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HFE C282Y homozygotes are at increased risk of breast and colorectal cancer

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Abstract

The evidence that mutations in the *HFE* gene are associated with increased cancer risk is inconsistent.

The Melbourne Collaborative Cohort Study is a prospective cohort study that commenced recruitment in 1990. Participants of born in Australia, New Zealand, the United Kingdom or Ireland (n = 28,509) were genotyped for the *HFE* C282Y variant. Incident cancers were ascertained from Australian cancer registries during an average of 14 years follow up. Hazard

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ratios, confidence intervals and p-values were obtained from separate Cox regression analyses for colorectal, breast and prostate cancers, all other solid cancers and all cancers.

Compared with those with no C282Y variant, C282Y homozygotes were at increased risk of colorectal cancer (HR 2·28; 95% CI 1·22, 4·25; p = 0.01) and female C282Y homozygotes were at increased risk of developing breast cancer (HR 2·39; 95% CI 1·24, 4·61; p = 0.01) but male C282Y homozygotes were not at increased risk for prostate cancer (HR 0·96; 95% CI 0·43, 2·15; p = 0.92). C282Y/H63D compound heterozygotes were not at increased risk for colorectal cancer, (HR 1 27; 95% CI 0·80, 2·01); breast cancer, (HR 1·16; 95% CI 0·74, 1·84); or prostate cancer (HR 1·08; 95% CI 0·68, 1·70).

Conclusion—*HFE* C282Y homozygotes have twice the risk of colorectal and breast cancer compared with those without the C282Y variant.

Keywords

Haemochromatosis; neoplasm; prospective cohort; iron

The essential trace element iron can be carcinogenic through a variety of mechanisms including catalysing the formation of mutagenic hydroxyl radicals,(1) suppression of the host immune response (2) and by acting as an essential nutrient for proliferating tumour cells.(3) Hereditary haemochromatosis is an inherited disorder of iron overload characterized by inappropriately elevated intestinal iron absorption. In HFE-associated hereditary haemochromatosis, mutations in the *HFE* gene can impair synthesis of the master iron-regulatory protein hepcidin. Reduced hepcidin levels leads to increased release of iron from intestinal cells and macrophages, elevating plasma transferrin saturation and causing deposition of iron in the liver and other tissues.(4) Individuals homozygous for the mutation that leads to the C282Y substitution in the HFE protein are at increased risk of iron overload (5) and account for 82–90% of clinical diagnoses of hereditary haemochromatosis for those of northern European descent.(6) We have recently shown that the majority of C282Y homozygotes (82% of men and 65% of women) have elevated serum ferritin and, based on objective criteria, 28% of male and 1% of female C282Y homozygotes develop iron overload-related disease by, on average, 65 years of age. People having a single copy of both the C282Y and H63D mutations in *HFE* (described as compound heterozygotes) have, on average, higher serum ferritin and transferrin saturation levels than people with neither *HFE* mutation, although they are not at increased risk of iron overload-related disease.(7) Previous studies of the association between *HFE* genotype and risk of colorectal cancer and breast cancer have provided inconsistent results,(8–17) possibly related to the small numbers of C282Y homozygous participants (see Supplementary Materials).

We assessed the relationships between the risk of cancer including breast, colorectal and prostate cancers and the C282Y variant of the *HFE* gene using a prospective cohort study.

Experimental Procedures

Participants

From 1990 to 1994 the Melbourne Collaborative Cohort Study enrolled 41,514 people (24,469 women) aged between 27 and 75 years (99·3% 40–69 years) in Melbourne, Australia. Participants were recruited using Electoral Rolls (voting is compulsory for Australian citizens), and by advertisements and community announcements.

Approximately one quarter of the participants were born in Greece, Italy or Malta, but as the prevalence of the C282Y variants in the *HFE* gene was low in this group,(18) genotyping

was restricted to the 31,181 participants born in Australia, New Zealand, the United Kingdom or Ireland.

Because cancer diagnosis was ascertained prospectively, 1245 participants who had been diagnosed with any cancer before enrollment in the study were excluded from the analysis. A further 41 were excluded because their baseline blood samples were missing or they had insufficient DNA for genotyping, leaving 29,895 eligible participants. The study protocol was approved by the Cancer Council Victoria's Human Research Ethics Committee (Project No. HREC0105). Participants gave written consent for participation and for the investigators to obtain their medical records.

