Research Article
The Chromosome 9p21 CVD- and T2D-Associated Regions in a Norwegian Population (The HUNT2 Survey)

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Background. Two adjacent regions upstream CDKN2B on chromosome 9p21 have been associated with type 2 diabetes (T2D) and progression of cardiovascular disease (CVD). The precise location and number of risk variants have not been completely delineated and a possible synergistic relationship between the adjacent regions is not fully addressed. By a population based cross-sectional case-control design, we genotyped 18 SNPs upstream of CDKN2B tagging 138kb in and around two LD-blocks associated with CVD and T2D and investigated associations with T2D, angina pectoris (AP), myocardial infarction (MI), coronary heart disease (CHD; AP or AMI), and stroke using 5,564 subjects from HUNT2.

Results. Single point and haplotype analysis showed evidence for only one common T2D risk haplotype (rs10757282|rs10811661; OR=1.19, 𝑃=2.0×10−3) in the region. We confirmed the strong association between SNPs in the 60kb CVD region with AP, MI, and CHD (𝑃<0.01). Conditioning on the lead SNP in the region, we observed two suggestive independent single SNP association signals for MI, rs2065501 (𝑃=0.03) and rs3217986 (𝑃=0.04).

Conclusions. We confirmed the association of known variants within the 9p21 interval with T2D and CHD. Our results further suggest that additional CHD susceptibility variants exist in this region.

1. Introduction

One interesting region associated with type 2 diabetes (T2D) and cardiovascular disease (CVD) is on chromosome 9p21 in a gene desert ~130 kb upstream of CDKN2B. Several SNPs in the 9p21 interval are strongly associated with MI [1–4], vascular disease [5–7], and cancer [8], all highly correlated (𝑟2 > 0.8) and to be found in a ~60 kb region in high linkage disequilibrium (LD). The 9p21 region also contains two adjacent, but separate, T2D signals; a strong signal mapped to a 2 kb LD-block (represented by rs10811661 and rs10757282) and a putatively independent second signal (rs564398) located ~100 kb from the T2D interval [9–11].

After the initial genome-wide association studies (GWASs), several investigations confirmed the association with the 9p21 candidate SNPs in T2D [12–17] and CVD [18–24] and extended the number of CVD phenotypes associated with the region [25–30]. A shared mechanistic link might...
therefore exist within this region increasing risk of both CVD and T2D through a common pathway. In patients with T2D, a variant within 9p21 showed significant interaction between poor glycemic control and risk of angiographically verified coronary artery disease (CAD) [31]. However, the effects of the disease susceptibility variants for the two major disease loci have shown to be independent, since T2D risk variants do not seem to confer increased risk of cardiovascular disease or the other way around [5, 32].

A multilocus analysis of the 9p21 region suggested a haplotype-effect on T2D risk rather than an effect from one single SNP [33], indicating that the bona fide locus could be situated somewhere in the vicinity of the test SNPs. However, a comprehensive sequencing study of the 9p21 locus that assessed rare variants and their association with T2D and MI did not discover any variants with stronger association than what was found in the initial GWASs [8]. Thus, we chose to evaluate the distribution of common tagSNPs within the region in individuals with overlapping T2D, angina pectoris (AP), previous MI, or stroke from the Norwegian population-based HUNT2 survey to assess the distribution of T2D and CVD risk alleles in HUNT 2.

2. Materials and Methods

2.1. Study Subjects and Ethics Statement. The second Nord-Trøndelag Health Study (HUNT2) is an extensive population-based health survey conducted in a Norwegian county with 127,000 inhabitants of which 60,000 participated [34]. HUNT2 is a subset of HUNT and was carried out in 1995–97. We had access to all subjects with diabetes (n = 1,850), in addition to 600 individuals selected for incident MI and/or stroke, but without diabetes, and 3,456 population-based random controls drawn from the same study population. After excluding 206 subjects with T1D, eight with genetically verified MODY [35], and 128 subjects with missing BMI data, 5,564 subjects were eligible for analysis (Figure 1). Diagnosis of diabetes, angina pectoris, previous MI, and stroke (ischemic or hemorrhagic strokes grouped as one phenotype) was self-reported. Written informed consent was obtained.
from all participants. This population based cross-sectional case-control study was approved by the Regional Committee for Research Ethics and the Norwegian Data Inspectorate, and was performed according to the latest version of the Helsinki Declaration.

