

Hemochromatosis risk genotype is not associated with colorectal cancer or age at its diagnosis

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Summary

Homozygotes for the higher penetrance hemochromatosis risk allele, *HFE* c.845G>A (p.Cys282Tyr, or C282Y), have been reported to be at a 2- to 3-fold increased risk for colorectal cancer (CRC). These results have been reported for small sample size studies with no information about age at diagnosis for CRC. An association with age at diagnosis might alter CRC screening recommendations. We analyzed two large European ancestry datasets to assess the association of *HFE* genotype with CRC risk and age at CRC diagnosis. The first dataset included 59,733 CRC or advanced adenoma cases and 72,351 controls from a CRC epidemiological study consortium. The second dataset included 13,564 self-reported CRC cases and 2,880,218 controls from the personal genetics company, 23andMe. No association of the common hereditary hemochromatosis (HH) risk genotype and CRC was found in either dataset. The odds ratios (ORs) for the association of CRC and *HFE* C282Y homozygosity were 1.08 (95% confidence interval [CI], 0.91–1.29; $p = 0.4$) and 1.01 (95% CI, 0.78–1.31, $p = 0.9$) in the two cohorts, respectively. Age at CRC diagnosis also did not differ by *HFE* C282Y/C282Y genotype in either dataset. These results indicate no increased CRC risk in individuals with HH genotypes and suggest that persons with HH risk genotypes can follow population screening recommendations for CRC.

Introduction

The majority of adult-onset hereditary hemochromatosis (HH; MIM: 235200) cases are associated with pathogenic

variants in the *HFE* gene (MIM: 613609; GenBank: NM_000410.3). The highest penetrance pathogenic variant is *HFE* c.845G>A (p.Cys282Tyr, or C282Y; rs1800562), which has a minor allele frequency (MAF)

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~4%–6% in mixed European-ancestry populations.^{1,2} This allele is more common in Northern European ancestry populations and less frequent in Southern European ancestry populations.³ Homozygosity for this variant accounts for ~85% of individuals with HH.^{4,5} A more common European ancestry allele is rs1799945C>G (p.His63Asp, or H63D; rs1799945; MAF = 17% in the 1000 Genomes European population); however, it has lower penetrance and is infrequently associated with HH, unless found in the compound heterozygous form C282Y/H63D. *HFE*-HH has incomplete penetrance; most C282Y homozygotes do not develop HH. Penetrance is markedly higher in males. The Electronic Medical Records and Genomics (eMERGE) network recently reported a diagnostic rate of HH in males of 24.4% for p.C282Y homozygotes and 3.5% for p.C282Y/H63D compound heterozygotes; these rates were 14.0% and 2.3%, respectively, for females. A study from Melbourne found iron-overload-related disorders in 28.4% of males and 1.2% of females with the *HFE* C282Y/C282Y genotype and found <1% of C282Y/H63D compound heterozygotes to be similarly affected.⁵ Rarely, other genes^{6,7} or other *HFE* variants⁸ are associated with HH.

Dietary iron is absorbed in the proximal intestine in a regulated fashion. The C282Y pathogenic variant leads to abnormal folding and cellular localization of the *HFE* protein, which ultimately leads to decreased production of the iron regulatory hormone hepcidin, resulting in inappropriately increased absorption of iron from the duodenum.⁹ Increased tissue iron deposition results in organ damage, particularly to the liver and heart, which has been well described in HH.¹⁰ Most circulating iron is bound to transferrin, while iron in cells is bound to ferritin. Serum ferritin is generally considered the best marker of total body iron stores and is evaluated and followed in individuals with HH.¹⁰ Individuals with iron overload are treated with therapeutic phlebotomy to reduce iron stores, decreasing risk of organ damage.¹¹

The relationship between iron and colorectal cancer (CRC) and other cancers remains under investigation. Free iron has been proposed to be linked to carcinogenesis. It has been hypothesized that the association of red meat consumption with CRC¹² is due in part to heme iron promoting reactive oxygen species through the Fenton reaction¹³ and lipid peroxidation.¹⁴ Dietary iron was found to be associated with CRC risk in a meta-analysis;¹⁵ however, the same authors found an inverse relationship between serum ferritin and CRC risk. Increased iron load in individuals with HH, who over-absorb iron, has been hypothesized to lead to a higher constitutive free-radical burden, promoting carcinogenesis. Thus, the relationship between cancer and *HFE* genotypes that increase iron absorption offers another approach to understanding the relationship between iron and cancer.

