Review

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Gilbert's syndrome revisited

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Summary

Gilbert's syndrome, also known as benign hyperbilirubinaemia, was described more than 100 years ago. It has usually been considered a physiological abnormality characterised by a mild elevation of the systemic level of unconjugated bilirubin, in the absence of any underlying liver or overt haemolytic disease. However, since the re-discovery of the potent antioxidant effects of bilirubin in the late 1980s, as well as multiple intracellular signalling pathways affected by bilirubin, an ever-increasing body of evidence suggests that individuals with Gilbert's syndrome may benefit from the mild hyperbilirubinaemia and are actually protected from the development of a wide variety of "diseases of civilisation" such as cardiovascular diseases, certain cancers, and autoimmune or neurodegenerative diseases. This review analyses the current state of medical knowledge given recent discoveries in this rapidly developing field, as well as their possible clinical significance, and provides a new perspective on this condition.

History

The original name for the condition, *La cholemie simple familiale*, was first reported by Gilbert, Castaigne, and Lereboullet as early as 1900, and described as an asymptomatic elevation of systemic levels of bilirubin in the absence of overt haemolytic disease. In subsequent years, it was studied by several famous physicians including the Dutch biochemist van den Bergh (who developed the diazo determination of serum/plasma bilirubin, still used in clinical chemistry today) and the Danish physician Jens Einar Meulengracht (after whom the term Gilbert-Meulengracht syndrome was coined in the German literature) – see[1] for a more comprehensive historical overview.

Since 1900, many names have been proposed for this clinical condition including *icterus intermittens juvenilis*, hereditary non-haemolytic bilirubinaemia, familial non-haemolytic jaundice, and constitutional hepatic dysfunction. All of these names have already been abandoned and Gilbert's syndrome (or benign hyperbilirubinaemia) is the only name now used for this condition.

Physiological ranges of serum bilirubin, definition, and epidemiology of Gilbert's syndrome

Gilbert's syndrome is defined phenotypically, and therefore not according to predisposing genetic markers, as the elevation of serum unconjugated bilirubin concentration above the upper limit of normal, with no laboratory signs of haemolysis or liver damage.

However, the physiological range of serum/plasma bilirubin concentration is only inaccurately defined.[2] Based on data from the Tietz Clinical Guide to Laboratory Tests, the physiological values of serum bilirubin in the general population encompass a wide range of 3.4-20.5 μmol/L (0.2-1.2 mg/dl), while those for the North American population based on the 2.5–97.5th percentile values are 5.1-17.1 μmol/L (0.3-1.0 mg/dl). Similarly, the Royal College of Pathologists of Australasia uses the upper limit of normal bilirubin concentration of 20 μmol/L (based, however, on two irrelevant reference sources (https://www.rcpa.edu.au/Manuals/RCPA-Manual/Pathology-Tests/B/Bilirubin, accessed March 30, 2023) – for a detailed discussion of all the aspects of bilirubin reference intervals see.[2] Hence, a bilirubin concentration of 17.1 μmol/L (1.0 mg/dl) is usually used as a conventional cut-off value to distinguish individuals with Gilbert's syndrome from the 'normobilirubinaemic' population. However, if this upper reference cut-off value is true, this would imply that Gilbert's syndrome is present in only 2.5% of the general population (individuals with bilirubin concentrations in the top 2.5%) while the prevalence of Gilbert's syndrome in the general population is commonly stated to be around 5%.[3] The whole problem is further complicated by the unacceptable interlaboratory variability and inaccuracy of serum bilirubin determination across individual laboratories,[4] as well as the fact that serum bilirubin concentrations are affected by sex, age, and ethnicity, to mention just the most important factors that must be considered when re-evaluating reference intervals of bilirubin concentrations in general populations. In our study, the 95% (2.5-97.5%) reference interval for serum bilirubin in the overall Czech general population covering almost 2,600

