Don’t MISS the mark: Rare missense variants in *APC* associated with polyposis phenotypes.

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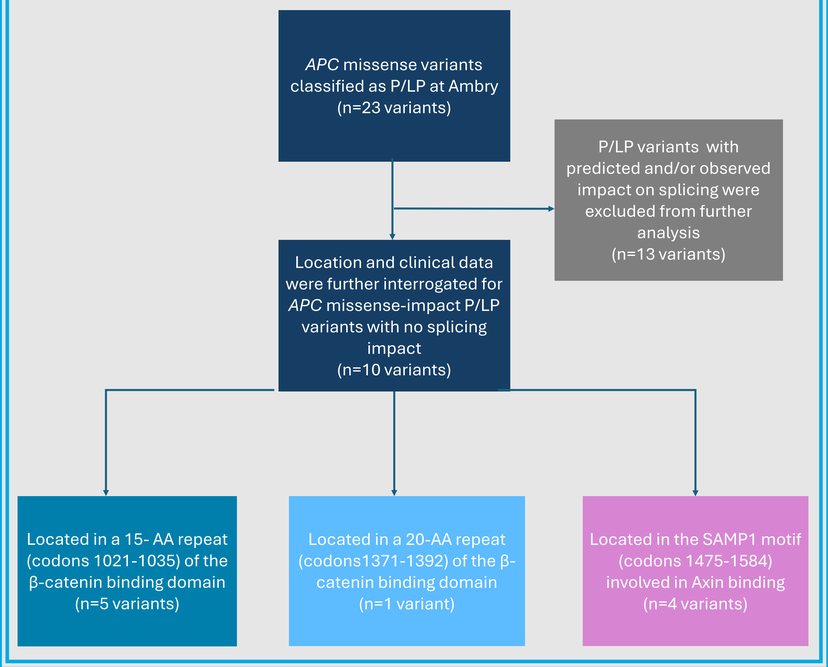
Background

Genetic variants in *APC* are associated with familial adenomatous polyposis (FAP), characterized by hundreds to thousands of colorectal polyps, or attenuated FAP (AFAP), characterized by fewer than 100 polyps. *APC* encodes a tumor suppressor protein that forms a complex with GSK3β and Axin to negatively regulate Wnt/β-catenin signaling. The Wnt/β-catenin pathway plays a critical role in many cellular processes, including cell cycle progression, proliferation, differentiation, migration, and apoptosis. Most disease-causing variants in *APC* introduce a premature termination codon (PTC) or are expected to cause aberrant splicing that results in premature truncation of the protein. It is thought that truncating variants impair the ability of the protein to bind to GSKβ, leading to uninhibited Wnt/β-catenin signaling, however, missense variants are rarely considered to be deleterious. For this reason, the ClinGen Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel (VCEP) recommends application of “BP1” evidence toward benign classification for *APC* missense variants. Current recommendations are to apply this line of evidence for all *APC* missense variants outside of one 15-amino acid repeat within the β-catenin binding domain (codons 1021-1035), where multiple likely pathogenic true missense variants have been identified[1]. We investigated whether there are any other regions in *APC* where missense variants are associated with phenotype and, thus, should also be considered for exclusion from application of BP1 as a line of evidence.

Methods

We retrospectively reviewed clinical data for missense *APC* variants classified as Pathogenic/Likely Pathogenic (P/LP) based on ACMG/AMP criteria in individuals undergoing multigene panel testing for hereditary cancer at a clinical diagnostic laboratory.



