When clinical meets molecular: why, when and how do *CTNNA1* germline variants cause hereditary diffuse gastric cancer development

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ABSTRACT:

Introduction: Rare CTNNA1/αE-catenin germline truncating variants were found in Hereditary diffuse gastric cancer (HDGC) patients, however, full disease spectrum and variant-type causality are understudied. We aim to explore genotype-phenotype associations in *CTNNA1* variant carriers and molecular pathways causing CTNNA1-driven diffuse gastric cancer (DGC).

Methods: Using a clinical database of 1388 individuals (1577 phenotypes) from 364 CTNNA1 variant carrier families (Testing cohort: 71 European; Validation cohort: 290/293 American), we analyzed genotype–phenotype associations with multivariable logistic regression. Variants functional impact was assessed with in vitro/in vivo models. Transcriptomic profile of 11 DGC was analyzed.

Results: From 71 European carrier families (61% ascertained for HDGC), 26 carried truncating variants from which 21 (81%) met HDGC criteria. DGC, occurring on average at 47.3±13.7, was significantly more likely to occur in truncating families than in non-truncating (OR=8.33; 95%CI [3.125-25]; $p<0.001$). While not statistically significant, lobular breast cancer (LBC) followed the same trend in truncating carriers (OR=4.76;

95%CI [0.98-50]; p=0.053). From a validation cohort of 293 families (24% ascertained for HDGC) enriched in truncating variants (271/293), 32 (12%) had HDGC. Here, LBC occurring on average at 55.2±12.3 was more frequent than DGC (40.6±17.0). We created CRISPR/Cas9 edited gastric cancer cells bearing a CTNNA1 truncating variant with complete CTNNA1/αE-catenin loss. Nonsense Mediated mRNA Decay (NMD) blockade increased CTNNA1 mRNA expression by 13-fold, recovering to wild-type (WT) levels. We created a Drosophila α-cat knockout (KO), in which organ development/lethality was rescued with overexpression of human WT/missense αE-catenin, but not with truncated αE-catenin. Paired normal/tumor transcriptomic analysis of DGC from carriers revealed 67 upregulated genes in tumors, including HIF1α and PIK3R3, two cancer therapy targets and drug repurposing candidates in CTNNA1-driven DGC.

Conclusion: We provide a rational to test CTNNA1 specifically in families meeting HDGC criteria, showing a DGC association with truncating, but not missense variants. Consolidation of LBC association to truncating variants requires larger series. We highlight NMD as a prime mechanism for CTNNA1 truncated transcripts degradation and created an in vivo model to assess variants functional impact. CTNNA1 DGC overexpress molecules worth exploring as therapy targets.

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