Assessment of Risk Factors at Baseline

At baseline, a structured interview schedule was used to obtain information about potential risk factors including country of birth, education, smoking history, alcohol consumption, and for women, reproductive history and use of hormone replacement therapy. Current usual diet was assessed by a 121-item food frequency questionnaire.(19) A blood sample was collected and weight, height, waist and hip circumferences were measured.(20)

Ascertainment of Cancer Cases

Addresses and vital status of the subjects were determined by record linkage to electoral rolls, the National Death Index, Victorian death records and from electronic phone books and responses to mailed questionnaires and newsletters. Cancer cases were identified by linkage to population-based cancer registries in all Australian states.

Assessment of HFE Genotype

Blood samples were stored either in liquid nitrogen or dried on Guthrie cards. DNA was extracted from Guthrie cards by the Chelex method and from buffy coats using a guanidinium isothiocyanate-based method (Corbett Buffy Coat CorProtocol™ 14102). All samples were genotyped for the single nucleotide polymorphism (SNP) in the *HFE* gene that is responsible for the C282Y substitution in the HFE protein (rs1800562) using real time PCR. Those samples with one copy of the variant leading to C282Y were also genotyped for the variant leading to the H63D substitution (rs1799945).(21) Therefore, there were four *HFE* genotype groups: (i) C282Y homozygotes, (ii) simple heterozygotes with one copy of the C282Y variant and no copies of the H63D variant, (iii) compound heterozygotes with one copy each of the C282Y and H63D variants and (iv) other *HFE* genotype with no copies of the C282Y variant and unknown number of copies of the H63D variant. The H63D and C282Y variants have only very rarely been reported to occur together on a single chromosome.(22)

For participants classified as C282Y homozygotes by this genotyping, additional genotyping was performed for confirmation. All participants homozygous for the C282Y variant (as part of an HFE-genotype stratified random sample) were invited to participate in a study of iron and health ("HealthIron") from 2004–7, where we collected a cheek swab, with subsequent genotyping for C282Y and H63D in an independent laboratory. For those who did not participate in HealthIron, additional genotyping was done on a baseline plasma sample. Only those participants classified as C282Y homozygotes by the initial and confirmatory genotyping were considered to be homozygotes for this analysis, otherwise they were classified according to the results of the confirmatory genotyping.

Statistical Analysis

Hazard ratios were estimated using Cox regression with age as the time axis. Follow up began at baseline and ended at death, date of diagnosis, date left Australia, or end of follow-

up, whichever came first. Follow-up ended on 31 December 2007 for colorectal cancer and 31 December 2006 for all other cancers. For participants who moved to other states, end of follow-up ended on 31 December 2003 because linkage to other cancer registries was complete to 2003 only. Separate analyses were performed for the three of the four most common cancers in this cohort (female breast, prostate and colorectal). Melanoma was the only other cancer common enough to be considered separately, but we did not analyze it because we had no data on sun exposure, its major cause. Separate analyses were also performed for all other solid cancers combined and all cancers. Analyses of prostate cancer and breast cancer were restricted to men and women, respectively. The primary exposure variables were combinations of SNP genotypes of the *HFE* gene (C282Y and H63D variants). The proportional hazards assumption was examined visually from plots of the Nelson-Aalen estimate of the cumulative hazard, and formally by tests based on Schoenfeld residuals. Statistical analyses were performed with Stata 10·1 (Stata Corporation, College Station, TX, USA).

Several variables were assessed as potential confounders of the association between *HFE* genotype and cancer risk. These included those listed in Table 1: age, height, weight, waist circumference, body mass index (BMI), smoking, alcohol consumption, physical activity, education, dietary intake of fresh red meat, processed meat, folate, calcium and multi-vitamin use. For women, all analyses considered adjustment for current hormone replacement therapy use, age at menarche, history of pregnancy (yes/no) and menstruation at baseline. A change of any estimated hazard ratio of 10% or more after inclusion of a potential confounding variable in the statistical model was considered to be indicative of confounding. None of the variables met this criterion, and hence the final hazard ratios were unadjusted. For the analyses of colorectal cancer, all cancer and all non-haematological cancer, statistical models based on females only were analysed first to examine possible confounding by reproductive and hormonal factors. None were found and the definitive models for these cancers were stratified by sex to account for differences by sex in the underlying hazard rates.