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Keypoints

- Gilbert's syndrome is the most common cause of moderate unconjugated hyperbilirubinemia (prevalence around 10% in the general population).
- It is primarily due to reduced activity of the conjugating enzyme bilirubin UDP glucuronosyl transferase (UGT1A1), in Caucasians commonly related to a UGT1A1*28 polymorphism (a TA repetition in the TATA box element in the promoter region of the *UGT1A1* gene).
- Reduced hepatic uptake of bilirubin is also present in Gilbert's syndrome. As the bilirubin carrier molecule(s) is still unknown, no genetic studies are available.
- Apart from mild hyperbilirubinaemia, Gilbert's syndrome is asymptomatic and no treatment is either needed or recommended.
- The diagnosis of Gilbert's syndrome involves exclusion of overt haemolysis and/or underlying liver disease as major differential diagnostic factors. Genetic assessment of the *UGT1A1* polymorphism is not necessary for diagnosis.
- Due to the potent antioxidant and hormone-like effects of bilirubin, individuals with Gilbert's syndrome are protected from the "diseases of civilisation" such as cardiovascular diseases, certain cancers, and autoimmune or neurodegenerative diseases.
- An 'iatrogenic' increase of bilirubin (induced Gilbert's syndrome), *e.g.* using nanoparticles to deliver bilirubin to tissues and organs of interest, may be an option to treat inflammatory, metabolic, and oncological diseases.

individuals was 4.3-23.6 μmol/L (5.5-27.5 μmol/L for males and 3.8-19.9 μmol/L for females[2] – these values are currently used as reference ranges at the workplace of one of the authors [L.V.]). The prevalence of Gilbert's syndrome in this Caucasian population (defined as hyperbilirubinemia >17 μmol/L with no laboratory signs of haemolysis or liver damage) was 8.9% (11.6% in men, and 6.1% in women),[2] thus within the range of previously reported data in other populations.[5]

Bilirubin concentrations in both the general population and individuals with Gilbert's syndrome often fluctuate depending on several factors such as sex, ethnicity, age, smoking status, circadian rhythms, seasonal period, nutritional influences, or physical activity.[2,6,7] Therefore, since there are substantial differences in the physiological serum bilirubin concentrations between men and women, independent physiological ranges must be re-established for each sex (and for different ethnicities), including a lower diagnostic cut-off value for Gilbert's syndrome in women.

The molecular basis of Gilbert's syndrome

The molecular basis of Gilbert's syndrome lies in the impairment of the conjugation of bilirubin with glucuronic acid in the liver. This is because the rate-limiting metabolic step in the transfer of unconjugated bilirubin from the systemic circulation into bile is bilirubin glucuronosylation in hepatocytes, which is mediated by a specific hepatic enzyme named bilirubin-UDP glucuronosyl transferase (UGT1A1, OMIM *191740) that forms bilirubin diglucuronoside (which is the correct name according to the IUBMB Enzyme Nomenclature rather than the oft-used term bilirubin diglucuronide).

In genome-wide association studies, the *UGT1A1* gene was found to be the major gene that controls intravascular levels of bilirubin.[8,9] Accordingly, specific mutations in the *UGT1A1* gene are responsible for the manifestation of mild unconjugated chronic hyperbilirubinaemia (Gilbert's syndrome).[10] *UGT1A1* is highly polymorphic, with more than 150 allelic genotypes identified so far. In most Caucasians, Gilbert's syndrome is associated with the UGT1A1*28 polymorphism ($A(TA)_7TAA$ promoter sequence, rs8175347) substantially reducing the glucuronosylation of bilirubin. Importantly, the penetrance of the UGT1A1*28 homozygous mutation is incomplete due to the interaction with other genes and possible post-translational modifications of certain regions of the promoter of the *UGT1A1* gene, which is believed to explain inter-individual variability in the expression of the *UGT1A1* gene (for more details, see[10]).

Interestingly, compared to Caucasians, Asians have higher bilirubin levels, but the $(TA)_7$ allele in this population is much less common. In these populations, various heterozygous variants in the coding regions of the *UGT1A1* gene are common and explain the decreased expression of *UGT1A1*.[11]

It is interesting to note that the frequency of the UGT1A1*28 genotype is comparable between men and women. Hence, the lower serum bilirubin concentrations in females are believed to be due to the modulation of UGT1A1 activity by sex steroids or by sex-dependent variation in the penetrance of the *UGT1A1* gene.[10]

Altered hepatic uptake of organic anions, bilirubin included, has been found in Gilbert's syndrome, possibly explaining increased serum bilirubin concentrations in individuals without a genetic defect in *UGT1A1*. As the carrier molecule that accounts for the hepatic uptake of bilirubin is still uncertain, the contribution of a genetic defect in the putative carrier in Gilbert's syndrome remains undefined.

