Supplementary information

Penetrance, cancer incidence and survival in HFE hemochromatosis – A population-based cohort study

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# Supplementary methods

To calculate the number of expected p.C282Y homozygous individuals in Tyrol, demographic data of all Tyrolean individuals from the National resident registry (Table S1; Zentrales Melderegister – Statistik Austria, <https://www.statistik.at/statistiken/bevoelkerung-und-soziales/bevoelkerung/bevoelkerungsstand/bevoelkerung-nach-alter/geschlecht>) and genotype frequencies (*HFE* p.C282Y homozygosity) from the KORA cohort were used. The KORA cohort (Table S2-S4) is a population-based study conducted in the nearby region of Augsburg, Germany. Frequencies for the HFE SNP (rs1800562) were analyzed in the KORAF3 (n=3796) and KORAF4 (n=3628) studies (Holle R, Happich M, Lowel H, Wichmann HE, Group MKS. KORA-a research platform for population based health research. Gesundheitswesen 2005;67 Suppl 1:S19-25). Incident cancer diagnoses were assessed for our hemochromatosis cohort from the regional cancer registry (Tiroler Tumorregister, which is ‘Gold certified’ by the North American Association of Central Cancer Registries), where all incident cancer diagnoses (including HCC disgnosis independent of etiology of liver disease) for the state Tyrol are recorded. Comorbidities were extracted from patient records if available.

Parametric variables were expressed as medians with first and third quartiles. Frequencies are reported as absolute numbers or percentages as indicated. Quantitative variables were compared using the nonparametric Mann-Whitney U test. Contingency tables were tested for significance using the Fisher's exact or χ2 tests. A p value <0.05 was considered as statistically significant. Cox regression analyses were carried out to estimate their association with patient lifetime.

Statistical analyses were performed using R Studio and R version 4.0. All packages used were retrieved from CRAN (Comprehensive R Archive Network). Data management was performed using the „dplyr“ package. Baseline statistics and tables were summarised using the ‘arsenal’ package. Graphs were created using the ‘ggplot2’ package. The ‘survival’ and ‘survminer’ package were used for survival analysis and the creation of Kaplan-Meier curves. Additional cohort data and R scripts are available on request. The graphical abstract was created with BioRender.com.

# Supplementary Tables and Figures

**Table S1:** Population data from Tyrol for the year 2021

|  |  |  |
| --- | --- | --- |
|  | **female** | **male** |
| **0 - 9 years** | 36122 | 38365 |
| **10 - 19 years** | 35495 | 37397 |
| **20 - 29 years** | 47686 | 50656 |
| **30 - 39 years** | 52400 | 53492 |
| **40 - 49 years** | 50190 | 49503 |
| **50 - 59 years** | 60364 | 60161 |
| **60 - 69 years** | 44206 | 40935 |
| **70 - 79 years** | 33950 | 28491 |
| **80 - 89 years** | 20040 | 13958 |
| **90+ years** | 4524 | 2047 |
| **total** | 385077 | 375028 |

**Table S2:** Genotype frequencies in the KORA cohorts F3 and F4.

|  |  |  |  |
| --- | --- | --- | --- |
| **SNP** | **Genotype** | **KORAF3** | **KORAF4** |
| **number** | **proportion** | **number** | **proportion** |
| **rs1800562** | **GG** | 1732 | 0.913 | 1634 | 0.9008 |
| **AG** | 160 | 0.084 | 171 | 0.094 |
| **AA** | 6 | 0.003 | 9 | 0.005 |
| **rs1799945** | **CC** | 1408 | 0.742 | 1292 | 0.712 |
| **CG** | 460 | 0.242 | 476 | 0.262 |
| **GG** | 30 | 0.016 | 46 | 0.025 |

**Table S3:** Allele frequencies in the KORA cohorts F3 and F4

|  |  |  |  |
| --- | --- | --- | --- |
| **SNP** | **Allele** | **KORAF3** | **KORAF4** |
| **number** | **proportion** | **number** | **proportion** |
| **rs1800562** | **G** | 3624 | 0.955 | 3439 | 0.948 |
| **A** | 172 | 0.045 | 189 | 0.052 |
| **rs1799945** | **C** | 3276 | 0.863 | 3060 | 0.843 |
| **G** | 520 | 0.137 | 568 | 0.157 |