Because data on use of non-steroidal anti-inflammatory drugs and aspirin at baseline were coded only for a random sample of 5,268 participants, these variables were assessed only for their association with genotype. Table 1 shows that there were only small differences in use of NSAIDs and aspirin when assessed by genotype group and, therefore, confounding by use of aspirin or NSAIDs is unlikely.

To obtain a quantitative summary of the literature on *HFE* genotypes and risk of breast, prostate and colorectal cancer, where possible we performed fixed effects meta-analyses that included the present study (see Supplementary Material).

Results

Samples from 28,509 (95%) of the 29,895 eligible participants were successfully genotyped for the major mutation in *HFE* (leading to the C282Y substitution). Of these, 24,339 (85.4%) were wild-type, 3977 (13.3%) were C282Y heterozygotes and 193 (0.64%) were C282Y homozygotes. The expected numbers under Hardy-Weinberg equilibrium are 24,313 wild type, 4029 heterozygous and 167 homozygous ($p = 0.03$), thus there was an excess of homozygotes and a deficit of heterozygotes.

Genotyping for H63D was successful for 3882 (98%) of the 3977 C282Y heterozygotes, of which 690 (17.8%) were heterozygous and thus were heterozygous for both the C282Y and H63D variants. We excluded from further analysis the 95 participants for whom the H63D genotyping failed, because it was not known whether they were simple C282Y

heterozygotes or compound heterozygotes. It was not possible to check for Hardy-Weinberg equilibrium because of the restriction of H63D genotyping to C282Y heterozygotes.

A summary of baseline characteristics for participants successfully genotyped is presented in Table 1. C282Y homozygotes had the lowest mean BMI, which would mean that any confounding of the association would be towards the null. A higher proportion of male participants than female participants were C282Y homozygotes. Only minor differences were seen for other risk factors.

Between initial attendance and 31 December 2007, 84 participants had left Australia and 3,365 were confirmed dead. There were 620 participants diagnosed with colorectal cancer (mean age at diagnosis 68·3, range 42·0–83·3 years), 664 women diagnosed with breast cancer (mean age at diagnosis 62·8, range 41·3–82·0 years), 758 men diagnosed with prostate cancer (mean age at diagnosis 67·6, range 47·6–83·6 years), 3755 with non-haematological cancers (mean age at diagnosis 66·1, range 41·3–84·4 years) and 4025 with any cancer (mean age at diagnosis 66·1, range 41·3–84·4 years).

Table 2 presents the results of the Cox regression analysis of the association between the C282Y and H63D variants of the *HFE* gene and the risk of cancer. C282Y homozygotes had an increased risk of colorectal cancer compared with those with no C282Y variant (HR 2·28; 95% CI 1·22, 4·25). Similarly, female C282Y homozygotes had increased risk of breast cancer compared with those with no C282Y variant (HR 2·39; 95% CI 1·24, 4·61). There was no evidence of increased risk of prostate cancer for male C282Y homozygotes (HR 0•96; 95% CI 0•43, 2•15), although the wide confidence interval does not preclude the possibility of an association of similar magnitude to those seen for breast and colorectal cancer. The hazard ratio for all other non-haematological cancers was 1·15 (95% CI0·73, 1·81). Hazard ratios for simple and compound heterozygotes were close to unity for each of the cancers in Table 2; the strongest association was for compound heterozygotes and risk of colorectal cancer (HR 1·27; 95% CI 0·8, 2·1). Exclusion of the first two years of follow-up made no material difference to any of the results (data not shown).

Meta-Analyses

No meta-analysis of studies of the association between C282Y homozygosity and the risk of colorectal cancer was performed because two studies had no C282Y homozygous cases;(11, 12) the other two studies had only two C282Y homozygous cases each.(8, 15) Two studies of colorectal adenomas had one and five C282Y homozygous cases respectively.(13, 14) The pooled estimate of the hazard ratio from three studies of breast cancer was 2•1 (95% CI 1•13, 3•90), although the other two studies each had only one homozygous case.(9, 16) The pooled estimate of the hazard ratio for prostate cancer, from two studies only, was 1•12 (95% CI 0•56, 2•21).