2.2. SNP Selection, Genotyping, and Quality Control. We selected 98 SNPs across 9p21 from the interval between Chr9:21,995,330 and 22,133,570 (NCBI Build 36). We selected 18 SNPs tagging a 138kb region using the Haploview implementation of the Tagger algorithm [36] using the following criteria: minor allele frequency (MAF) of >5% and pairwise $r^2 > 0.80$. In addition, we added two previously GWAS-identified T2D susceptibility variants (rs564398 and rs10811661) and three confirmed CVD susceptibility variants (rs13330490, rs10757278, and rs13330491). The genotyping was carried out by the multiplex MassARRAY iPLEX System (SEQUENOM Inc., San Diego, CA, USA). Figures displaying regional information such as the strength and extent of the association signals relative to genomic position, local linkage disequilibrium (LD), and recombination patterns and the positions of genes in the region were created using a combination of LocusZoom web interface [38], R package SNP Plotter [39], and Haploviz [36]. We had >80% power to detect high-frequency alleles with ORs of 1.20 to 1.30 for both T2D and CVD phenotypes, but only around 50% and 30% power for T2D and CVD phenotypes, respectively, if the true ORs were 1.0. These estimates were performed using the Genetic Power Calculator [40]. All P values are presented without correction for multiple testing.

2.3. Statistical Analysis. We used logistic regression to model single-point and haplotype association for the 18 SNPs with T2D, MI, angina pectoris, coronary heart disease, and stroke positive cases assuming additive effect of allele dosage. Gender, age, and BMI were used as covariates in the regression model in the analysis of T2D. Diabetes status and smoking were added to the list of covariates while analyzing AP, previous MI, CHD, and stroke. Individuals with a history of either AP, previous MI, or stroke were excluded as control subjects in the regression models when analyzing CVD traits. For T2D, AP, MI, and CHD, we carried out tests conditioning on the lead SNPs (MI, angina pectoris, CHD: rs13330490 and rs10757278, T2D: rs10811661) to look for secondary signals of association. Multimarker haplotype analyses, haplotype frequency estimates, and haplotype comparisons for all phenotypes were performed using PLINK [37]. The sliding window approach used for multimarker haplotype analysis associates direct neighboring SNPs, generating 17 pairs of SNPs in the two-point analysis. All SNPs frequencies were consistent with Hardy-Weinberg equilibrium (HWE, $P > 0.01$). All analyses were carried out using PLINK version 1.07 software [37] and/or Stata SE v10.0 for Windows (Stata Corp LP, Brownsville, TX, USA). Figures displaying regional information such as the strength and extent of the association signals relative to genomic position, local linkage disequilibrium (LD), and recombination patterns and the positions of genes in the region were created using a combination of LocusZoom web interface [38], R package SNP Plotter [39], and Haploviz [36].

3. Results

Table 1 shows the clinical characteristics for the 5564 individuals enrolled in the present study.