**Table S4:** Frequencies of genotypes in the KORA cohorts F3 and F4.

|  |  |  |
| --- | --- | --- |
| **Genotype combination** | **KORAF3** | **KORAF4** |
| **number** | **proportion** | **number** | **proportion** |
| **GG-CC** | 1265 | 0.666 | 1138 | 0.627 |
| **GG-CG** | 473 | 0.230 | 450 | 0.248 |
| **GG-GG** | 30 | 0.016 | 46 | 0.025 |
| **AG-CC** | 137 | 0.072 | 145 | 0.080 |
| **AG-CG** | 23 | 0.012 | 26 | 0.014 |
| **AG-GG** | 0 | 0.000 | 0 | 0.000 |
| **AA-CC** | 6 | 0.003 | 9 | 0.005 |
| **AA-CG** | 0 | 0.000 | 0 | 0.000 |
| **AA-GG** | 0 | 0.000 | 0 | 0.000 |

**Table S5:** Comparison of patients homozygous for p.C282Y with and without provisional iron overload (pIOL). \*As allocation f patients into the pIOL and non-pIOL group was based on serum iron parameters no statistical test for group differences was performed.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Provisional iron overload**n=296 | **No provisional iron overload**n=155 | **p** |
| **Females** (n) | 102 | 67 | 0.085 |
| **Median age at genotyping** (y) | 49.9(35.5 – 60.5) | 46.7(34.5 – 59.9) | 0.301 |
| **Iron** (µmol/L) | 294 | 154 | n.a.\* |
| 36.9(32.2 – 41.2) | 25.5(17.5 – 33.1) |
| **Ferritin** (µg/L) | 296 | 155 | n.a.\* |
| 821(496 – 1495) | 163(60-307) |
| **Transferrin** (mg/dL) | 289 | 155 | n.a.\* |
| 186(167 – 208) | 204(182 – 232) |
| **Transferrin saturation** (%) | 296 | 155 | n.a.\* |
| 81(69 – 88) | 48(35 – 67) |
| **C-reactive protein** (mg/dl) | 219 | 136 | 0.066 |
| 0.2(0.1 – 0.7) | 0.3(0.1 – 0.7) |
| **FIB-4 score**<1.30≥1.30 | 128 (61%)83 (39%) | 87 (66%)45 (34%) | 0.388 |
| **Any cancer diagnosis** (n) | 16 (5.41%) | 10 (6.45%) | 0.810 |
| **HCC (C22.0) diagnosis** (n) | 3 (1.01% of all pat, 18.75% of cancer diagnosis) | 5 (3.45% of all pat, 50% of cancer diagnosis) | 0.189 |
| **Median follow up** (y) | 11.9 | 14.5 | <0.001 |
| **Cumulative follow up** (y) | 3428.003 | 2167.499 | - |

Parametric variables are expressed as medians (25th and 75th percentile). Frequencies are reported as absolute numbers (percentages).

**Table S6:** Causes of death are known in 55 of 71 cases.

|  |  |  |
| --- | --- | --- |
|  | **n** | **%** |
| Cardiovascular | 19 | 35% |
| Cancer | 25 | 45% |
| Infection | 1 | 2% |
| Trauma | 3 | 5% |
| Lung disease | 1 | 2% |
| Liver disease | 3 | 5% |
| Neurodegenerative disease | 3 | 5% |

**Table S7:** Number of cancer diagnosis in p.C282Y homozygous individuals.

|  |  |
| --- | --- |
| **Type of malignancy** | **Frequency** |
| Malignant neoplasm of central nervous system | 1 |
| Malignant neoplasm of bladder | 2 |
| Malignant neoplasm of breast | 4 |
| Malignant neoplasm of bronchus and lung | 2 |
| Malignant neoplasm of colon | 1 |
| Malignant neoplasm of corpus uteri | 1 |
| Malignant neoplasm of esophagus | 1 |
| Malignant neoplasm of liver and intrahepatic bile ducts | 10 |
| Malignant neoplasm of ovary | 2 |
| Malignant neoplasm of pancreas | 3 |
| Malignant neoplasm of prostate | 1 |
| Malignant neoplasm of stomach | 1 |
| Malignant neoplasm without specification of site | 1 |

