Gilbert's syndrome, circulating bilirubin and lung cancer: a genetic advantage?

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Cigarette smoking is the leading cause of lung cancer (all histological types), accounting for about 89% and 70% of lung cancer deaths in men and women, respectively, in Europe and other regions where cigarette smoking is common.[1] Oxidative damage to DNA by free radicals and oxidants in cigarette smoke (figure 1) is one of the major pathways that can lead to lung cancer development.[2]

As the heme pathway plays an important role against oxidative stress, uridine diphosphate-glucuronosyl-transferase 1-1 (*UGT1A1*) gene polymorphisms might be expected to protect against oxidative stress-induced cancer initiation.[3] Congenital underexpression of hepatic *UGT1A1* causes mild chronic unconjugated hyperbilirubinaemia, known as 'Gilbert's syndrome, GS'. Individuals with GS have mildly raised total bilirubin concentrations in the blood (>17 µmol/L) with normal serum activities of liver transaminases, biliary damage markers and red blood cell counts.[4] The frequency of the Gilbert's polymorphism is 30%–45%, however, phenotypic hyperbilirubinaemia is estimated to be 5%–10% in Caucasians.[5 6] A remarkable body of evidence from experimental and clinical studies has demonstrated that bilirubin has substantial anti-inflammatory and antioxidative properties.[4] The hypothesis that genetically raised bilirubin plays a role in lung cancer development, and may interact with cigarette smoking—a major source of oxidants—is therefore compelling.

In their article published in this issue of *Thorax*, Horsfall and colleagues[7] used data from the UK Biobank that included more than 350 000 men and women aged 40–69 years and recruited between 2006 and 2010 in different regions in the UK, to study potential causal relationships between serum total bilirubin and lung cancer incidence.

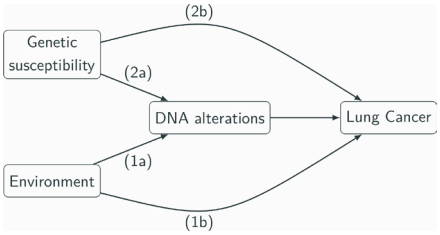

Figure 1 Causal pathways to lung cancer development. In this context, DNA alteration is influenced by 'environment', including cigarette smoke, (pathway 1a), and 'genetic susceptibility', including *UGT1A1*—uridine diphosphate-glucuronosyl-transferase—genotypes that are less susceptible to oxidants (and carcinogens) in cigarette smoke (pathway 2a), and their interaction. Pathways (1b) and (2b) refer to other possible effects of the environment and genetic susceptibility not through DNA alterations.

It is biologically plausible, and supported by previous smaller-scale observational studies,[8 9] to expect stronger associations between circulating bilirubin and lung cancer incidence among cigarette smokers. This hypothesis is also concordant with the results of a multi-omics systems toxicology study in mice, where cigarette smoke exposure was related with cellular oxidative stress responses in the lungs, which led to a drastic activation of the heme–biliverdin–bilirubin pathway.[10]

Hence, the aetiology of lung cancer can be conceptualised as reflecting the joint consequences of the interrelationship between (1) exposure to environment (here: cigarette smoke) and (2) genetic susceptibility (here: *UGT1A1* genotypes susceptible to oxidants in smoke), and their interaction.

In their exemplarily well-designed observational study, Horsfall and colleagues report that each 5 µmol/L increment in circulating bilirubin was associated with 1.2/10 000 person-years decrease (95% CI: 0.7 to 1.8) in overall lung cancer incidence.[7] After stratification by smoking status, a clear dose–response association became evident with the strongest reduction in predicted lung cancer incidence (−18.2/10 000 (95% CI: −33.3 to −3.4)) observed among current heavy smokers, who reported smoking 20 or more cigarettes per day. Another remarkable observation was that lung cancer incidence among current smokers with a bilirubin level >17 µmol/L, indicative of GS, was about 50% lower compared with a similar group of smokers in the lowest bilirubin quintile (<5 µmol/L). No association between circulating bilirubin and lung cancer incidence was observed among never smokers. This interaction was observed on the additive scale, which is of particular public health relevance, however it may have been also of interest to assess multiplicative interaction, where the magnitude in the interaction would be probably less pronounced.

Although all these analyses were controlled for a range of known predictors of lung cancer, one could argue that these associations could still be confounded by unobserved risk factors. Furthermore, despite the prospective design, reverse causation could also have distorted these findings.

Horsfall and colleagues[7] addressed these potential shortcomings by complementing the serological analysis with an instrumental variable approach, in this context also referred to as Mendelian randomisation (MR),[11] using single nucleotide polymorphisms (SNPs) robustly associated with circulating bilirubin concentrations. Provided that the main assumptions of MR hold, the obtained associations should be unbiased with regard to confounding and reverse causation. Nevertheless, MR is prone to other weaknesses such as horizontal pleiotropy, where the genetic instrument affects cancer risk also through other pathways than through raised bilirubin.[12] However, in case of coherence between estimates obtained by MR and serological analyses, robust evidence for observed associations can be obtained. This is the main strength of the work by Horsfall and colleagues, because their estimates of the genetic approach mirror the results of the serological approach as described above. Their conclusion that '*adult smokers in the UK Biobank with genetically raised bilirubin have lower rates of lung cancer*'[7] is therefore based on strong evidence and consistent with the endogenous antioxidant hypothesis of bilirubin.

A few uncertainties remain. One is selection bias, which as with traditional epidemiological analysis, can also adversely affect MR studies.[13] Selection bias is a recognised issue in UK Biobank, where participants differ from the UK general population in several

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characteristics. Horsfall *et al*[7] acknowledge that UK Biobank could be enriched with heavy smokers, who are less susceptible to adverse health effects. One would, however, expect that observed associations would then be rather attenuated than spurious. Indeed, in a comparison of risk factor associations in UK Biobank against general population-based studies, the magnitude of the association of cigarette smoking with lung cancer were weaker for UK Biobank.[14]

Replication in other populations seems therefore warranted. Coincidentally, we are investigating bilirubin in relation to risk of several cancers independently from Horsfall and colleagues. In a two-sample MR analysis, we observed an inverse association between circulating bilirubin predicted by a set of 109 SNPs and lung cancer risk among individuals who ever smoked (OR 0.86; 95% CI: 0.76 to 0.96, per 1SD increment), whereas no association was observed among never smokers (OR 1.01; 95% CI: 0.76 to 1.34; N Seyed Khoei, personal communication). These findings are very much inline with those of Horsfall *et al*,[7] although we did not yet formally test for interaction by smoking status. Importantly, our results are based on a different set of genetic instruments and a different (Caucasian) study population from an international genetic consortium (N lung cancer cases=29 266, N controls=56 450).

Taken together, it appears justified to test the utility of bilirubin in future studies as low-cost marker for lung cancer risk stratification. Research should also be expanded to other cancer sites, where bilirubin may also act as an endogenous antioxidant. Promising results are emerging from our own research, for example for colorectal cancer.[15]

We can conclude with some confidence that raised bilirubin may confer a genetic advantage in terms of protecting people exposed to smoke oxidants against lung cancer.

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