All these facts emphasise that Gilbert's syndrome is a phenotypic diagnosis and that UGT1A1*28 homozygosity is only a predisposing factor. Hence, molecular testing should not be used to confirm the diagnosis or to redefine the diagnostic criteria of Gilbert's syndrome (advocated by some authorities) as it can be misleading.

Diagnosis of Gilbert's syndrome

Gilbert's syndrome manifests as mild unconjugated asymptomatic hyperbilirubinaemia, usually found in young adults during routine laboratory check-ups or after an intercurrent illness. The diagnosis is typically made *per exclusionem*, *i.e.* by excluding other causes of hyperbilirubinaemia. In individuals with isolated asymptomatic unconjugated hyperbilirubinaemia (usually 17–70 μmol/L [1-4 mg/dl]), a careful past medical history should be taken, including a review of previous laboratory

records (often showing intermittent unconjugated hyperbilirubinaemia), the use of xenobiotics (certain drugs may selectively affect hepatic bilirubin metabolism) and family history (Gilbert's syndrome is often found within family members). This is followed by a physical and laboratory workup searching for underlying haemolytic or chronic liver disease, determination of complete blood count (overt haemolysis excluded by reticulocyte count <1.5% and negative Coombs test), liver enzyme activities (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyltransferase – within a physiological range) together with liver ultrasound in selected cases where co-existing structural liver disease or splenomegaly (a possible cause of haemolysis) are suspected. If these examinations are normal, the individual should be re-examined after 6 to 12 months. If neither symptoms, nor new haematological or biochemical abnormalities appear, the diagnosis of Gilbert's syndrome is made. According to our clinical experience, a diagnosis based on such a conservative medical approach rarely needs to be revised. Since the prevalence of elevated liver enzyme activities in the general population varies between 10-20%, Gilbert's syndrome may coincide with some underlying liver disorder. In these cases, a careful and detailed examination of possible causes of altered liver function tests is needed and treatment/follow-up is guided by the specific liver disease.

Although overt haemolysis needs to be excluded in suspected cases of Gilbert's syndrome, the life span of red blood cells was found to be shortened by 30% in individuals with Gilbert's syndrome[12] confirming previous observations. Mild dyserythropoiesis, with defective incorporation of iron into erythrocytes, was reported in the past in the majority of individuals with Gilbert's syndrome, providing an indication of the complexity of impaired bilirubin metabolism in this condition.

Several exposure tests used in the past in the diagnosis of Gilbert's syndrome, such as nicotinic acid, phenobarbital, rifampicin, or fasting tests, are currently considered obsolete.

Neither liver biopsy nor *UGT1A1* genotyping is required. Genetic testing is reserved for differentiating Gilbert's syndrome from Crigler-Najjar syndrome type 2 (more severe hereditary unconjugated hyperbilirubinaemia [>100 μmol/L/6 mg/dl]), which is important for family counselling, or when considering treatment with drugs that interfere with UGT1A1 activity (Table 1).

The dark *vs.* the light side of Gilbert's syndrome

The dark side of Gilbert's syndrome

Fatigue, asthenia, and variable vaguely defined dyspeptic complaints attributed to Gilbert's syndrome in the past are no longer considered a part of this condition, and proper evaluation of possible causes is warranted in these cases. Although Gilbert's syndrome is generally benign, several clinical conditions need to be considered (Table 1).

Alongside the increased prevalence of pigment gallstone disease, the risk of drug toxicity is also higher in individuals with Gilbert's syndrome. Gilbert's syndrome can be unravelled by some drugs that have a suppressive effect on *UGT1A1* gene expression or that act as substrates that compete with bilirubin for the UGT1A1 enzyme.[7] In both cases, xenobiotic biotransformation can be impaired, leading to potentially toxic side effects. Typical examples of such drugs include irinotecan (a topoisomerase 1 inhibitor used in patients with colon cancer), and atazanavir (an antiviral agent used for the treatment of HIV infection).[7] Currently, the Dutch Pharmacogenetics Working Group and the French Network of Pharmacogenetics guidelines[16,17] consider *UGT1A1* genotyping essential before the initiation of treatment with irinotecan, and the same is true for atazanavir (https://cpicpgx.org/guidelines/guideline-for-atazanavir-and-ugt1a1/Last updated March 30, 2023, accessed on 23 Feb 2023).