Results and Discussion

		Ambry Internal Clinical Data									Classifications				
Missense Variant	Protein motif	Total # of internal probands	Individuals reporting family hx of polyposis and/or CRC	Individuals <50 with unknown scope hx (age range)	individuals ≥50 with unknown scope hx (age range)	individuals with 0-9 polyps by c-scope (age range)	individuals with 10-19 polyps by c-scope (age range)	Individuals with 20-99 polyps by c-scope (age range)	Individuals with ≥100 polyps by c-scope (age range)	Individuals with polyps and/or CRC listed in indications for testing, but exact # of polyps unknown (age range)	ACMG evidence codes used by ClinGen VCEP	Classification by ClinGen VCEP	ACMG evidence codes used (Ambry)	Classification (Ambry)	Other ClinVar Classifications (excluding Ambry and ClinGen VCEP)
c.3077A>G p.N1026S	15-AA repeat 1 (β-catenin binding)	2	2	0	0	1 (30s)	0	0	0	1 (60s)	PP1_strong, PS1_supp, PS4_mod, PM2_supp	P	PS4_strong (+ external data), PP1_strong (+ external data)	LP	Px1 LPx1
c.3077A>C p.N1026T	15-AA repeat 1 (β-catenin binding)	4	2	1 (40s)	0	0	0	3 (40s-50s)	0	0	PS4_supp, PM2_supp, PM5_supp	VUS	PS4_strong, PM5_mod	LP	VUS x2
c.3083G>T p.S1028I	15-AA repeat 1 (β-catenin binding)	5	5	2 (30s)	0	0	0	3 (30s-60s)	0	0	PS4_strong, PM5_supp, PM2_supp	LP	PS4_strong, PM5_mod, PM1_mod	LP	VUS x3
c.3083G>A p.S1028N	15-AA repeat 1 (β-catenin binding)	8	8	2 (20s)	0	2 (40s)	0	3 (40s-60s)	0	1 (50s)	Not yet reviewed	N/A	PS4_strong, PM5_mod, PM1_mod, PP1_supp	P	VUS x5
c.3084T>A p.S1028R	15-AA repeat 1 (β-catenin binding)	15	14	4(<10)	0	1 (30s)	1 (30s)	6 (40s-50s)	0	3 (40s-70s)	PS4_mod, PP1_mod, PS3_supp, PM5_supp, PM2_supp	LP	PS4_strong, PM1_mod, PM5_mod	P	LP x1, VUS x2
c.4139C>T p.T1380I	20-AA-repeat 2 (β-catenin binding)	6	5	2 (20s-30s)	0	0	0	2 (50s-60s)	2 (50s-60s)	0	Not yet reviewed	N/A	PS4_very strong (+ external data)	LP	LP x1, VUS x1
c.4732T>G p.C1578G	SAMP1 (axin binding)	58	41	9 (<10- 40s)	5 (50s-70s)	1 (40s)	5 (30s-70s)	25 (30s-80s)	6 (40s-70s)	7 (40s-70s)	PS4, BS3_supp, BP1	VUS	PS4_very strong, PM1_mod	P	LP x2, VUS x5
c.4732T>C p.C1578R	SAMP1 (axin binding)	6	6	1 (20s)	1 (50s)	0	1 (30s)	3 (50s)	0	0	Not yet reviewed	N/A	PS4_strong, PM5_mod	LP	LP x1, VUS x1
c.4735A>T p.I1579F	SAMP1 (axin binding)	5	5	0	1 (60s)	0	0	4 (40s-50s)	0	0	Not yet reviewed	N/A	PS4_very strong (+ external data)	P	VUS x3
c.4747A>G p.M1583V	SAMP1 (axin binding)	5	4	1 (40s)	0	1 (30s)	0	2 (50s-70s)	0	1 (40s)	Not yet reviewed	N/A	PS4_strong (+ external data), PM1_mod	LP	VUS x1

Table 1: Missense variants with no predicted splicing impact that were classified as P/LP in our cohort. These variants were identified in a total of 114 individuals undergoing multigene panel testing. 79 individuals (~69%) reported a significant personal history of >10 polyps and/or CRC, with the majority of these reporting 20-100 polyps. 9/10 of these variants were located in either a specific 15-AA-repeat (dark blue) or the SAMP1 motif (pink). 1 was located in a 20-AA-repeat (light blue). Over 80% of all individuals with these variants reported a significant family history of polyps and/or CRC in first- and/or second-degree relatives.

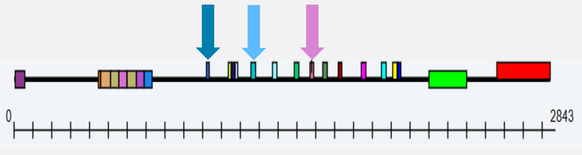


Figure 1: Diagram of protein structure of APC and motifs in which P/LP missense variants were identified. P/LP missense variants were found primarily in the 15-AA repeat (dark blue arrow, codons 1021-1035) that has been reported as critical for β-catenin binding[2,3], and the SAMP1 motif (pink arrow, codons 1475-1584) that has been reported as critical for axin binding[4]. In addition, one P variant was found in a 20-AA repeat of the β-catenin binding domain (light blue arrow, codons 1371-1392) that has not yet been well-studied.

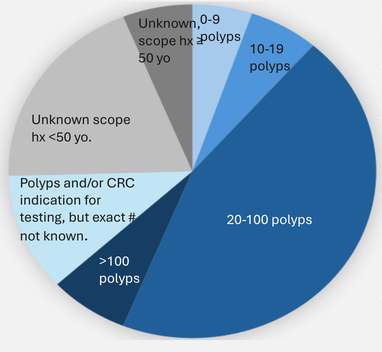


Figure 2: Distribution of reported phenotypes in 114 individuals with P/LP Missense variants in *APC*.

- 0-9 polyps, n=6, ~5% *(all individuals <45 yrs of age)*
- 10-19 polyps, n=7, ~6%
- 20-99 polyps, n=51, ~45%
- ≥100 polyps, n=8, ~7%
- Reported polyps and/or CRC as indication for testing, but exact no. is unknown, n=13, ~12%
- Unknown: no c-scope history reported:
  - <50 yo, n=22, ~19%
  - ≥50 yo, n=7, ~6%

TAKE HOME POINTS

1. Although rare, missense variants in *APC* can be pathogenic and are associated with attenuated FAP phenotypes- they should not be overlooked as potentially causative.

2. Specific regions of the APC protein may be more susceptible to a deleterious impact by missense variation- we recommend that these motifs be excluded from BP1 eligibility in variant classification.

References

1. Spier I, Yin X, Richardson M, et al; InSiGHT-ClinGen Hereditary Colon Cancer/Polyposis Variant Curation Expert Panel. Gene-specific ACMG/AMP classification criteria for germline APC variants: Recommendations from the ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel. *Genet Med.* 2024 Feb;26(2):100992.
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4. Spink KE, Polakis P, Weis WI. Structural basis of the Axin-adenomatous polyposis coli interaction. *EMBO J.* 2000 May 15;19(10):2270-9.
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