The association of HH and hepatocellular carcinoma is well established.^{16,17} Associations of HH and CRC and breast cancer are less clear. Possible associations of HH in

other cancers, including colon cancer, are less clear. Increased CRC risk for carriers of either the *HFE* C282Y or the H63D HH risk-associated variants versus wild-type homozygotes (WT/WT) was reported in 2003; this was observed in a relatively small sample size of 475 CRC cases and 833 controls, and no increased risk was found for the variants separately or for any genotype.¹⁸ A 2013 meta-analysis, which included 9 studies of European ancestry participants, with 7,588 CRC cases and 81,571 controls, reported an increased risk of CRC (odds ratio [OR], 2.00; 95% confidence interval [CI], 1.32–3.04) in *HFE* C282Y homozygotes, although, notably, 7 of the 9 included studies did not provide significant evidence of association, and publication bias was not evaluated.¹⁹ A second 2016 meta-analysis also reported that *HFE* C282Y homozygosity was associated with excess CRC risk (OR, 1.69; 95% CI, 1.04–2.75, not adjusted for multiple contrasts, including 5 different cancer phenotypes and a sixth other cancer group).²⁰ It considered 10 studies with 286 CRC cases and 36,263 controls across 7 studies and included all of the papers considered by the 2013 meta-analysis except the large Asberg et al.²¹ dataset discussed below, plus three additional papers.²⁰

The association of clinical HH and CRC has not been studied in large samples, but one smaller study of 1,847 individuals with HH and 5,973 of their first-degree relatives found no association.¹⁷ The reported *HFE* genotype association with CRC is based on small sample sizes^{18–21} and has not been incorporated into clinical genetics or gastroenterology care. Additionally, the possible relationship between hemochromatosis risk genotypes or phenotype and CRC raises the question of appropriate CRC screening ages for individuals with the risk of genotype. Previous studies have not evaluated the association of *HFE* genotype or clinical HH phenotype with the age at CRC diagnosis. Therefore, we evaluated both the association of *HFE* genotype with both CRC case versus control status and age at CRC diagnosis in two large datasets, one a large CRC epidemiological study consortium and the other a personal genomics company. Specifically, our primary analyses contrasted *HFE* C282Y homozygotes with participants with no C282Y or H63D allele, which we refer to as wild-type homozygotes or WT/WT genotype.

Subjects and methods

Participants, phenotyping, and genotyping

The first dataset, CRC case-control (CRC C-C), includes data from The Colon Cancer Family Registry (CCFR),^{22,23} Colorectal Cancer Transdisciplinary Study (CORECT),^{24,25} and Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO).^{26,27} Details about the studies, genotyping quality, and imputation are described elsewhere.²⁷ The CRC case-control and genotype status of this dataset is reported by platform in [Table S1](#). All studies were approved by their respective institutional review boards, and proper consent was obtained. Given that hemochromatosis primarily affects individuals of European ancestry, we limited our

Table 1. OR for association of CRC status with HFE C282Y/C282Y genotype versus WT/WT

	Beta	SE	OR	95% CI	p value
CRC C-C					
Overall	0.079	0.091	1.08	(0.91–1.29)	0.386
Male	–0.061	0.129	0.94	(0.73–1.21)	0.638
Female	0.200	0.127	1.22	(0.95–1.57)	0.116
23andMe					
Overall	0.010	0.132	1.01	(0.78–1.31)	0.938
Male	0.017	0.188	1.02	(0.70–1.47)	0.930
Female	0.005	0.187	1.00	(0.70–1.45)	0.981

analyses to that ancestry group to reduce risk of population stratification. The dataset included 59,733 CRC or advanced adenoma cases (46.5% female, 8.5% advanced adenoma), of which 54,211 had age-at-diagnosis data, and 72,351 controls (49.6% female), who had European ancestry assigned by imputation. CRC phenotype was confirmed as invasive colorectal adenocarcinoma or advanced adenoma by medical record, pathology report, or death certificate. Henceforth this phenotype will be referred to as CRC. Control participants were selected based on study-specific eligibility and matching criteria (e.g., sex and age). HH diagnosis was not available for this dataset. Ancestry was determined genetically, using principal component analysis. *HFE* variants rs1800562 and rs1799945 were either directly genotyped or imputed using the TOPMed²⁸ panel as reference. Imputed allele dosages were converted to genotype calls using dosage <0.5 and >1.5 thresholds.