Meta analyses of compound heterozygotes gave pooled estimates of the hazard ratio of 1•36 (95% CI 0•92, 2•01) for colorectal cancer, 1•41 (95% CI 0•97, 2•06) for colorectal cancer and adenomas together, and 0•95 (95% CI 0•79, 1•16) for breast cancer. No other studies published data for prostate cancer. For simple C282Y heterozygotes, the pooled estimates of the hazard ratio were 1•00 (95% CI 0•84, 1•19) for colorectal cancers, 0•99 (0•86, 1•15) for colorectal cancers and adenomas together, 0•95 (95% CI 0•79, 1•16) for breast cancer and 0•94 (95% CI 0•78, 1•13) for prostate cancer.

Discussion

HFE C282Y homozygotes had a two-fold increased risk of breast and colorectal cancer compared with those who had no C282Y variant. They had no increased risk of prostate

cancer or of all other cancers combined, but moderate associations cannot be ruled out with confidence.

Our study has several strengths. Recruitment was not based on the presence or absence of haemochromatosis and occurred prior to the discovery of the *HFE* gene, thus reducing the potential for selective recruitment bias or reverse causation. We had almost complete ascertainment of cancers because all Australian states have high-quality population-based cancer registries and few participants left the country. We had extensive information on diet and other risk factors that might confound the associations, none of which showed great variation between *HFE* genotypes (see Table 1). There are also several limitations. We were unable to determine whether the associations with genotype were mediated through body iron stores because data on baseline serum ferritin and transferrin saturation were not available for most cases of cancer. Surveillance of participants known to have haemochromatosis may have contributed to the apparent increased risk of breast and colorectal cancer. Because we had incomplete information on diagnoses of haemochromatosis for the C282Y homozygotes, we were unable to undertake sensitivity analyses to address this issue. If iron is involved in the causal pathway, we might have underestimated some associations if some C282Y homozygotes had therapeutic venesection, thus depleting their iron stores. Finally, there was deviation from Hardy-Weinberg equilibrium for C282Y. Genotyping errors are unlikely to be the cause of this deviation because of the additional genotyping of C282Y homozygotes using a second, independent DNA sample. We note also that genotyping errors such that people with no or only one C282Y variant were classified as C282Y homozygotes, would have attenuated the associations.

The present study had more cases homozygous for the C282Y substitution than other studies of breast and colorectal cancer, and provides most of the evidence regarding whether C282Y homozygotes have increased risks for these cancers. The pooled estimates for breast and prostate cancer were both close to the estimate derived from our study. No pooled estimate was calculated for colorectal cancer because two studies had no homozygous cases. For compound heterozygotes the pooled estimate was consistent with a small increase in risk for colorectal cancer, albeit not significant. For breast cancer, the pooled estimate was close to one, but a modest association cannot be excluded. For C282Y heterozygotes the pooled estimates for breast cancer, colorectal cancer and prostate cancer were all one or close to one and had narrow confidence intervals, suggesting that C282Y heterozygotes have no increase in risk for breast, colorectal or prostate cancer.

Elevated body iron stores is one potential explanation for an association between *HFE* genotype and risk for cancer. The strongest evidence for a direct role of body iron stores comes from a secondary analysis of a randomised controlled trial of phlebotomy for patients with peripheral arterial disease.(23) The risk for cancer was lower for the phlebotomy group (ferritin 79.7 ng/mL versus 122.5 ng/mL) with a HR of 0·65 (95% CI 0•43, 0•97). Results from several cohort studies have also been reported. Positive associations were found between serum ferritin and risk for liver cancer and for combined all other cancers combined in a Taiwanese cohort study.(24) In an analysis of participants in a French antioxidant trial, women with serum ferritin levels above 160 g/L had 1·88 (95% CI 1·05, 3·35) times the cancer risk of those with levels below 30 g/L, but no association was seen for men.(25) Other studies found little evidence of positive associations with colorectal adenomas,(13, 26) or some evidence of inverse associations with colorectal cancer.(27, 28)