However, the list of drugs and other compounds that affect UGT1A1 activity is much longer, and this UGT1A1 inhibitory activity should be considered in the clinical setting[7,13] (Table 1). Hyperbilirubinemia induced by various xenobiotics, which is often viewed as an adverse effect or even as a sign of drug-induced liver injury, must be regarded as a sign of delayed biotransformation of the drug with all possible clinical consequences, as described for the anticonvulsant lamotrigine, paracetamol, or silymarin complex flavonolignans.[7,13]

In this respect, the European Medicines Agency and US Food and Drug Administration now recommend studying the inhibition of UGT1A1 when testing new drugs (European Agency Medicines. Guideline on the investigation of drug interactions. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf.2012; US Food and Drug Administration. Guidance for industry: drug interaction studies—study design, data analysis, implications for dosing and labeling recommendations; Draft guidance. Available from: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf.2012).

Furthermore, clearance of various xenobiotics (which are not substrates for glucuronosylation), such as the antidiabetic drug tolbutamide that competes with bilirubin for binding sites on glutathione S-transferase, is known to be impaired in individuals with Gilbert's syndrome. Additionally, the clearance of bile acids, such as ursodeoxycholic acid, is impaired in individuals with Gilbert's syndrome and, *vice versa,* ursodeoxycholic acid has been found to increase serum bilirubin concentrations significantly, indicating dysfunctional hepatic uptake of bilirubin[7,8]. Considering the endocrine and metabolic effects of bile acids,[19] these changes could be of clinical relevance.

The light side of Gilbert's syndrome

The benefits associated with mild elevations of serum bilirubin concentrations outweigh the negative effects indicated above (Table 1).

The most pronounced beneficial effect seems to be the protection from atherosclerotic diseases, as proven by numerous studies (for a review, see[20]). However, the spectrum of medical conditions that are beneficially affected by the mild elevation of serum bilirubin is much wider, covering the majority of "diseases of civilisation", including metabolic (such as overweight and obesity, metabolic syndrome, non-alcoholic fatty liver disease or diabetes), oncological, inflammatory, autoimmune and neurodegenerative diseases (such as inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis, psoriasis, schizophrenia, multiple sclerosis, osteoporosis, or pre-eclampsia).[2,6]

Interestingly, Gilbert's syndrome is associated with a reduced tendency to gain body fat in later life.[21,22] Individuals with Gilbert's syndrome in the younger age cohort have a

Table 1. Gilbert's syndrome – overview.

General	
Definition	Isolated, asymptomatic, unconjugated hyperbilirubinaemia (17-70 μmol/L [1-4 mg/dl]) with no signs of overt haemolysis or impaired liver enzyme activities
Aetiology	Mutations in the promoter or structural gene of *UGT1A1* responsible for conjugation of bilirubin with glucuronic acid in the liver
Epidemiology	Prevalence 2-12% Male-to-female preponderance: 3:1 Marked inter-ethnic differences in prevalence
Diagnosis	Isolated, asymptomatic, unconjugated hyperbilirubinaemia (17-70 μmol/L [1-4 mg/dl]) Hepatic enzyme activities (ALT, AST, ALP, GGT) within the physiological range No signs of overt haemolysis (reticulocyte count <1.5%, negative Coombs test) Genetic testing is reserved for those with higher serum bilirubin concentrations to differentiate Gilbert's syndrome from Crigler-Najjar syndrome type 2, or when considering treatment with drugs that interfere with UGT1A1 activity
Therapy	Not required
Prognosis	Very good, drug interactions being the most important health issue
Factors affecting systemic bilirubin concentrations in Gilbert's syndrome	
Natural factors	Age, sex, ethnicity, seasonal variations, circadian rhythms, altitude
Lifestyle factors	Smoking, BMI, overweight and obesity, alcohol intake, diet (fasting), regular aerobic activity/sedentary lifestyle
Xenobiotics	Drugs, herbals
Other factors	Psychological stress, hormonal influences, intercurrent illness
Compounds suppressing or competing with UGT1A1 activity	
Chemical drugs	Anticancer drugs (irinotecan, antiviral agents (atazanavir, indinavir), tyrosine kinase inhibitors (ragorafenib, sorafenib, lapatinib, nilotinib, sunitinib, pazopanib), NSAIDs (ibuprofen, ketoprofen), statins (simvastatin, atorvastatin, cerivastatin), other lipid-lowering drugs (ezetimibe), acetaminophen, oxazepam, lorazepam, ethinylestradiol, cyclosporin A, rifampicin, buprenorphine, tocilizumab, tranilast[7,13]
Natural compounds	Silymarin complex flavonolignans, quercetin, epigallocatechin gallate, echinacea, saw palmetto, Japanese and Chinese herbs (*Kampo*, *Daio*, *Kanzo*, *Kaihi*, or *Ogon),* valerian, hops extracts[7,14,15]
Association of Gilbert's syndrome with clinical conditions	
Health risks	Drug interactions[7,13]; More severe course of jaundice in neonatal age and inherited haemolytic diseases Increased prevalence of pigment gallstone disease
Health benefits	Lower risk of atherosclerotic diseases Lower risk of diabetes and associated diseases Positive effects on body mass status Lower risk of cancer Lower risk of autoimmune diseases Lower risk of neurodegenerative diseases

