The field of epidemiology has seen many parameters enter the arena as risk factors. Many of them left the scene without leaving a mark, which was often caused by chance findings caused by small sample sizes or spurious associations resulting from confounding by other risk factors. Others became firmly established as risk factors, and therefore drug targets, but finally were dethroned by exclusion of causality, as it was recently reported for C-reactive protein. Such targets, but finally were dethroned by exclusion of causality, became firmly established as risk factors, and therefore drug targets, but finally were dethroned by exclusion of causality, as it was recently reported for C-reactive protein. Such parameters are then no longer handled as risk factors but as markers of the diseases, which might still be quite helpful for diagnostic purposes.

The mendelian randomization approach is not only used to see stronger evidence for causality that under certain circumstances might be provided by a mendelian randomization approach. The original idea for this approach was developed by Katan almost 25 years ago, and is based on the fact that it is randomly determined at the time of conception which of the 2 alleles from the father as well as from the mother will be transmitted to the child. In the case that certain alleles result in higher concentrations of, for example, LDL cholesterol, as it is the case for apolipoprotein e4, these alleles determine to a certain extent whether a person is exposed, for example, to high LDL cholesterol levels. Because the transmitted alleles are of life-long persistence, the carriers of those apolipoprotein e4 alleles are also exposed to life-long increased LDL cholesterol, and therefore a higher frequency of those alleles can be expected and is observed in patients with cardiovascular disease (CVD). The association between the polymorphism and CVD is less likely to be influenced by reverse causation or confounding. This is in line with the observation that the calculated estimate for an association of a polymorphism with CVD outcomes often changes only marginally if an extended adjustment for other CVD risk factors is performed.

The first time this mendelian randomization approach was applied was 20 years ago for lipoprotein(a) \( \text{Lp}(a) \), at a time when a lively discussion was held as to whether \( \text{Lp}(a) \) causes CVD or whether the elevated levels are a consequence of CVD. Finally, Sandholzer et al showed that isoforms in the apolipoprotein(a) gene that result in high \( \text{Lp}(a) \) levels are significantly more frequent in patients with CVD when compared with control subjects. This clearly demonstrated that the apolipoprotein(a) gene locus primarily determines the risk for CVD by its strong influence on \( \text{Lp}(a) \) concentrations. These and following studies made \( \text{Lp}(a) \) a drug target, but, despite tremendous efforts, no easily applicable method is available to lower this highly atherogenic lipoprotein.

The mendelian randomization approach is not only used to support causality. Recent studies used this approach to exclude causality with a very high likelihood. A large study in 4 independent populations observed a strong association between various polymorphisms within the CRP gene and CRP levels, and increased levels of CRP were associated with ischemic heart disease and cerebrovascular disease. However, no increased risk for the outcomes was observed for the genetic variants within the CRP gene region that are associated with life-long increases in CRP levels. This observation in more than 50 000 individuals supports that high CRP levels are not causally related to outcomes but are rather a marker of the disease.

Is Bilirubin the Next Candidate to Inthrone or Dethrone?

The antioxidative and cytoprotective properties of bilirubin made this product of the heme metabolism an interesting candidate for investigation of CVD outcomes. Many studies showed an association between low bilirubin levels and CVD. Early segregation analyses suggested a major gene control-

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To prove causality of a parameter with a certain disease is often a hard task. Cross-sectional case-control studies are not evidenitary because the demon of the “reverse causation” is omnipresent, and one never knows whether it is spook or reality. Reverse causation means that the parameter of interest is not causing the disease, but the disease itself results secondarily in a change of the parameter. Prospective, observational studies might support a higher probability of causality, especially when they are long-term studies in which individuals are included long before events and subclinical disease develops. If these data are supported by experimental evidence from, for example, animal models, one might consider as the next line of evidence interventional studies to see stronger evidence for causality that under certain circumstances might be provided by a mendelian randomization approach. The original idea for this approach was developed by Katan almost 25 years ago, and is based on the fact that it is randomly determined at the time of conception which of the 2 alleles from the father as well as from the mother will be transmitted to the child. In the case that certain alleles result in higher concentrations of, for example, LDL cholesterol, as it is the case for apolipoprotein e4, these alleles determine to a certain extent whether a person is exposed, for example, to high LDL cholesterol levels. Because the transmitted alleles are of life-long persistence, the carriers of those apolipoprotein e4 alleles are also exposed to life-long increased LDL cholesterol, and therefore a higher frequency of those alleles can be expected and is observed in patients with cardiovascular disease (CVD). The association between the polymorphism and CVD is less likely to be influenced by reverse causation or confounding. This is in line with the observation that the calculated estimate for an association of a polymorphism with CVD outcomes often changes only marginally if an extended adjustment for other CVD risk factors is performed.

