

Title: Hereditary Gastric Cancer: Beyond E-cadherin (*CDHI*) mutation-causes

Importance. Familial aggregation occurs in approximately 10% of gastric cancers, which are generally sub-classified histologically as intestinal-type and diffuse gastric cancers.

Though the genetic basis of familial intestinal-type gastric cancers is not known, in <50% of families clinically defined as hereditary diffuse gastric cancer, germline mutations in the E-cadherin gene, *CDHI*, are detected. This has led to management guidelines and prevention strategies for mutation carriers.

Objectives. To determine whether pathogenic germline mutations in genes alternative to *CDHI* can be found in hereditary gastric cancer families using a multiplex panel sequencing approach.

Design, Setting and Participants. One hundred fifteen probands from families who met the International Gastric Cancer Linkage Consortium clinical criteria for hereditary diffuse gastric cancer (n=106) or familial intestinal-type gastric cancer (n=9) were included. All diffuse gastric cancer probands tested negative for *CDHI*-mutations. Germline DNA was screened against a custom panel of 55 genes, including 14 prospective gastric cancer susceptibility genes, using a multiplexed amplicon-based next generation sequencing assay. Candidate mutations were validated via Sanger sequencing. Tumours from pathogenic mutation-positive probands were evaluated by immunohistochemistry.

Results. Of 115 probands, four clearly pathogenic truncating mutations were identified in unrelated families, including two different mutations in *CTNNA1* (alpha-catenin) and two different mutations in *BRCA2*. Previously described, functionally pathogenic

missense mutations in *SDHB* (2 families) and *STK11* (2 families) were also seen.

Additional truncating mutations of likely lower penetrance were identified in *ATM* (4 families), *MSR1* (2 families) and *PALB2* (1 family). Cancers from carriers of *CTNNA1* truncating variants had prominent loss of protein expression, further supporting their pathogenicity.

Conclusion and Relevance. Using a multi-gene panel, we showed that families with hereditary gastric cancer carry pathogenic mutations in genes commonly associated with other cancer susceptibility syndromes. In addition, this data suggests that familial gastric cancers, specifically hereditary diffuse gastric cancer subtype, should be genetically and not clinically defined. The genetic basis of the remaining families is likely attributable to mutations in genes yet to be implicated in gastric cancer or, in the true diffuse gastric cancer families, atypical aberrations in the non-coding regions of *CDH1*.