The second dataset was drawn from research participants among clients of the personal genetics company, 23andMe (Sunnyvale, CA, USA). Participants provided informed consent and answered online surveys under a protocol approved by Ethical and Independent Review Services, an independent Association for the Accreditation of Human Research Protection Programs (AAHRPP)-accredited institutional review board. CRC phenotype was determined by self-report response to the online survey question “What type(s) of cancer were you diagnosed with or treated for? Please select all that apply:” with the selection of “Colon/colorectal cancer.” Self-report of colon cancer has been reported to be reasonably accurate in prior studies.^{29,30} Similarly, HH diagnosis phenotype was assessed by self-report (“Have you ever been diagnosed with, or treated for, hemochromatosis?”) and contrasted with *HFE* genotype and self-report of phlebotomy (“Did your doctor ever remove your blood regularly in order to treat hemochromatosis?”). The 23andMe CRC dataset was restricted to 2,893,782 participants inferred to be of greater than 97% European ancestry, based on local ancestry, with age between 30 and 90 years. It included 13,564 CRC cases (50.1% female) and 2,880,218 non-CRC controls (55.5% female). A total of 11,270 participants reported HH, of whom 89 also reported CRC. *HFE* genotypes for rs1800562 and rs1799945 variants were determined by five different Illumina genotyping platforms (call rate > 99.95% for both variants). DNA extraction and genotyping were performed on saliva samples by LabCorp.

Statistical analyses

We conducted a CRC case-control analysis in the CRC C-C dataset using logistic regression to estimate the OR of CRC associated with

the exposure of C282Y/C282Y genotype versus no HH risk allele genotype (WT/WT), adjusted for age, sex, genotyping platform, and 3 ancestry principle components. Our analysis assumes the known recessive model for HH and only considers the highest penetrance risk genotype, which is expected to have the largest effect size. A secondary analysis contrasted participants with either C282Y/H63D or C282Y/C282Y risk genotypes to participants with no risk allele (WT/WT). Given that hemochromatosis penetrance is higher in males, sex-specific sensitivity analyses were also performed. We tested whether *HFE* C282Y/C282Y genotype is associated with age at CRC diagnosis using a two-sample t test.

Similar analyses were undertaken in the 23andMe dataset. Logistic regression was used to predict the outcome of prevalent CRC status for the exposure of C282Y homozygosity versus no risk allele (WT/WT); covariates included age, sex, genotyping platforms, and ancestry principle components. To test the association of C282Y homozygosity versus WT/WT on age at CRC diagnosis, Cox proportional hazard analysis was performed.

Results

CRC C-C dataset

The OR of CRC risk, including covariates age, sex, genotyping platform, and 3 ancestry principle components, was 1.08 (95% CI, 0.91–1.29; $p = 0.39$; see Table 1 for summary of CRC-*HFE* genotype association tests). The analyses included 580 participants who were *HFE* C282Y homozygotes and 114,053 participants who had no C282Y or H63D alleles (WT/WT genotype; Table S1). The analysis had 80% power to detect an OR of 1.24. Stratified analyses by sex identified no increased risk in males (OR, 0.94; 95% CI, 0.73–1.21; $p = 0.64$) or females (OR, 1.22; 95% CI, 0.95–1.57; $p = 0.12$). Secondary analyses combining $N = 2,307$ participants who were C282Y/H63D compound heterozygotes (967 CRC cases and 1,340 controls) with the C282Y homozygotes versus WT/WT also detected no association of genotype with CRC risk; this yielded an OR of 1.00 (95% CI, 0.92–1.08; $p = 0.99$).

The distribution of age at CRC diagnosis also did not differ between *HFE* C282Y/C282Y and WT/WT genotype; the mean age at CRC diagnosis was 64.2 years for 199 C282Y/C282Y genotype individuals and 64.7 years for 34,832 non-carriers ($p = 0.6$; age at CRC diagnosis for all genotypes is reported in Table 2). Genotype was not associated with age at CRC diagnosis in either males or females in stratified analyses.