### Table 1: Clinical characteristic of the 5564 subjects included in the study and eligible for analysis.

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>T2D</th>
<th>AP</th>
<th>MI</th>
<th>Stroke</th>
<th>No T2D and/or CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individuals (n)</td>
<td>5,564</td>
<td>1,590</td>
<td>769</td>
<td>672</td>
<td>500</td>
<td>3,027</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>2,754/2,810</td>
<td>754/836</td>
<td>435/334</td>
<td>745/197</td>
<td>256/244</td>
<td>1,424/1,603</td>
</tr>
<tr>
<td>Age (years at examination)</td>
<td>60.4 ± 17.1</td>
<td>68.1 ± 12.0</td>
<td>72.4 ± 9.2</td>
<td>70.7 ± 10.3</td>
<td>70.8 ± 11.0</td>
<td>53.2 ± 17.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ± 4.4</td>
<td>29.2 ± 4.8</td>
<td>28.0 ± 4.3</td>
<td>27.5 ± 3.9</td>
<td>27.4 ± 3.9</td>
<td>26.4 ± 4.1</td>
</tr>
<tr>
<td>Ever smoked (yes/no)</td>
<td>2,600/2,964</td>
<td>647/943</td>
<td>345/424</td>
<td>367/305</td>
<td>241/259</td>
<td>1,468/1,559</td>
</tr>
<tr>
<td>Nonfasting glucose b (mmol/L)</td>
<td>6.6 ± 3.1</td>
<td>9.6 ± 4.2</td>
<td>7.6 ± 3.6</td>
<td>7.2 ± 3.5</td>
<td>6.6 ± 2.7</td>
<td>5.4 ± 1.2</td>
</tr>
<tr>
<td>Serum triglyceride (mmol/L)</td>
<td>2.0 ± 1.3</td>
<td>2.5 ± 1.6</td>
<td>2.4 ± 1.6</td>
<td>2.3 ± 1.3</td>
<td>2.2 ± 1.5</td>
<td>1.8 ± 1.1</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>6.1 ± 1.3</td>
<td>6.2 ± 1.3</td>
<td>6.3 ± 1.3</td>
<td>6.2 ± 1.3</td>
<td>6.4 ± 1.3</td>
<td>6.0 ± 1.3</td>
</tr>
<tr>
<td>Serum HDL cholesterol (mmol/L)</td>
<td>1.3 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>1.2 ± 0.4</td>
<td>1.3 ± 0.4</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>73.6 ± 13.6</td>
<td>75.5 ± 14.5</td>
<td>6.8 ± 13.6</td>
<td>6.7 ± 13.3</td>
<td>72.1 ± 13.4</td>
<td>74.1 ± 12.8</td>
</tr>
<tr>
<td>Type 2 diabetes (n, %)</td>
<td>1,590 (28.6%)</td>
<td>1,590 (100%)</td>
<td>326 (42.4%)</td>
<td>212 (31.5%)</td>
<td>110 (22%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Myocardial infarction (n, %)</td>
<td>672 (12.1%)</td>
<td>212 (13.3%)</td>
<td>357 (46.9%)</td>
<td>672 (100%)</td>
<td>83 (16.6%)</td>
<td>n/a</td>
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<tr>
<td>Stroke (n, %)</td>
<td>500 (9.0%)</td>
<td>110 (6.9%)</td>
<td>115 (15.1%)</td>
<td>357 (53.1%)</td>
<td>500 (100%)</td>
<td>n/a</td>
</tr>
<tr>
<td>Angina pectoris (n, %)</td>
<td>769 (13.7%)</td>
<td>326 (20.5%)</td>
<td>769 (100%)</td>
<td>83 (12.4%)</td>
<td>115 (23%)</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Values are presented as means ± SD or number (%). Some individuals have more than one outcome (for example MI + diabetes); hence, the sum of these column counts does not match the total counts of individuals. Only nonfasting glucose measurements were available for participants in the HUNT2 cohort. MI denotes previous myocardial infarction. Abbreviations: T2D, Type 2 diabetes; AP, angina pectoris; MI, myocardial infarction; CVD, cardiovascular disease; bpm, beats per minute.
significant results; the strongest association was found for haplotypes incorporating both rs10757282 and rs10811661.

