Genetic testing for Gilbert’s syndrome: how useful is it in determining the cause of jaundice?

Aram S. Rudenski and David J. Halsall

Gilbert’s syndrome is a benign condition causing hyperbilirubinemia, which is also a symptom of liver or hemolytic disease. A genetic test may be possible for Gilbert’s syndrome because an associated gene defect has been isolated. Here we present a mathematical analysis of the use of this test in excluding harmful causes of hyperbilirubinemia. The effectiveness of the test depends on a low prevalence and high penetrance of the gene defect and a low prevalence of harmful hyperbilirubinemia. If the gene defect has a 12% prevalence and 10% penetrance, then the prevalence of harmful hyperbilirubinemia needs to be <1.5% for a positive test to reduce this risk fivefold. Estimates of the prevalence of harmful hyperbilirubinemia and of the prevalence and penetrance of the Gilbert genotype are required to assess the value of the genetic test in determining a cause of hyperbilirubinemia. Until these values for prevalence and penetrance are known, genetic testing for Gilbert’s syndrome cannot be recommended on a routine basis.

As more genes are identified and the techniques for sequencing genes improve, it becomes easier to carry out diagnostic genetic tests routinely. Identification of a penetrant mutant allele of a gene can confirm a disease. Examples are the testing for genetic abnormalities that lead to Huntington’s chorea or cystic fibrosis (1).

In Gilbert’s syndrome, however, a benign genetic polymorphism causes jaundice, a symptom that may also result from a harmful disorder, such as liver or hemolytic disease.

Recent studies (2) suggest that Gilbert’s syndrome is caused by an alteration in the promoter sequence for the gene for the enzyme uridyl diphosphate glucuronosyl phosphotransferase (UDPGT), thus raising the possibility of a genetic test for the condition. Gilbert’s syndrome appears to be benign apart from the risk of invasive investigations prompted by the finding of jaundice.

Traditionally, the diagnosis of Gilbert’s syndrome has been a diagnosis of exclusion, made when moderately raised concentrations of unconjugated bilirubin have been observed in a patient with no evidence of hemolysis and in whom other tests of liver function are within reference values. Bilirubin is usually measured as part of a panel of liver function tests such as liver alkaline phosphatase and a liver transaminase. In patients with liver disease, abnormalities in these analytes often accompany an increase in serum bilirubin and thereby exclude Gilbert’s syndrome as the cause of jaundice. The diagnosis is similarly excluded when hematological investigations show a hemolytic disorder. Diagnoses of exclusion are always less satisfactory than positive confirmation, and particularly when the patient has other symptoms, a physician may seek harder to exclude liver problems.

Methods for measuring the conjugated bilirubin fraction are often used to differentiate between hepatic and other causes of jaundice. Gilbert’s syndrome and hemolytic disorders result solely in a rise of the unconjugated fraction of bilirubin, whereas liver damage is usually associated with varying degrees of intrahepatic cholestasis, with impaired bilirubin excretion leading to a rise in the conjugated fraction of bilirubin. In theory then, an accurate estimation of conjugated bilirubin could be a sensitive indicator of liver disease. However, most hospital automated analyzer methods for determining conjugated bilirubin concentrations lack the required specificity, giving a detectable concentration in healthy individuals in whom none should be present. HPLC methods for measuring conjugated and unconjugated bilirubin are more specific, but the special care needed for storage and handling of samples and calibrators makes them less appropriate for routine analysis (3).

A genetic test for Gilbert’s syndrome would provide a positive diagnosis for this condition and may, in theory, aid in the management of a patient found to have an isolated increased bilirubin. Here, we examine the effect that possession of the Gilbert genotype has on the likelihood of jaundice being caused by a harmful disorder. We
take into account the effect of penetrance of the benign genotype because the reported 12% frequency of the Gilbert genotype (4) is greater than the prevalence of jaundice in the general population.

Our calculations indicate that the presence of the Gilbert genotype reduces the risk of harmful disease in the jaundiced individual but cannot rule it out. The degree of reduction of risk is small if the penetrance of Gilbert's is low or if Gilbert's syndrome and liver/hemolytic disorders are common.

**Materials and Methods**

Set theory and probability were used to define the different possible categories of phenotype and genotype. An assumption of statistical independence between the Gilbert genotype and other causes of jaundice was made.