23andMe dataset

HFE C282Y homozygosity versus WT/WT genotype was not associated with history of CRC (OR, 1.01; 95% CI, 0.78–1.31; $p = 0.94$). The analyses included 11,678 participants who were *HFE* C282Y homozygotes and 1,806,905 participants who had no C282Y or H63D alleles (WT/WT genotype). Secondary analyses considering both C282Y homozygotes and C282Y/H63D compound heterozygotes combined versus WT/WT showed no association with CRC as well (OR, 0.93; 95% CI, 0.83–1.05; $p = 0.25$). Similarly, no association of C282Y homozygote genotype with age

Table 2. Age at CRC diagnosis stratified by HFE C282Y and H63D genotype and HH phenotype

Genotype	Sample size	Mean CRC onset age	Onset age SD
CRC C-C all			
C282Y/C282Y	199	64.2	10.5
C282Y/H63D	839	64.5	11.9
H63D/H63D	1,168	64.7	11.5
C282Y/WT	4,630	64.4	11.1
H63D/WT	12,543	64.5	11.3
WT/WT	34,832	64.7	11.5
CRC C-C male			
C282Y/C282Y	97	63.6	9.16
C282Y/H63D	440	64.1	11.3
H63D/H63D	625	64.7	11.2
C282Y/WT	2,388	64.1	10.7
H63D/WT	6,565	64.5	10.8
WT/WT	18,687	64.6	11.1
CRC C-C female			
C282Y/C282Y	102	64.9	11.6
C282Y/H63D	399	64.9	12.4
H63D/H63D	543	64.8	11.2
C282Y/WT	2,242	64.7	11.5
H63D/WT	5,978	64.6	11.9
WT/WT	16,145	64.8	11.8
23andMe all			
C282Y/C282Y	58	52.3	12.6
C282Y/H63D	234	56.0	11.4
C282Y/WT	1,376	56.4	12.3
H63D/H63D	287	55.6	11.6
H63D/WT	3,137	56.2	12.1
WT/WT	8,472	56.5	12.2
23andMe male			
C282Y/C282Y	29	51.9	12.1
C282Y/H63D	118	57.0	11.2
H63D/H63D	144	55.5	12.1
C282Y/WT	676	57.1	12.1
H63D/WT	1,541	56.6	11.8
WT/WT	4,257	57.1	12.0
23andMe female			
C282Y/C282Y	29	52.6	13.2
C282Y/H63D	116	55.1	11.6
H63D/H63D	143	55.8	11.1
C282Y/WT	700	55.6	12.4

Table 2. Continued

Genotype	Sample size	Mean CRC onset age	Onset age SD
H63D/WT	1,596	55.8	12.4
WT/WT	4,215	55.8	12.4

at diagnosis of CRC was detected using survival analysis (OR, 1.07; 95% CI, 0.82–1.40; $p = 0.63$; Table 2).

We considered testing the association of self-reported HH with CRC in the 23andMe dataset; however, evaluation of the self-reported data suggested poor phenotype quality. 23andMe participants' self-report of HH phenotype was compared with *HFE* C282Y and H63D allele counts. Of the 89 participants who reported having both CRC and HH, only 39 had genotypes consistent with an HH diagnosis (20 C282Y/C282Y, 12 C282Y/H63D, and 7 H63D/H63D), and 50 (52.2%) did not have genotypes consistent with the HH diagnosis (20 WT/WT, 20 WT/H63D, and 10 WT/C282Y). Of the 11,178 reporting HH but not CRC, 6,131 (54.9%) did not have compatible genotypes. While there are rare non-*HFE* causes of HH in adults and rare *HFE* variants that were not genotyped here that can cause HH, it is likely that the majority of these participants without a risk genotype are misreporting HH or misdiagnosed, as HH rarely occurs in WT/WT European ancestry individuals.³¹ We evaluated whether use of a supplemental question regarding treatment with phlebotomy would improve accuracy of self-report of HH; however, even in genotypes not consistent with HH, over 40% of participants self-reporting HH also reported phlebotomy. Therefore, the self-report of HH and phlebotomy were considered to poorly reflect actual HH to analyze further.

Discussion

Our analyses, in two very large datasets, detect no association of *HFE* C282Y/C282Y genotype with CRC. The upper limit on the 95% CI of the OR in both cases was ~1.3. While we cannot rule out a more modest association of this genotype with CRC, these results conflict with prior reports of 2- to 3-fold excess risk or the 2016 meta-analysis estimate of an OR of 1.7.²⁰ Our secondary analyses of *HFE* C282Y/C282Y or C282Y/H63D genotype versus WT/WT genotype also did not find an *HFE* genotype association with CRC. The less-penetrant H63D allele has generally not been associated with CRC risk,^{20,32} and we also did not see any association of this allele with CRC in our analyses. Additionally, we found no association of HH risk genotype with age at CRC diagnosis, consistent with the lack of *HFE* CRC association.