3.2 Cardiovascular Diseases: Angina Pectoris, Myocardial Infarction, and Stroke. Figure 2 and Additional file 1 (in Supplementary Material available online at http://dx.doi.org/10.1155/2014/164652) show the association results for each of the 18 SNPs with AP, previous MI, CHD (AP or previous MI), and stroke positive cases after adjustment for age, gender, BMI, diabetes status, and smoking. We report replication of the strong association between SNPs in the 60 kb CVD region (defined by rs8181047 to rs10757278, Figure 2) with AP (rs10757278: OR = 1.22; P = 1.1 × 10⁻³, Figure 2), MI (rs1333040: OR = 1.23, P = 1.8 × 10⁻³, Figure 2), and CHD (rs10757278: OR = 1.37; P = 2.0 × 10⁻³, Figure 2). Subanalyses showed that the effect of the CHD-associated SNPs was strongest in those having the most severe phenotype including both AP and previous MI. None of the SNPs in the CVD region demonstrated association with stroke, but one marker (rs10757282) in the previously implicated T2D region did show nominal evidence for association with stroke (OR = 1.2 (1.04–1.38), P = 0.01, Figure 2).

In exploratory analysis, we observed several nominally significant potentially novel single SNP associations for angina pectoris, previous MI, individuals with both AP and previous MI, and stroke in the 138 kb interval (Additional file 1). After conditioning upon the highly confirmed CVD susceptibility SNPs rs1333040 and rs10757278, only two remaining SNPs (rs2065501, OR = 1.32, P = 0.04; and rs3217986, OR = 1.15, P = 0.04) showed nominal P values < 0.05 and only for MI (Table 4).

4. Discussion

Our findings highlight the genetic complexity of the chromosome 9p21 region. We found a weak but consistent single-locus association between marker rs10811661 and T2D, as previously found in several studies [9–11, 41]. This was in agreement with our former results obtained for this marker in a replication study performed in the same material from the HUNT2 population [13]. However, in the present study, we demonstrate a stronger association with a haplotype tagged by rs10811661 and rs10757282 and T2D. These results are in line with other studies [8]. Thus, these SNPs may tag a risk haplotype harboring an allele important for development of T2D. Alternatively, the 11 kb candidate region could harbor several variants associated with the disease.

Published data are conflicting regarding any additional T2D-associated signals in the 9p21 region [9–11]. Our data do not support the existence of additional signals. The role of rs564398 as a T2D susceptibility variant is disputed [9, 12, 42]. Ethnicity may play a role, although our data are not supporting that this marker has a particularly strong effect in Caucasians [43].