We consider how the probabilities of Gilbert's syndrome and/or liver disease change when the population is narrowed down first to those with jaundice, that is, the patient group, and then to those in whom the Gilbert genotype has been characterized. We are interested in the use of the test for the Gilbert genotype in investigating the patient group, as opposed to screening the general population. The distinction is illustrated in Fig. 1.

**Definitions**

The starting point for this analysis is formed by the probabilities of liver disease (or hemolytic disorder) and of the Gilbert genotype in the general population.

We define

- $G$ as the proportion of the general population with the Gilbert genotype, that is, the proportion of the population who are homozygous for the proposed UDPGT promoter insertion mutation;
- $\pi$ as the penetrance of the Gilbert genotype, that is, the proportion of those people with the Gilbert genotype who develop jaundice in the absence of any other cause; and
- $D$ as the proportion of the general population with a pathological cause of jaundice, such as liver disease or hemolysis. For simplicity in the rest of this section, we shall use the term “liver disease” to denote all harmful causes of jaundice, including hemolytic disorders.

The way the general population can be subdivided into those with and without harmful disease and those without and with the benign genotype, penetrant or non-penetrant, is shown in Fig. 2.

**Probabilities of Gilbert's Syndrome and of Liver Disease in the Patient Group (Those with Jaundice)**

The probabilities of having the Gilbert genotype or of having liver disease or both are higher in the patient group, those with jaundice, than in the general population.

The probability of liver disease in someone with jaundice (symptom), that is, the patient group probability of liver disease is given by the equation:

$$Pr\{\text{liver disease } | \text{ symptom} \} = \frac{D}{D + \pi G(1-D)}$$

The value of this expression tends to 1 as the prevalence and/or penetrance of the Gilbert genotype diminishes.

*Example 1.* If the prevalence of liver disease is 1.2% and of the Gilbert genotype 12%, and if the penetrance of the genotype is 10%, then the probability of liver disease in someone with a high bilirubin alone is:

$$\frac{0.012}{0.12 + 0.1 \times 0.12 \times 0.988}$$

that is, 0.503, or 50.3%. This percentage falls to 9.2% if the penetrance is 100% or falls to 14.3% if the prevalence of liver disease is only 0.2%.

Dividing by the prevalence in the general population gives the factor by which the risk of disease is increased by virtue of having a symptom of the disease and thus being in the patient group. The expression for this risk ratio is:

$$\frac{Pr\{\text{liver disease } | \text{ symptom} \}}{Pr\{\text{liver disease} \}} = \frac{1}{D + \pi G(1-D)}$$

![Fig. 1. Flow chart illustrating how disease probabilities vary among groups on the basis of the presence of or absence of symptoms, and before and after genotyping.](https://academic.oup.com/clinchem/article-abstract/44/8/1604/5642969)
The denominator corresponds to the proportion of the general population with isolated jaundice.

Example 2. The value of this expression for $D = 1.2\%$, $G = 12\%$, and $\pi = 10\%$ is:

$$\frac{1}{0.12 + 0.1 \times 0.12 \times 0.988}$$

giving a value of 41.9. Thus, someone with a high bilirubin would be 41.9 times more likely to have liver disease than a random member of the general public.

Similarly, the probability of the Gilbert genotype (benign genotype) being present in someone with jaundice is as follows:

$$\Pr(\text{benign genotype} | \text{symptom}) = \frac{G(D + \pi(1 - D))}{D + \pi G(1 - D)}$$

This is different from the probability of the cause of the jaundice being solely Gilbert’s syndrome (benign cause):

$$\Pr(\text{benign cause} | \text{symptom}) = \frac{\pi G(1 - D)}{D + \pi G(1 - D)}$$

This distinction arises because the Gilbert genotype, whether expressed or not, can be found in people with liver disease.

Example 3. With $D = 1.2\%$, $G = 12\%$, and $\pi = 10\%$, the probability of the Gilbert genotype being present in someone with a raised bilirubin is:

$$\frac{0.12 \times (0.012 + 0.1 \times 0.988)}{0.12 + 0.1 \times 0.12 \times 0.988}$$

that is, 55.7%, whereas the probability of jaundice being solely caused by Gilbert’s is:

$$\frac{0.1 \times 0.12 \times 0.988}{0.12 + 0.1 \times 0.12 \times 0.988}$$

equal to 49.7%.