A positive CRC-*HFE* association found in the larger 2013 meta-analysis appears to be primarily driven by a disproportionately large single report of a Norwegian cohort by Asberg et al.²¹ In that study, Asberg et al. did not genotype all participants but only genotyped 622 individuals who

had a “high” non-fasting serum transferrin saturation (TS 55% [men, normal 15%–50%] or TS 50% [women, normal 12%–45%])³³ and also a subsequent “high” fasting serum TS. Of participants with two “high” TS measures, 49% tested positive for *HFE* C282Y homozygosity; of participants homozygotes, mean serum TS was 70% in females and 79% in males. Participants without such abnormal iron studies (64,616 individuals) were assigned a “non-homozygous” genotype without any genetic testing. Thus, with respect to genotype classification, their study relied more on iron studies than measured *HFE* genotype, as non-penetrant C282Y homozygotes would be misclassified for genotype, and other causes of elevated iron would be misclassified as positive for genotype. This appears to be the reason it was excluded from the 2016 meta-analysis.²⁰

The only other study that concluded excess risk of CRC with the *HFE* C282Y/C282Y genotype, Osborne et al.,³⁴ was included by both meta-analyses.^{19,20} Osborne et al. reported an OR of 2.31 (95% CI, 1.24–4.32), finding 10 incident CRC cases with that genotype. The supplemental material of a recent phenome-wide association study (PheWAS) has reported no positive association of CRC (International Classification of Diseases, Ninth Revision [ICD9] 153.2) with any iron-related SNPs, specifically reporting negative results for rs1800562 (*HFE* p.C282Y) as $\beta = 0.01$ and $p = 0.8$, and rs1799945 (*HFE* H63D) of $\beta = -0.07$ and $p = 0.04$, uncorrected for 909 tests.³⁵

A limitation of this work is that the 23andMe history of CRC diagnosis status and age at diagnosis are self-reported. Prevalent CRC status is susceptible to survival bias. While they differ in the design and assessment of CRC, the results from the two datasets are consistent in finding no association of *HFE* genotype with CRC or age at CRC diagnosis. Lack of reliable data on HH diagnosis is an additional limitation, as we could not assess risk of CRC in penetrant HH. Given the incomplete penetrance of HH, a true association that relies on elevated iron stores as a mechanism might be very small and, thus, difficult to detect, even in this large sample size; however, the CIs here rule out a large increased risk of CRC. Our analyses are limited to individuals of European ancestry and may not be generalizable to other ancestry groups. Strengths of the current study include the large sample sizes and the use of two very different ascertainment systems: a consortium of existing CRC case-control studies and nested case-control studies and the large population-based 23andMe dataset, where enrollment is likely not correlated with CRC status or HH risk.

In summary, we do not find any evidence of an *HFE* C282Y/C282Y genotype association with CRC in two large and complementary datasets, the first ascertained by CRC case-control status and the second ascertained independently of either CRC or HH diagnoses status. Further, we see no association of HH risk genotypes on CRC age at diagnosis. These results indicate no increased CRC risk in individuals with HH genotypes and suggest that persons with HH risk genotypes can follow population screening recommendations for CRC.^{32,36,37}

Data and code availability

Genotype data for GECCO and CORECT have been deposited in the database of Genotypes and Phenotypes (dbGaP) under accession number phs001078.v1.p1, phs001415.v1.p1, and phs001315.v1.p1. The UK Biobank resource is available upon application to the Access Management Team of UK Biobank Limited.

The variant-level data from the 23andMe dataset are fully disclosed in the manuscript. Individual-level data are not publicly available due participant confidentiality and in accordance with the IRB-approved protocol under which the study was conducted.

Supplemental Information

Supplemental Information can be found online at <https://doi.org/10.1016/j.xhgg.2020.100010>.

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Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

Declaration of interests

D.R.C. is a paid consultant for UnitedHealth Group Research and Development. H.H. is on the Scientific Advisory Boards of Invitae Genetics, Genome Medical, and Promega. She is a consultant for 23and Me. She conducts collaborative research with Invitae Genetics, Myriad Genetic Laboratories, and Ambry Genetics. She holds stocks in Genome Medical. J.M., P.F., E.K., and members of the 23andMe Research Team are current or former employees of 23andMe and hold stock or stock options in 23andMe. S.B.G. is a founder of Brogent International with equity.

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Web resources

dbGaP, <https://www.ncbi.nlm.nih.gov/gap/>
Ethical and Independent Review Services, <http://www.eandireview.com>
GenBank, <https://www.ncbi.nlm.nih.gov/genbank/>
OMIM, <https://www.omim.org>

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