The 9p21 risk variants are located in non-protein coding regions; their effects possibly influencing expression of nearby genes. The region contains two cyclin-dependent kinase inhibitors, CDKN2A (p16INK4a) and CDKN2B (p15INK4b), and
<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor allele</th>
<th>Single point OR (95% CI)</th>
<th>P</th>
<th>Two-point SNPs</th>
<th>Omnibus P</th>
<th>Haplotype</th>
<th>Frequency</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3217986</td>
<td>C</td>
<td>1.03 (0.86–1.22)</td>
<td>7.74 × 10⁻¹</td>
<td>rs3217986/rs523096</td>
<td>4.69 × 10⁻¹</td>
<td>AC/CT/AT</td>
<td>0.48/0.08/0.44</td>
<td>1.05/1.05/0.95</td>
<td>0.31/0.61/0.22</td>
</tr>
<tr>
<td>rs523096</td>
<td>C</td>
<td>1.04 (0.95–1.14)</td>
<td>3.97 × 10⁻¹</td>
<td>rs523096/rs10965215</td>
<td>4.70 × 10⁻¹</td>
<td>CA/TA/CG/TC</td>
<td>0.03/0.40/0.46/0.12</td>
<td>0.83/0.97/1.06/0.98</td>
<td>0.23/0.53/0.24/0.76</td>
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<td>rs10965215</td>
<td>A</td>
<td>0.96 (0.88–1.05)</td>
<td>5.64 × 10⁻¹</td>
<td>rs10965215/rs564398</td>
<td>5.64 × 10⁻¹</td>
<td>GG/AA/TA/GC</td>
<td>0.46/0.42/0.12/0.01</td>
<td>0.97/1.05/0.96/0.98</td>
<td>0.71/0.29/0.41</td>
</tr>
<tr>
<td>rs564398</td>
<td>G</td>
<td>1.05 (0.96–1.15)</td>
<td>9.32 × 10⁻¹</td>
<td>rs564398/rs1333034</td>
<td>9.32 × 10⁻¹</td>
<td>AA/GG/AT/GC</td>
<td>0.34/0.11/0.55/0.05</td>
<td>1.02/0.98/1.00/0.97</td>
<td>0.76/0.74/0.97</td>
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<td>rs1333034</td>
<td>G</td>
<td>0.97 (0.84–1.12)</td>
<td>6.02 × 10⁻¹</td>
<td>rs1333034/rs1881047</td>
<td>6.02 × 10⁻¹</td>
<td>GG/AC/CC/GC</td>
<td>0.40/0.34/0.26/0.12</td>
<td>0.96/1.01/1.04/0.98</td>
<td>0.35/0.78/0.44</td>
</tr>
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<td>rs8181047</td>
<td>A</td>
<td>1.01 (0.92–1.12)</td>
<td>4.69 × 10⁻¹</td>
<td>rs8181047/rs10811647</td>
<td>4.69 × 10⁻¹</td>
<td>GG/AA/TC/TC</td>
<td>0.12/0.46/0.43/0.26</td>
<td>0.97/1.05/0.96/0.98</td>
<td>0.71/0.29/0.41</td>
</tr>
<tr>
<td>rs10811647</td>
<td>G</td>
<td>0.96 (0.87–1.05)</td>
<td>0.03/0.40/0.46/0.12</td>
<td>rs10811647/rs564398</td>
<td>0.03/0.40/0.46/0.12</td>
<td>CA/TA/CG/TG</td>
<td>0.03/0.40/0.46/0.12</td>
<td>0.97/1.05/0.96/0.98</td>
<td>0.71/0.29/0.41</td>
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<tr>
<td>rs1333039</td>
<td>G</td>
<td>1.04 (0.95–1.14)</td>
<td>4.69 × 10⁻¹</td>
<td>rs1333039/rs16905599</td>
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<td>CA/CG/CG/CG</td>
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<td>rs16905599</td>
<td>A</td>
<td>1.05 (0.87–1.26)</td>
<td>2.75 × 10⁻¹</td>
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<td>2.75 × 10⁻¹</td>
<td>AC/TC/CT/CT</td>
<td>0.06/0.39/0.56/0.78</td>
<td>1.03/1.00/0.99/0.86</td>
<td>0.78/0.99/0.86</td>
</tr>
<tr>
<td>rs1333040</td>
<td>C</td>
<td>1.00 (0.91–1.09)</td>
<td>9.72 × 10⁻¹</td>
<td>rs1333040/rs10757278</td>
<td>9.72 × 10⁻¹</td>
<td>AC/TC/CT/CT</td>
<td>0.06/0.39/0.56/0.78</td>
<td>1.03/1.00/0.99/0.86</td>
<td>0.78/0.99/0.86</td>
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<tr>
