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The effect of hematocrit and hemoglobin on the risk of ischemic heart disease: A Mendelian randomization study

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ABSTRACT

Hematocrit and hemoglobin affect viscosity, and have been considered as risk factors for ischemic heart disease (IHD), although observations are inconsistent; randomized controlled trials targeting hematocrit or hemoglobin have not been definitive. To clarify their role, the risk of IHD was assessed according to genetically determined hematocrit and hemoglobin. We applied single nucleotide polymorphisms (SNPs) strongly determining hematocrit and hemoglobin, from a genome wide association study, to a large case (64,746) control (130,681) study of coronary artery disease, CARDIoGRAMplusC4D, to obtain unconfounded estimates using instrumental variable analysis by combining the Wald estimators for each SNP taking into account any correlation between SNPs using weighted generalized linear regression. Hematocrit was positively associated with IHD, odds ratio (OR) 1.07 per %, 95% confidence interval (CI) 1.03 to 1.11, before and after excluding SNPs from gene regions directly functionally relevant to IHD. However, hematocrit was not associated with IHD (OR 0.99, 0.94 to 1.04) after also excluding SNPs associated with lipids at genome wide significance. Hemoglobin was not associated with IHD (OR 1.06 per g/dL, 0.97 to 1.15) which was similar (OR 1.02, 0.94 to 1.11) after excluding SNPs from gene regions directly functionally relevant to IHD. Hemoglobin was negatively associated with IHD after also excluding SNPs associated with lipids at genome wide significance (OR 0.86, 0.78 to 0.94). In conclusion, hematocrit shares genetic determinants with IHD, but whether the genes contribute to IHD via hematocrit or other mechanisms is not entirely clear. Higher Hemoglobin is unlikely to cause IHD.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in developed and developing countries. (Lozano et al., 2012) Substantial progress has been made in teasing out the underlying causes of CVD from numerous risk factors. However, recently unexpected findings from randomized controlled trials (RCTs) and Mendelian randomization (MR) studies have revealed that some CVD risk factors may not be as causal as expected, such as high-density lipoprotein cholesterol, (HPS2-Thrive-Group, 2014; Schwartz et al., 2012) C-reactive protein, (Yousuf et al., 2013) or do not appear to be as strongly causal as expected, such as fasting glucose. (Boussageon et al., 2011) CVD was initially conceptualized by Virchow in terms of factors concerning vessels, flow and viscosity. (RLK, 1856) Atherosclerotic disease and hypertension are proven causes of CVD routinely targeted in primary and secondary prevention. Aspects of viscosity, such as coagulation, are well established targets of secondary prevention, but the role of other factors related to viscosity, have not been clearly established. Hematocrit (Hct), a major determinant of viscosity, (Lee et al., 1998) has been thought to be a risk factor for CVD since the 1960s. (Burch and Depasquale, 1962) Observationally higher Hct or hemoglobin (Hgb) has been associated with higher risk of ischemic heart disease (IHD), (Kunnas et al., 2009; Toss et al., 2013; Wannamethee et al., 1994) but this association is not always evident (Gagnon et al., 1994) perhaps because of reverse causality or confounding by poor health status. Moreover, it is increasingly realized that causality is difficult to establish from observational studies. RCTs have suggested that Hct and Hgb might play a causal role in CVD. An RCT of anemia treatment in chronic kidney disease found a higher Hgb target increased hospitalization and death from CVD events. (Coyne, 2012) Another RCT of a lower Hct target in polycythemia vera patients found a lower rate of CVD death and major thrombosis. (Marchioli et al., 2013) However, no RCT has examined the effect of reducing Hct or Hgb in the normal range on IHD. As such the role of Hct and Hgb in CVD is unclear because the observational studies are inconsistent and the experimental studies tend to be in specific patient groups.

Abbreviations: CAD/MI, Coronary artery disease/myocardial infarction; CI, confidence interval; CVD, cardiovascular disease; GWAS, genome wide association study; Hct, hematocrit; Hgb, hemoglobin; IHD, ischemic heart disease; LDL-C, low-density lipoprotein cholesterol; MR, Mendelian randomization; OR, odd ratio; RCTs, randomized controlled trials; SNPs, single nucleotide polymorphisms; CHARGE, Cohort for Heart and Aging Research in Genomic Epidemiology.