Other cohort studies have considered risk of cancer in relation to transferrin saturation and total iron binding capacity, which examine an iron transport compartment and hence not iron stores. Three analyses from follow-up of the first National Health and Nutrition Examination

Survey (NHANES) have been reported.(29–31) Stevens *et al.* reported a relative risk for all cancer of 1·81 (95% CI 1·21–2·71) comparing people with a baseline transferrin saturation of 60% or higher with people with a transferrin saturation of 30% or less.(29) The risk was only slightly elevated for those with transferrin saturation between 50% and 60% (relative risk 1·38 (95% CI 1·00–1·90)) and not elevated at lower levels. The latest analysis with more cases found weakly elevated risks for colorectal cancer.(31) Positive associations between risk for cancer and transferrin saturation were also found during follow-up of the second NHANES cohort.(32) Selby et al. reported an inverse association between total iron binding capacity and subsequent risk for lung cancer, but little evidence of an association for other cancers.(33) A Finnish study found increased risks for colorectal and lung cancer associated with high transferrin saturation.(34)

For several of the prospective studies that did not find positive associations, the highest categories of transferrin saturation or serum ferritin were low and it is possible that associations restricted to high body iron stores might have been missed.

Other mechanisms might also explain the association between *HFE* genotype and risk of cancer. HFE is a non-classical major histocompatibility protein and has been purported to have an immunological function whereby individuals with *HFE* variants have abnormal expression of MHC class I molecules and an impaired class I antigen presentation pathway (35), as well as also having an altered CD4/CD8 ratios.(36) This may be responsible for the finding that *HFE* variants have increased risk of sustained viral response in chronic hepatitis C.(37) Studies reviewed by Santos et al. found genes that occur in the commonly amplified (DNA copy number aberration) regions of chromosome 6p (the most commonly amplified genomic interval is 6p21–p23.) have helped to identify molecular pathways that become deregulated during tumour progression in diverse tumour types.(38) It has been proposed that chromosome 6p harbours one or more oncogenes that are in the same chromosomal region as the *HFE* gene,(39) and are directly involved in tumour progression, with a bias toward solid tumours (the *HFE* gene has been mapped to the locus 6p21.3).(40) Similarly, Motokura et al. have mapped the human cyclin D3 gene (CCND3) to chromosome 6p→q13, and members of this family of genes have been implicated as possible protooncogenes for parathyroid, lymphoid, and mammary tumors.(41) Alternatively, there may be an as yet undiscovered interaction of *HFE* with other genes accounting for the increased cancer risk.

In conclusion, people homozygous for the C282Y variant of the *HFE* gene are at a two-fold increased risk for colorectal cancer, and female breast cancer, but not for prostate cancer. Clinicians caring for patients with hereditary haemochromatosis should take this into account when deciding on screening recommendations for colorectal and breast cancer or evaluation of relevant or suggestive clinical signs and symptoms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Summary of cohort characteristics