<td>rs10757278</td>
<td>G</td>
<td>0.97 (0.89–1.07)</td>
<td>3.46 × 10⁻¹</td>
<td>rs10757278/rs10811659</td>
<td>3.46 × 10⁻¹</td>
<td>GA/AA/AG/AG</td>
<td>0.16/0.14/0.32/0.38</td>
<td>0.89/0.94/1.04/1.06</td>
<td>0.10/0.42/0.49/0.28</td>
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<tr>
<td>rs10811659</td>
<td>A</td>
<td>0.92 (0.83–1.02)</td>
<td>4.60 × 10⁻¹</td>
<td>rs10811659/rs10811659</td>
<td>4.60 × 10⁻¹</td>
<td>AC/CG/AT/AT</td>
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<td>0.93/0.98/0.95/1.08</td>
<td>0.20/0.87/0.49/0.11</td>
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<td>0.93 (0.83–1.04)</td>
<td>1.98 × 10⁻¹</td>
<td>rs1081659/rs10757282</td>
<td>1.98 × 10⁻¹</td>
<td>TC/TC/CT/CT</td>
<td>0.44/0.21/0.35/0.10</td>
<td>0.97/0.92/0.98/0.98</td>
<td>0.14/0.14/0.72</td>
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<tr>
<td>rs10757282</td>
<td>T</td>
<td>1.08 (0.99–1.18)</td>
<td>2.05 × 10⁻³</td>
<td>rs10757282/rs10811661</td>
<td>2.05 × 10⁻³</td>
<td>CT/TT/CT/CT</td>
<td>0.16/0.28/0.56/0.19</td>
<td>0.93/0.89/0.98/0.98</td>
<td>7.63 × 10⁻⁴/1.11/5.71 × 10⁻²</td>
</tr>
<tr>
<td>rs10811661</td>
<td>C</td>
<td>0.89 (0.78–1.00)</td>
<td>8.58 × 10⁻²</td>
<td>rs10811661/rs1333051</td>
<td>8.58 × 10⁻²</td>
<td>CT/TA/TA/TA</td>
<td>0.11/0.05/0.08/0.95</td>
<td>0.98/0.85/1.11/1.45</td>
<td>0.45/0.04/6.8 × 10⁻¹</td>
</tr>
<tr>
<td>rs1330301</td>
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<td>5.04 × 10⁻¹</td>
<td>rs1330301/rs2065501</td>
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<td>TA/TA/TA/TA</td>
<td>0.03/0.29/0.08/0.60</td>
<td>0.92/1.08/0.95/0.96</td>
<td>0.61/0.15/0.57/0.38</td>
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<td>1.06 (0.96–1.17)</td>
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<td>rs2065501/rs10757267</td>
<td>6.15 × 10⁻¹</td>
<td>AT/CT/AA/CA</td>
<td>0.09/0.04/0.23/0.64</td>
<td>1.07/1.02/0.95/0.94</td>
<td>0.41/0.91/0.36/0.19</td>
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<td>rs10757287</td>
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<td>4.43 × 10⁻¹</td>
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<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Association results for T2D from single and two-point haplotype analysis after correction for gender, age, and BMI. Top associated haplotypes are outlined.
CDKN2BAS, a large antisense noncoding RNA gene. Expression of these genes is coregulated and most of the confirmed CVD risk variants correlate with decreased expression of CDKN2BAS and furthermore to atherosclerosis [44, 45]. Recent follow-up studies show correlation between the number of risk alleles and atherosclerotic CAD progression, but no predisposition to MI in patients with preexisting atherosclerotic CAD nor increased reoccurrence of MI [46–48]. This suggests 9p21 risk variants promote atherosclerosis rather than triggering MI [49]. Our associations with angina pectoris as well as MI and with the strongest associations in those having both AP and previous MI at the time of screening may thus likely be mediated through increased propensity for atherosclerosis.

