ADDITIONAL FILE 1

Supplementary Results

Population allele frequency threshold (AFT) calculation

To obtain a *POLE* and *POLD1* specific AFT we used the Whiffin/Ware calculator[1] (http://cardiodb.org/allelefrequencyapp/). We applied a conservative penetrance estimate (on the lower range) of 30%, and a prevalence of familial CRC of 1 in 770 in the general population (calculated from the Swedish nationwide cohort data: 12,829,251 people, 16,679 of whom are CRC patients with 1st or 2nd-degree relatives affected with CRC).[2] Data obtained by our group showed that the prevalence of *POLE* and *POLD1* pathogenic variants among familial CRC patients is ~0.6% (5/795 nonrelated CRC-affected individuals)[3-5]. Other relatively large studies[6-9] also detected prevalence values for *POLE* and *POLD1* pathogenic variants among familial CRC patients below 1%, only one[10] slightly exceeding that figure (~1.1%) (calculations made considering the pathogenic variants listed in Table 2). Based on these data, a value of 1% for the genetic contribution of *POLE* and *POLD1* pathogenic variants was considered (genetic heterogeneity: 0.01). Taking into consideration a 95% confidence interval (CI), the inferred AFT obtained for BA1, with allele heterogeneity set at 1, was 2.16 x 10^{-5} (0.002%) and for BS1, with allele heterogeneity set at 0.1, 2.16 x 10^{-6} (0.0002%).

Table S1. Standard ACMG/AMP combination rules to define pathogenic, likely pathogenic, likely benign and benign variants.[11]

Pathogenic	Likely pathogenic	Likely benign	Benign
≥ 2 Strong	1 Strong AND 1-2 Moderate	1 Strong AND 1 Supporting	1 Stand Alone (BA)
1 Strong AND: a. ≥ 3 Moderate OR b. 2 Moderate AND ≥ 2 Supporting OR c. 1 Moderate AND ≥ 4 Supporting	1 Strong AND ≥ 2 Supporting	≥ 2 Supporting	≥ 2 Strong
	≥ 3 Moderate		
	2 Moderate AND ≥ 2 Supporting		
	1 Moderate AND ≥ 4 Supporting		

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