**Table S8:** Cox regression of life expectancy for all for HFE C282Y homozygous individuals.

|  |  |  |
| --- | --- | --- |
|  | **Univariate** | **Multivariate**(n=317, events=54) |
| **Variable** | **n (events)** | **HR [95% CI]** | **p** | **HR [95% CI]** | **p** |
| **Sex (female)** | 498(71) | 0.527[0.32-0.87] | 0.012 | 0.606[0.35-1.06] | 0.077 |
| **Age at genotyping (years)** | 498(71) | 0.967[0.94-0.99] | 0.021 | 0.934[0.90-0.97] | <0.001 |
| **Iron (µmol/L)** | 419(61) | 0.997[0.97-1.02] | 0.802 |  |  |
| **logFerritin (µg/L)** | 422(62) | 1.097[0.90-1.34] | 0.355 |  |  |
| **Transferrin (mg/dL)** | 411(61) | 0.997[0.99-1.00] | 0.399 |  |  |
| **Transferrin Saturation (%)** | 414(62) | 1.000[0.99-1.01] | 0.973 |  |  |
| **CRP (mg/dL)** | 361(61) | 1.052[1.01-1.10] | 0.021 | 1.069[1.02-1.12] | 0.004 |
| **FIB-4** | 350(62) | 1.240[1.12-1.38] | <0.001 | 1.257[1.12-1.41] | <0.001 |
| **Any cancer diagnosis** | 498(71) | 2.629[1.63-4.25] | <0.001 | 2.501[1.43-4.38] | 0.001 |
| **Provisional iron overload at genotyping** | 498(71) | 0.887[0.62-1.27] | 0.512 |  |  |

**Table S9:** Univariate Cox regression of life expectancy and cardiovascular or hepatic comorbidities for all for HFE C282Y homozygous individuals. Parameters were not included into a multivariate analysis due to insufficient number of events.

|  |  |  |  |
| --- | --- | --- | --- |
| **Variable** | **n (events)** | **HR [95% CI]** | **p** |
| BMI [kg/m²] | 207(32) | 1.00[0.93 - 1.07] | 0.91 |
| Arterial hypertension | 217(44) | 0.46[0.24-0.87] | 0.02 |
| Diabetes mellitus II | 217(44) | 1.63[0.58-4.61] | 0.36 |
| Steatosis | 27241 | 4.00[1.81-8.83] | <0.01 |
| Cirrhosis | 385(67) | 6.41[3.72-11.04] | <0.01 |
| History of harmful alcohol consumption | 285(47) | 6.48[3.04-13.82] | <0.01 |
| History of smoking | 291(45) | 5.96[3.12-11.42] | <0.01 |

**Table S10:** Comorbidities of individuals homozygous for HFE p.282Y. N depicts the number of individuals with available data.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **n** | **All** | **n** | **Female** | **n** | **Male** |
| **BMI [kg/m2]** | 224 | 25.0(22.3-28.4) | 91 | 24.1(21.3 – 28.5) | 133 | 25.5(23.1 – 28.4) |
| **BMI >25 kg/m2 [n]** | 224 | 114 (50.9%) | 91 | 37 (40.7%) | 133 | 77 (57.9%) |
| **Smoking [n]** | 313 | 123 (39.3%) | 127 | 37 (29.1%) | 186 | 86 (46.2%) |
| **Alcohol use disorder [n]** | 308 | 26 (8.4%) | 191 | 23 (12%) | 117 | 3 (2.6%) |
| **Arterial Hypertension [n]** | 419 | 156 (37,2%) | 159 | 60 (37.7) | 260 | 96 (36.9%) |
| **Diabetes mellitus 2 [n]** | 406 | 38 (9.4%) | 149 | 11 (7.4%) | 257 | 27 (10.5%) |
| **Steatosis [n]** | 297 | 161 (54.2%) | 111 | 48 (43.2%) | 186 | 113 (60.8%) |
| **Cirrhosis [n]** | 415 | 28 (6.7%) | 158 | 5 (3.2%) | 257 | 23 (8.9%) |
| **MASLD cirrhosis [n]****ALD cirrhosis [n]****HCV cirrhosis [n]** | 415 | 3 (0.7%)3 (0.7%)1 (0.2%) | 158 | 2 (1.3%)1 (0.6%)0 (0%) | 257 | 1 (0.4%)2 (0.8%)1 (0.4%) |
| **Decompensated cirrhosis [n]** | 415 | 10 (2.5%) | 158 | 1 (0.6%) | 257 | 9 (3.5%) |
| **Liver transplantation [n]** | 467 | 2 (0.4%) | 179 | 0 (0%) | 288 | 2 (0.7%) |
| **Phlebotomy treatment after diagnosis [n]** | 354 | 221 (62.6%) | 140 | 76 (54.3%) | 213 | 145 (68.1%) |

**Figure S1:** Life expectancy of p.C282Y homozygotes compared to a modelled propensity score-matched control population. p.C282Y homozygotes are stratified according to sex.