The rs10757278 SNP has been highlighted as a potential functional variant for the association with atherosclerotic disease based on effects on expression of the \textit{INK4a/ARF} locus (p15\textit{INK4b}, p16\textit{INK4a}, \textit{ARF} and \textit{CDKN2BAS}) [50–52]. In the present study, we confirmed the associations for SNPs in the CVD region with AP and MI. The associations were strongest among subjects having both AP and previous MI. This could be a marker for early progression of atherosclerotic CAD, supporting the aforementioned association between 9p21 risk variants and early progression. Moreover, the rs10757278 SNP has been mapped to one of 33 identified enhancers in the 9p21 interval, in which the risk variant disrupts a transcription factor binding site, which could have functional relevance for an atherosclerosis-associated pathway in human endothelial cells [53].

We found no association between SNPs in the CVD region and stroke. Our results are in accordance with some studies [5, 54], but not with others [52, 55]. Several investigations aiming to address this discrepancy have confirmed 9p21 as a risk factor for stroke, but with evidence for heterogeneity of effect across stroke subtypes. The strongest association has been shown for large vessel stroke [56]. Thus, lacking stroke subtyping in our study may be the reason we did not find this association. Participants of the HUNT2 survey were identified having stroke through a self-administered questionnaire, hence details regarding type of stroke, hemorrhagic versus ischemic, or subtypes like atherothrombotic or cardioembolic were not available. One could anticipate that SNPs in the 9p21 region associated with ischemic, but not hemorrhagic stroke. Studies have indicated that sequence variation in 9p21 influences atherosclerosis development and progression; the strongest association being seen for large vessels [29]. On the other hand, rs1333040 has recently been linked to sporadic brain arteriovenous malformations known to increase hemorrhagic stroke risk [7]. Moreover, the adja- cent rs10757278 has been linked to hemorrhagic stroke [52]. These results might suggest different pathways for ischemic and hemorrhagic stroke sharing common mechanisms linked to the same SNPs in the 9p21 region. Interestingly, when restricting the analysis to subjects with T2D, several SNPs in the 60 kb CVD region appeared associated with stroke, with the most significant being rs1333040 (OR = 1.44; \(P = 0.01\)). This association was not seen in stroke subjects without T2D. Interaction between variants within the 9p21 region and poor glycemic control increasing risk of CVD in patients with T2D has been suggested [31]. If similar associations were to be found for stroke risk in diabetics, it would be interesting to see whether poor glycemic control also affects different types of stroke differently.

Our exploratory results also highlights two potential novel CVD susceptibility variants, rs3217986 and rs2065501, which are located close to, but not in strong LD with the former and well-confirmed CVD region. The rs3217986 is located in the 3’ UTR of \textit{CDKN2B} as well as in intron 1 of the non-protein coding \textit{CDKN2BAS}; \textit{CDKN2BAS}. Although speculative, it could be hypothesized that the risk variant of rs3217986 might exert an effect on atherosclerotic CAD susceptibility by influencing expression of one or both of these two genes. To our knowledge, there are no reports on whether the risk variant of rs3217986 is correlated with expression of \textit{CDKN2B} and/or \textit{CDKN2BAS}; thus, this hypothesis needs to be further resolved.

The study must be viewed in light of its limitations. Although previous studies have confirmed highly significant associations between SNPs in the region and CVD and T2D, the many tests performed in this study could lead to a risk of false positive findings. Thus, while the primary single SNP associations and the T2D-risk haplotype are supported by previous studies, the more explorative findings of putative secondary signals need to be further investigated in much larger cohorts. The sparse risk increase associated with these common variants also renders our findings inadequate for clinical prediction. Fine-mapping studies of disease associated regions may still prove important to guide further investigation towards understanding the disease pathogenesis and possibly providing tools for cost-efficient risk stratification in the future.