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In the absence of definitive evidence, examining whether people with genetically higher Hct or Hgb are more prone to IHD may provide a way of answering the key question of whether Hct or Hgb might be a potential target of intervention for prevention of IHD. MR studies are less open to confounding than observational studies because genes are randomly allocated at conception, a process analogous to the randomization in RCTs. (Lawlor et al., 2008) To date, no MR study has examined the role of Hct or Hgb in IHD. Here, we assessed whether people with genetically higher Hct and Hgb had a higher risk of IHD using a genome wide association study (GWAS) to provide genetic determinants of Hct and Hgb, and a large case–control study of coronary artery disease/myocardial infarction (CAD/MI) (CARDIoGRAMplusC4D) with extensive genotyping to assess the effect of these genetic variants on IHD.

2. Methods

2.1. Genetically determined Hct and Hgb

Genetically determined Hct and Hgb were obtained from a GWAS of Hct and Hgb in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium, (Psaty et al., 2009) a population-based study of 24,167 people of European ancestry, mean age 61.2 years, with replication in the HaemGen Consortium, (Soranzo et al., 2009) a population-based study of 9456 people of European ancestry, mean age 51.1 years. (Ganesh et al., 2009) Only single nucleotide polymorphisms (SNPs) strongly associated at genome wide significance (p-value $\leq 5 * 10^{-8}$) with Hct or Hgb were used. Correlations between the SNPs (linkage disequilibrium) for Hct or Hgb were obtained from SNP Annotation and Proxy Search using the appropriate catalog (http://www.broad.mit.edu/mpg/snap/ldsearchpw.php). Where SNPs for Hct or Hgb were correlated ($r^2 \geq 0.8$), the SNP with larger p-value was discarded. Pleiotropic effects were obtained from a comprehensive genetic cross-reference system, Ensembl (http://www.ensembl.org/index.html).

2.2. Genetic determinants of IHD

Data on CAD/MI have been contributed by the CARDIoGRAMplusC4D (Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics) consortium, which have been downloaded from www.CARDIOGRAMPLUSC4D.ORG. (Deloukas et al., 2013; Mehta, 2011; Schunkert et al., 2011) CARDIoGRAMplusC4D is a case–control study of CAD/MI with genotyping for 63,746 cases and 130,681 controls mainly of European descent with some South Asians. CARDIoGRAM has fewer cases (22,233) and controls (64,762) but more extensive genotyping. (Deloukas et al., 2013; Mehta, 2011; Schunkert et al., 2011) We obtained the association of each SNP with IHD, using CARDIoGRAMplusC4D if possible and then CARDIoGRAM.

2.3. Statistical analyses

The effects of Hct and Hgb on IHD were obtained from separate sample instrumental variable analysis by combining the Wald estimates for each SNP using weighted generalized linear regression taking into account the correlation between SNPs, which gives an odd ratio (OR) with 95% confidence interval (CI). All statistical analyses were conducted using Stata version 13.1 (StataCorp LP, College Station, TX) and R version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). This analysis using publicly available summary data does not require ethical approval.

3. Results

We obtained 42 SNPs for Hct from a GWAS of the CHARGE consortium, comprising 24,167 people of European ancestry. (Ganesh et al., 2009) Only one SNP (rs4745982 from *HK1*) of these 42 SNPs was not available in CARDIoGRAMplusC4D or CARDIoGRAM. No highly correlated SNP to substitute could be found. Of the 41 remaining SNPs, 23 were excluded because they were highly correlated. Based on the remaining 18 SNPs in gene regions *ATXN2*, *BRAP*, *HBS1L*, *HFE*, *HK1*, *PRKAG2*, *PRKCE*, *PTPN11*, *SCGN*, *SLC17A3*, *TFR2*, *TMPRSS6*, *TRAFD1* and *ZAN*, Hct was positively associated with IHD (OR 1.07, 1.03 to 1.11) (Table 1). However, three of these 18 SNPs (rs11065987 (*BRAP*), rs1800562 (*HFE*) and rs11066301 (*PTPN11*)) are associated with lipids at genome wide significance and are from gene regions potentially functionally relevant to low-density lipoprotein cholesterol (LDL-C). (Willer et al., 2013) The *BRAP* gene region is the breast cancer suppressor protein associated protein region, which is highly related to lipids. (Avery et al., 2011; Teslovich et al., 2010; Willer et al., 2013) The *HFE* gene region relates to iron metabolism and lipid oxidation. (Roest et al., 1999; Tuomainen et al., 1999) The *PTPN11* gene region plays an essential role in blood development (Saxton et al., 1997; Tartaglia et al., 2003) and male fertility (Puri and Walker, 2016), but also encodes Src homology-2 domain-containing protein tyrosine phosphatase 2, which regulates apolipoprotein B secretion. (Phung et al., 1997; Ugi et al., 1996) Based on the remaining 15 SNPs, Hct was still positively associated with IHD (OR 1.07, 1.03 to 1.11) (Table 1). However, 3 of these 15 remaining SNPSs (rs10774625 (*ATXN2*), rs1408272 (*SLC17A3*) and rs11066188 (*TRAFD1*)) are associated with lipids at genome wide significance, (Willer et al., 2013) although not from gene regions thought to be functionally relevant to lipids. After further excluding these three SNPs potentially affecting IHD via lipids rather than Hct, Hct was not associated with IHD (OR 0.99, 0.94 to 1.04) (Table 1) based on the remaining 12 SNPs, with the SNPs from *ATXN2* and *TRADF1* making the difference (Table 1).