Risk Factor	Definition or Units	C282Y Homozygote	Compound Heterozygote	C282Y Heterozygote	No C282Y Mutation
Number		193	690	3,192	24,339
Male		94 (49.0%)	272 (39.4%)	1257 (39.4%)	9728 (40.0%)
Female		99 (51.0%)	418 (60.6%)	1935 (60.6%)	14611 (60.0%)
Age	Years (at baseline)	55.5 (8.8)	55.1 (8.9)	54.9 (9.0)	55.1 (8.9)
Male Height	Centimetres	174.8 (6.9)	175.7 (7.1)	174.6 (6.3)	174.4 (6.7)
Female Height	Centimetres	162.2 (5.8)	161.7 (6.1)	161.7 (6.1)	161.3 (6.2)
Male Weight	Kilograms	78.4 (10.2)	83.1 (13.7)	81.2 (11.6)	81.2 (12.2)
Female Weight	Kilograms	67.2 (11.1)	68.2 (12.7)	67.4 (12.1)	67.5 (12.4)
Male Waist circumference	Centimetres	90.9 (9.0)	94.0 (10.5)	92.5 (9.9)	92.5 (10.1)
Female Waist circumference	Centimetres	77.7 (11.1)	78.9 (11.4)	78.2 (11.3)	78.3 (11.3)
Male Body Mass Index		25.6 (2.8)	26.9 (3.9)	26.7 (3.5)	26.7 (3.6)
Female Body Mass Index		25.6 (4.3)	26.1 (5.0)	25.8 (4.6)	26.0 (4.6)
Smoking	Never smoked	102 (52.6%)	408 (59.1%)	1853 (58.1%)	13881 (57.0%)
	Former smoker	71 (36.8%)	219 (31.7%)	1001 (31.4%)	7900 (32.5%)
	Current smoker	20 (10.4%)	63 (9.1%)	338 (10.6%)	2556 (10.5%)
Alcohol	0 g/d	68 (35.3%)	267 (38.9%)	1266 (39.9%)	9842 (40.7%)
	1–39 g/d (men) 1–19 g/d (women)	115 (59.6%)	339 (49.4%)	1549 (48.8%)	11698 (48.4%)
	40–59 g/d (men) 20–39 g/d (women)	7 (3.6%)	50 (7.3%)	263 (8.3%)	1893 (7.8%)
	60+ g/d (men) 40+ g/d (women)	3 (1.6%)	30 (4.4%)	95 (3.0%)	734 (3.0%)
Country of Birth	Australia	179 (92.2%)	623 (89.3%)	2908 (90.3%)	22004 (84.7%)
	British Isles	14 (7.3%)	58 (8.4%)	260 (8.2%)	2493 (10.2%)
	New Zealand	0 (0%)	4 (0.6%)	25 (0.8%)	226 (0.9%)
Education	Primary School	8 (4.1%)	35 (5.1%)	143 (4.5%)	2026 (8.3%)
	Some High School	99 (51.3%)	352 (51.0%)	1764 (55.3%)	13209 (54.3%)
	Completed High School	32 (16.6%)	90 (13.0%)	499 (15.6%)	3817 (15.7%)
	Degree/diploma	55 (28.5%)	221 (32.0%)	911 (28.5%)	6917 (28.4%)
Calcium	Calcium from diet (mg/d)	883.8 (321.2)	920.9 (467.2)	899.6 (412.0)	885.6 (408.3)

Risk Factor	Definition or Units	C282Y Homozygote	Compound Heterozygote	C282Y Heterozygote	No C282Y Mutation
Folate	Folate from diet (mcg/d)	333.1 (112.0)	341.3 (139.1)	337.1 (137.3)	334.5 (138.6)
Multi-vitamin	Multi-vitamin used at least once a week	26 (13.4%)	139 (19.9%)	637 (19.2%)	4841 (18.6%)
Fresh meat	Fresh meat intake (times/week)	5.0 (3.0)	4.9 (4.3)	4.8 (3.0)	4.8 (3.4)
Processed meat	Processed meat intake (times/week)	3.2 (2.4)	3.0 (2.5)	2.9 (2.5)	2.9 (2.8)
Activity *	Physical activity score	4.5 (3.9)	4.6 (3.8)	4.5 (3.8)	4.5 (3.7)
aspirin use †		14%	10%	14%	14%
NSAIDs use †		10%	12%	9%	9%
Women Only					
Age at menarche	Years	13.1 (1.6)	13.1 (1.6)	13.1 (1.5)	13.0 (1.6)
Menstrual Periods at Baseline ‡	No	68 (68.7%)	273 (65.0%)	1205 (61.9%)	9697 (63.3%)
	Yes	31 (31.3%)	147 (35.0%)	741 (38.1%)	5633 (36.7%)
Parity	Parous	81 (81.8%)	353 (84.5%)	1673 (86.5%)	12664 (86.7%)
	Nulliparous	18 (18.2%)	65 (15.6%)	262 (15.5%)	1947 (13.3%)
Hormone replacement therapy	Never	67 (69.1%)	304 (72.6%)	1414 (72.9%)	10906 (71.4%)
	Former	6 (6.2%)	42 (10.0%)	174 (9.1%)	1391 (9.1%)
	Current	24 (24.7%)	73 (17.4%)	432 (17.9%)	2974 (19.5%)

*physical activity score is a calculated field - see Haydon et al. (39)

†in a random sample of 5268 participants

‡menstrual periods at baseline is a calculated field defined from the two questions “Have you had a menstrual period in the last 12 months?” and “Have you had a hysterectomy?”.

