-GERD control patient and (C and D) patient with BE and EAC shows narrow intercellular spaces and abundant desmosomes (yellow arrowheads, inset views). In comparison, the esophageal mucosa from (E and F) a *VSIG10L* S631G carrier in our family, individual III-4, shows dilated intercellular spaces and reduced

desmosome formation. BE indicates Barrett esophagus; EAC, esophageal adenocarcinoma; FBE, familial Barrett esophagus; GERD, gastroesophageal reflux disease.