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Table. Previous Studies Investigating the Association of UGT1A1 Polymorphisms With Bilirubin Levels and Cardiovascular End Points

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>CVD End Point</th>
<th>No. of Cases/Control Subjects</th>
<th>Genotype 7/7 Versus 6/6 TA Repeats</th>
<th>Association of Bilirubin With CVD End Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotterdam Study14</td>
<td>Retrospective</td>
<td>MI</td>
<td>185/255</td>
<td>+90%/+53%</td>
<td>1.3 (0.8–2.2)</td>
</tr>
<tr>
<td>ECTIM Study15</td>
<td>Retrospective</td>
<td>MI</td>
<td>366/314</td>
<td>ND</td>
<td>1.9 (1.1–3.4)</td>
</tr>
<tr>
<td>Lingenhel et al11</td>
<td>Retrospective</td>
<td>CAD Men: 365/397</td>
<td>102%/+94%</td>
<td>0.97 (0.61–1.54)</td>
<td>0.90 (0.86–0.94) per 0.1 mg/dL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Women: 112/222</td>
<td></td>
<td>0.93 (0.45–1.93)</td>
<td>0.77 (0.68–0.87) per 0.1 mg/dL</td>
</tr>
<tr>
<td>Rantner et al12</td>
<td>Retrospective</td>
<td>PAD</td>
<td>255/255</td>
<td>+117%/+52%</td>
<td>NS</td>
</tr>
<tr>
<td>Lin et al16</td>
<td>Retrospective</td>
<td>CAD</td>
<td>1320/1060</td>
<td>+71%</td>
<td>0.33 (0.15–0.75)</td>
</tr>
<tr>
<td>Ekblom et al13</td>
<td>Prospective case-referent study with 3.5-year lag time until event</td>
<td>MI</td>
<td>618/1184§</td>
<td><del>+100%/</del>+65%</td>
<td>0.95 (0.65–1.38)</td>
</tr>
<tr>
<td>Framingham Heart Study10</td>
<td>Prospective 24-year follow-up</td>
<td>CVD</td>
<td>156/1583</td>
<td>+81%</td>
<td>0.36 (0.18–0.74)</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; CVD, cardiovascular disease (includes fatal and nonfatal myocardial infarction, angina pectoris, coronary insufficiency, stroke, transient ischemic attack, intermittent claudication, congestive heart failure, or death from coronary heart disease); MI, myocardial infarction; PAD, peripheral arterial disease; OR, odds ratio; CI, confidence interval; NA, not applicable; ND, not done; and Q, quartile.

*Relative difference of bilirubin levels in carriers of 7/7 TA-repeats compared with wild-type 6/6 TA-repeats in the entire group. If available, changes are reported stratified for control subjects/cases.

†Serum bilirubin concentrations were available only for 114 cases and 162 control subjects.

‡This study did not investigate the TA-repeat polymorphism but rather the SNP rs887829, and compared the A/A versus the G/G genotype.

§Genotypes were available for 571 cases and 1074 control subjects for analysis.

CVD indicates coronary artery disease; MI, myocardial infarction; and CVD, cardiovascular disease (includes fatal and nonfatal myocardial infarction, angina pectoris, coronary insufficiency, stroke, transient ischemic attack, intermittent claudication, congestive heart failure, or death from coronary heart disease).
persons in the analysis might influence the results in the other direction. A survival bias as the result of older ages at recruitment is possible, because up to half of the incident cases do not survive the first half-year after a major cardiovascular event and thus are not available for inclusion into the studies. Events in those individuals could be due to stronger genetic effects and thus be more severe. All these influences and biases can be avoided by studies with an early recruitment phase long before the majority of the events take place.

Furthermore, studies up to now are heterogeneous in terms of the events ranging from peripheral arterial disease, MI, to a broad range of CVD events. For example, in the study by Ekblom et al., control subjects were only excluded when they had an MI, stroke, cancer, or death before the time of diagnosis of the index case. This means that a control subject was allowed to have a symptomatic coronary stenosis without having had an MI, which might have resulted in a dilution of the association because these events are not rare in a population of that age.

One might wonder why the UGT1A1 gene was not discovered in genome-wide association studies on CVD outcomes. First, almost all studies included in these studies were of a cross-sectional nature, with the same limitations as discussed above. Second, the SNP microarrays do not genotype the UGT1A1 TA-repeat polymorphism, but rather SNPs only weakly correlated with this functionally relevant polymorphism. In the Framingham Heart Study, we observed between this polymorphism and the nearby SNP rs1113193 only an \( r^2 \) of 0.12, and the association of these 2 polymorphisms with bilirubin levels was markedly less pronounced for rs1113193 compared with the TA-repeat polymorphism \( (P = 10^{-8} \text{ versus } 10^{-5}) \). Therefore, very large studies are required to detect this association by SNP analysis, considering the low significance threshold in genome-wide association studies.

Conclusions

Bilirubin is far from being intransferred but also far from being dethroned as a causal (genetically determined) risk factor for CVD. We are still lacking sufficient studies to answer these questions. Long-term observational cohorts with recruitment at early ages long before atherosclerosis becomes clinically detectable might be the most powerful design to address these questions. Because bilirubin was not as commonly measured in these studies as cholesterol levels were, and because the genotyping of the TA-repeat polymorphism is technically more sophisticated than a common SNP, the large and long-term observational studies must be encouraged to study this association.

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None.

References


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Florian Kronenberg

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