Similarly, we obtained 80 SNPs for Hgb, all of which were available in CARDIoGRAMplusC4D or CARDIoGRAM. Of these 80 SNPs, 54 were excluded because they were highly correlated, leaving 26 SNPs in gene regions *ATXN2*, *BRAP*, *HFE*, *HIST1H1A*, *HIST1H1C*, *HIST1H1T*, *HK1*, *LRRC16A*, *MPST*, *PRKAG2*, *PRKCE*, *PTPN11*, *SCGN*, *SLC17A*, *TMPRSS6*,*TRAFD1*, *TRIM38* and *TSHZ2*. Based on these remaining 26 SNPs, Hgb was unrelated to IHD (OR 1.06, 0.97 to 1.15) (Table 1). However, three of these 26 SNPs (rs11065987 (*BRAP*), rs1800562 (*HFE*) and rs11066301 (*PTPN11*)) are located in gene regions potentially functionally relevant to LDL-C. (Willer et al., 2013) Based on the remaining 23 SNPs, Hgb was unrelated to IHD (OR 1.02, 0.94 to 1.11) (Table 1). However, three of these 23 remaining SNPs (rs10774625 (*ATXN2*), rs1408272 (*SLC17A3*) and rs17630235 (*TRAFD1*)) are associated with lipids at genome wide significance, (Willer et al., 2013) although not from gene regions thought to be functionally relevant to lipids. After further excluding these three SNPs, potentially affecting IHD via lipids rather than via Hgb, Hgb was associated with lower IHD (OR 0.86, 0.78 to 0.94) (Table 1) based on the remaining 20 SNPs, with the SNPs from *ATXN2* and *TRADF1* making

Table 1
Unconfounded estimates of the association of Hematocrit(%) and Hemoglobin(g/dL) with Ischemic Heart Disease based on SNP-level Wald estimates combined using weighted generalized linear regression.

Instrument set	OR	95% CI
Hematocrit (n = 18)	1.07	(1.03, 1.11)
Hematocrit (excluding functionally pleiotropic SNPs, n = 15)	1.07	(1.03, 1.11)
Further exclude rs1408272 from *SLC17A3*, n = 14	1.09	(1.05, 1.14)
Further exclude rs10774625 from *ATXN2*, n = 14	1.03	(0.99, 1.07)
Further exclude rs11066188 from *TRAFD1*, n = 14	1.02	(0.98, 1.07)
Hematocrit (excluding all pleiotropic SNPs, n = 12)	0.99	(0.94, 1.04)
Hemoglobin (n = 26)	1.06	(0.97, 1.15)
Hemoglobin (excluding functionally pleiotropic SNPs, n = 23)	1.02	(0.94, 1.11)
Further exclude rs1408272 from *SLC17A3*, n = 22	1.02	(0.94, 1.12)
Further exclude rs10774625 from *ATXN2*, n = 22	0.95	(0.87, 1.04)
Further exclude rs17630235 from *TRAFD1*, n = 22	0.94	(0.86, 1.03)
Hemoglobin (excluding all pleiotropic SNPs, n = 20)	0.86	(0.78, 0.94)

CI, confidence interval; OR, odd ratio; SNP, single nucleotide polymorphism.