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase.

modestly but significantly lower BMI compared to matched controls (22.5 *vs*. 23.5 kg/m^2), while the difference in the older age group is much higher (23.8 *vs*. 27.2 kg/m^2). Accordingly, the mean body fat content in older individuals with Gilbert's syndrome was substantially lower than in age-matched controls.[21] Higher plasma bilirubin levels are associated with better insulin sensitivity and a lower risk of metabolic syndrome and type 2 diabetes, independently of BMI,[23,24] and the same is true for the protective role of bilirubin in the development of vascular diabetic complications.[25] Despite helping to maintain adipocyte insulin sensitivity (which theoretically should lead to maintenance of the adipose tissue), bilirubin decreases adiposity, most likely due to its role in preventing hypothalamic leptin sensitivity by inhibiting microglial activation.[22] All these facts may explain the more significant beneficial metabolic effects of bilirubin in individuals with Gilbert's syndrome of older age – and also explain the decrease in overall mortality and the prolonged lifespan of individuals with Gilbert's syndrome[26,27] (see also below).

Prognosis

Apart from the risk of impaired biotransformation of xenobiotics, there is almost no reason to label persons with Gilbert's syndrome as "patients", nor to recommend restrictions on diet[28] or physical activity. On the contrary, Gilbert's syndrome is more prevalent among elite athletes and elite athletes generally have higher serum bilirubin concentrations compared to the general population,[29] which, most likely, predisposes them to better physical performance.[30] It is noteworthy that individuals with Gilbert's syndrome have higher haemoglobin levels,[31] and lower markers of immune system activation such as white blood cells and platelets[31,32]; all factors that could contribute to a better tolerance of strenuous exercise.

Gilbert's syndrome and longevity: myth or fact?

As discussed in our recent report,[10] serum bilirubin concentrations have likely become elevated during evolution due to the acquisition of the TA repeat in the TATA box element in the promoter region of the *UGT1A1* gene. This is supported by the correlation between a shorter TATA box in great apes and low serum bilirubin levels, with the same association between the length of the TATA box and bilirubin levels in humans.[33]

It is tempting to speculate that longevity could be affected, at least in part, by serum bilirubin, as also supported by a negative association between serum bilirubin concentrations and all-cause mortality reported in our recent nested case-control study conducted within the Polish arm of the HAPIEE study.[26] Mortality rates were decreased by almost

50% in individuals with Gilbert's syndrome compared to the general normobilirubinaemic population in the UK Health Improvement Network primary care database.[34] A more recent Polish study found a positive association between lifespan and high-normal serum bilirubin concentrations in men, but not women,[27] which is consistent with human epidemiological studies that demonstrate powerful sex-dependent health-promoting effects of bilirubin,[26] as well as with experimental data on sexual dimorphism in lipid metabolism of hyperbilirubinaemic Gunn rats.[35]

These data are also consistent with studies demonstrating a significant correlation between serum bilirubin concentrations and polyunsaturated fatty acids (PUFAs) in Japanese centenarians with better performance status.[36] Considering the involvement of PUFAs in longevity,[37] serum bilirubin and PUFAs appear to play a protective role in the aging process. In addition, high-normal serum bilirubin concentrations negatively correlate with visceral obesity,[38] an important factor associated with decreased longevity.[39] Furthermore, a recent study reported that serum bilirubin concentrations are negatively associated with frailty and disability in activities of daily living in elderly Japanese patients with diabetes.[40] Taken together, these data strongly support the beneficial role of mildly elevated serum bilirubin concentrations in aging.

Iatrogenic Gilbert's syndrome: fantasy or reality?