Table 2

Hazard ratios for the associations between *HFE* genotype group and the risk of cancer

CANCER	allele	C282Y copies	cases	Person years	HR[§]	95 % CI	P[/]
COLORECTAL (stratified by sex)	Co-dominant	0	530	326,019	ref·		0·02
		1	80	53,275	0·88	0·67, 1·14	
		2	10	2,566	2·28	1·22, 4·25	
	Recessive	0 or 1	610	379,294	ref·		0·01
		2	10	2,566	2·31	1·24, 4·32	
	with H63D	0	530	326,019	ref·		0·04
		1 (no H63D)	61	44,017	0·92	0·73, 1·17	
		1 (& 1 H63D)	19	9,233	1·27	0·80, 2·01	
		2	10	2,566	2·28	1·22, 4·25	
BREAST (female only)	Co-dominant	0	565	188,991	ref·		0·03
		1	90	31,238	0·93	0·73, 1·20	
		2	9	1,245	2·39	1·24, 4·61	
	Recessive	0 or 1	655	220,229	ref·		0·01
		2	9	1,245	2·40	1·24, 4·63	
	with H63D	0	565	188,991	ref·		0·09
		1 (no H63D)	71	25,805	0·96	0·77, 1·20	
		1 (& 1 H63D)	19	5,433	1·16	0·74, 1·84	
		2	9	1,245	2·39	1·24, 4·61	
PROSTATE	Co-dominant	0	646	121,404	ref·		0·84
		1	106	19,585	1·04	0·83, 1·30	
		2	6	1,159	0·96	0·43, 2·15	
	Recessive	0 or 1	752	140,989	ref·		0·91
		2	6	1,159	0·96	0·43, 2·14	
	with H63D	0	646	121,404	ref·		0·96

CANCER	allele	C282Y copies	cases	Person years	HR[§]	95 % CI	P[/]
		1 (no H63D)	87	16,220	1·02	0·83, 1·26	
		1 (& 1 H63D)	19	3,364	1·08	0·68, 1·70	
		2	6	1,159	0·96	0·43, 2·15	
ANY OTHER NON-HAEMATOLOGICAL CANCER[♣] (stratified by sex)	Co-dominant	0	1510	289,434	ref·		0.58
		1	234	47,498	0.92	0.79, 1.07	
		2	12	2,163	1.01	0.57, 1.78	
	Recessive	0 or 1	1744	336,932	ref·		0.96
		2	12	2163	1.02	0.58, 1.79	
	with H63D	0	1510	289,434	ref·		0.39
		1 (no H63D)	188	39,419	0.94	0.82, 1.08	
		1 (& 1 H63D)	46	8,080	1.11	0.82, 1.48	
		2	12	2,163	1.01	0.57, 1.78	
ANY CANCER (stratified by sex)	Co-dominant	0	3441	320,421	ref·		0·11
		1	548	52,519	0·96	0·86, 1·06	
		2	36	2,451	1·30	0·93, 1·80	
	Recessive	0 or 1	3989	372,940	ref·		0·12
		2	36	2,451	1·30	0·94, 1·81	
	with H63D	0	3441	320,421	ref·		0·09
		1 (no H63D)	439	43,486	0·97	0·89, 1·06	
		1 (& 1 H63D)	109	9,033	1·13	0·93, 1·36	
		2	36	2,451	1·30	0·93, 1·80	

[§]Analysis used Cox regression with age as the primary timescale but included no other potential confounders

[♣]The catagory comprises all cancers bar colorectal, breast (female), prostate and haematological cancers

[/]P-values were generated from the likelihood ratio test, which was used to compare a model assuming a separate risk parameter for each HFE genotype to the null model assuming no HFE genotype effect on the risk of cancer. The “Co-dominant” model classifies HFE genotype based on the C282Y mutation alone (likelihood ratio test with 2 d.f.) whereas the “With H63D” model further classifies those with one copy of the C282Y mutation into those who do and do not have a copy of the H63D mutation (likelihood ratio test with 3 d.f..)