Despite the close proximity between the CVD and T2D risk regions, our study is in line with previous studies and indicates that there is no apparent overlap between the two risk regions. Theories with reference to the concrete disease mechanism mediated by the risk variants of the 9p21 interval have increased in numbers the last years. However, since most of them still remain exploratory, the exact nature of the disease associated variants and their targets require further elucidation. They may possibly differ between CVD and T2D. It is possible that large-scale genome sequencing efforts may aid by identifying the underlying risk variants in the 9p21 region.
Table 4: Top five association results for CVD after conditioning upon lead SNPs.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor allele</th>
<th>AP OR (95% CI)</th>
<th>P</th>
<th>MI OR (95% CI)</th>
<th>P</th>
<th>Both MI and AP OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs3217986</td>
<td>C</td>
<td>1.21 (0.95–1.53)</td>
<td>0.13</td>
<td>1.32 (1.01–1.71)</td>
<td>0.04</td>
<td>1.25 (0.89–1.75)</td>
<td>0.19</td>
</tr>
<tr>
<td>rs2066501</td>
<td>A</td>
<td>1.07 (0.94–1.21)</td>
<td>0.33</td>
<td>1.15 (1.01–1.32)</td>
<td>0.04</td>
<td>1.11 (0.94–1.33)</td>
<td>0.23</td>
</tr>
<tr>
<td>rs10757282</td>
<td>C</td>
<td>1.09 (0.96–1.24)</td>
<td>0.18</td>
<td>1.14 (1.00–1.31)</td>
<td>0.05</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>rs10811647</td>
<td>G</td>
<td>n/a</td>
<td></td>
<td>0.84 (0.69–1.03)</td>
<td>0.09</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>rs16905599</td>
<td>A</td>
<td>1.25 (0.95–1.63)</td>
<td>0.11</td>
<td>1.27 (0.95–1.71)</td>
<td>0.10</td>
<td>1.31 (0.89–1.94)</td>
<td>0.17</td>
</tr>
<tr>
<td>rs1333051</td>
<td>T</td>
<td>0.87 (0.71–1.07)</td>
<td>0.20</td>
<td>n/a</td>
<td>n/a</td>
<td>0.85 (0.64–1.13)</td>
<td>0.27</td>
</tr>
<tr>
<td>rs8181047</td>
<td>A</td>
<td>n/a</td>
<td></td>
<td>n/a</td>
<td>n/a</td>
<td>1.16 (0.89–1.53)</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Association results for the top five associated SNPs after conditioning upon the lead CVD SNPs rs3333040 and rs10757278 for individuals with angina pectoris (AP), myocardial infarction (MI), and both MI and AP.

5. Conclusions

In conclusion, we confirm the association between variants in the 9p21 interval with T2D and CHD. Our results suggest that there exist additional CVD susceptibility variants in this region, highlighting the genetic complexity of the 9p21 region and human disease.

Abbreviations

AP: Angina pectoris
CAD: Coronary artery disease
CHD: Coronary heart disease
CVD: Cardiovascular disease
MI: Myocardial infarction
GWAS: Genome-wide association study
HUNT: Helseundersøkelsen Nord-Trøndelag
LD: Linkage disequilibrium
MODY: Maturity onset diabetes of the young
SNP: Single-nucleotide polymorphism
T2D: Type 2 diabetes
UTR: Untranslated region.

Conflict of Interests

The authors declare that they have no competing interests.

Authors’ Contribution

Øyvind Helgeland wrote the paper with assistance from Jens K. Hertel, Helge Raeder, Anders Molven, Pål R. Njølstad, and Stefan Johansson. Carl G. P. Platou, Kristian Midthjell, and Ottar Nygård reviewed and edited the paper. Øyvind Helgeland and Jens K. Hertel performed statistical analysis and interpreted the data with assistance from Anders Molven, Helge Raeder, Ottar Nygård, Pål R. Njølstad, and Stefan Johansson. Pål R. Njølstad and Stefan Johansson conceived the study design with contribution from Øyvind Helgeland, Jens K. Hertel, and Anders Molven. Kristian Midthjell collected background data. Stefan Johansson directed genotyping and statistical analysis. Øyvind Helgeland, Jens K. Hertel, Helge Raeder, Carl G. P. Platou, Kristian Midthjell, Ottar Nygård, Pål R. Njølstad, and Stefan Johansson contributed to discussion. All authors read and approved the final paper.

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