Based on a large pile of recent clinical and experimental evidence, it seems reasonable to iatrogenically mimic Gilbert's syndrome to enhance protection against the diseases of civilisation.[7] It is necessary to emphasise that only a very mild increase in serum bilirubin concentration is biologically relevant, as the risk of various civilisation diseases decreases significantly with each micromolar increase in serum bilirubin concentrations.[2,20]

The first attempts to treat humans with bile pigments date back more than 2,000 years, as documented in the *Shen Nong Ben Cao Jing*, one of the earliest Chinese medical books.[41] *Calculus bovis* (artificial bezoar, or *Niu Huang* in Chinese), a pulverized ox pigment gallstone predominantly composed (>50%) of calcium bilirubinate and bile salts, was believed to '*clear the heart, resolve heat, disinhibit phlegm and suppress fright*'.[41] It is noteworthy to mention that the "bilirubin tolerance test" established by Eilbott back in 1927,[42] and used extensively thereafter,[43] was the first attempt to artificially elevate systemic bilirubin levels (in these cases for diagnostic reasons). Dozens of studies on parenteral bilirubin administration, covering almost 2,000 individuals, have been performed, indicating the feasibility of this approach.[44]

There are several options to mildly elevate systemic or intracellular concentrations of bilirubin and to augment bile pigment-mediated biological responses. The first one is the induction of HMOX1 (heme oxygenase 1), the key enzyme of the heme catabolic pathway, whose final product in the intravascular compartment is bilirubin. Various xenobiotics, both chemical drugs and natural compounds, have been found to efficiently induce HMOX1 with multiple biological benefits.[6,7,45]

Another possibility is to decrease the efficacy with which liver cells conjugate bilirubin to glucuronic acid via partial inhibition of UGT1A1, as demonstrated in HIV-infected patients treated with the antiviral agent atazanavir, whose administration results in mild elevation of serum bilirubin concentrations and reduced cardiovascular risk.[46] Non-selective inhibition of UGT1A1 has been reported for numerous other xenobiotics routinely used in clinical medicine,[6,7] but also for naturally occurring compounds such as plant flavonolignans.[14] A more elegant approach to enhancing the pool of bile pigments appears to be dietary supplementation with bilirubin-like structures that commonly occur in nature, such as chlorophylls from green plants[47] or phycocyanobilin from blue-green algae.[48]

The lifestyle modification approach is an optimal way to modulate bilirubin metabolism in the body since lifestyle factors that include ideal body composition, appropriate dietary factors (increased consumption of fruits and vegetables, increased carbohydrate intake, or mild caloric restriction), or sufficient aerobic activities have been associated with mildly elevated serum bilirubin concentrations.[6]

Last but not least is the nanotherapeutic approach to delivering bilirubin to the tissues and organs of interest, using various forms of bilirubin nanoparticles to treat inflammatory, metabolic, and oncological diseases.[6,49,50] Interestingly, bilirubin-coated vascular stents have been reported to attenuate the inflammatory response in a recent experimental animal study,[51] further expanding the therapeutic potential of this bile pigment.

Perspectives and conclusions

It is no longer in doubt that bilirubin cannot be considered simply an end product of haemoglobin degradation but must be regarded as a true hormone.[10] A systematic approach to Gilbert's syndrome, involving the collation of data in international registries, is warranted to uncover unknown associations and to determine the natural course of serum bilirubin concentrations over childhood and adulthood, the heritability of Gilbert's syndrome, gene penetrance (including assessment of biological factors modulating UGT1A1*28 gene penetrance), and the impact on lifespan, physical fitness and frailty in older age, just to mention some of the most important research issues.

Since the associations between serum bilirubin and clinical conditions are valid, it is also important to determine the decision limits for low serum bilirubin concentrations that predict the risk of civilisation disease development. Hence, validated cut-off values to determine clinical decision limits for serum bilirubin concentrations should be carefully established individually for men and women, as well as different ethnicities. It is also necessary to stress that a similar approach should be used to determine the reference ranges and decision limits for children and adolescents to predict future health risks.

Finally, the role of bilirubin on gut microbiome-associated metabolic consequences, both in individuals with Gilbert's syndrome or in the normobilirubinaemic population, is yet to be uncovered, with possible important clinical implications, as suggested by recent reports.[50,52–54]

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Abbreviation

PUFAs, polyunsaturated fatty acids.

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Conflicts of interest

The authors declare no conflicts of interest that pertain to this work.

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Authors' contributions

Both authors contributed equally to the data search, design of the paper layout, writing, and critical reading.

Supplementary data

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