**Postinvestigation Probabilities**
The probability of liver disease changes once the presence or absence of the Gilbert genotype has been confirmed. In the absence of the Gilbert genotype, there must be another cause for the jaundice. In mathematical notation:

$$\Pr(\text{disease} | \text{normal genotype} | \text{symptom}) = 1$$

A pathological cause, however, can co-exist with the Gilbert genotype, so that the probability of liver disease given the presence of the Gilbert genotype type is not zero:

$$\Pr(\text{disease} | \text{benign genotype} | \text{symptom}) = \frac{D}{D + \pi(1 - D)}$$

Conversely, the probability of there being no other cause for jaundice than Gilbert’s is:

$$\Pr(\text{no disease} | \text{benign genotype} | \text{symptom}) = \frac{\pi(1 - D)}{D + \pi(1 - D)}$$

The value of this expression increases towards 1 as the penetrance $\pi$ approaches 1 and as the prevalence $D$ of liver disease approaches 0. The dependence of this prob-
ability on genotype penetrance and on prevalence of liver disease is illustrated in Fig. 3.

Example 4. For \( D = 1.2\% \), \( G = 12\% \), and \( \pi = 10\% \), the probability that someone with a high serum bilirubin with the Gilbert genotype also has liver disease is:

\[
\frac{0.012}{0.012 + 0.1 \times 0.988}
\]

that is, 10.8%. This probability falls to 1.2%, the value of \( D \), if the penetrance \( \pi \) is 100%. The probabilities that Gilbert’s syndrome is the sole explanation for the raised bilirubin are then:

\[
\frac{0.1 \times 0.988}{0.012 + 0.1 \times 0.988}
\]
equal to 89.2%, and:

\[
\frac{0.988}{0.012 + 0.988}
\]
equal to 98.8%, respectively.

A positive test for the Gilbert genotype reduces the risk of liver disease being the cause of jaundice. The ratio of this reduction is \( \frac{D + G\pi(1-D)}{D + \pi(1-D)} \).

This quantity can be obtained by dividing the probability of liver disease in someone with jaundice after a positive test for the Gilbert genotype by the probability in such a patient before genotyping. These two probabilities and their ratio are plotted in Fig. 4, using different values of harmful disease prevalence \( D \), with benign genotype frequency \( G \) and its penetrance \( \pi \) being set to 12% and 10%, respectively. Unlike most tests, where one is looking for a marker of the presence of disease, so that the post-to-pretest probability ratio is >1, the ratio here is \( \leq 1 \). The ratio is 1 when \( D \) is 1. It decreases, tending to \( G \), as \( D \) decreases and tends to zero.

Example 5. For \( D = 1.2\% \), \( G = 12\% \), and \( \pi = 10\% \), a positive test for the Gilbert genotype reduces the risk of liver disease being the cause of a raised bilirubin to:

\[
\frac{0.012 + 0.12 \times 0.1 \times 0.988}{0.12 + 0.1 \times 0.988}
\]

that is, 19.7% of what it was in an untested person with jaundice. This figure is 13.7% if \( D \) is only 0.2%, and is 13.1% (the inverse of the risk increase due to jaundice in Example 2) if \( \pi \) is 100%.

The likelihood ratio (LR) for liver/hemolytic disease of a positive test for Gilbert’s syndrome in patients with jaundice is given by the expression:

\[
LR \{ \text{benign genotype} \mid \text{symptom} \} = \frac{\Pr \{ \text{benign genotype} \mid \text{disease} \mid \text{symptom} \}}{\Pr \{ \text{benign genotype} \mid \text{no disease} \mid \text{symptom} \}} = \frac{GD/D}{\pi G(1-D)/\pi G(1-D)} = \frac{GD}{\pi G}
\]

It is easily seen that the likelihood ratio for liver disease of a negative test for Gilbert’s is infinite.

Discussion
Detection of a genotype known to cause a disorder, such as mutations that cause cystic fibrosis, can give a definitive diagnosis. In the case of Gilbert’s, however, it is the absence of the Gilbert genotype that implies that individuals have a harmful cause for their jaundice. The presence of the gene cannot exclude liver or hemolytic disease, because these can co-exist with Gilbert’s syndrome. Intuitively, one might assume that the presence of the Gilbert genotype makes another cause less likely. This study shows how the extent of this reduction depends on

![Fig. 3. How the probability of Gilbert’s syndrome being the sole cause of jaundice changes with different values of penetrance of the Gilbert genotype and with the prevalence of jaundice-inducing liver/hemolytic disease in the general population.](https://academic.oup.com/clinchem/article-abstract/44/8/1604/5642969)
prevalence and penetrance of the Gilbert genotype and on the prevalence of harmful diseases that cause jaundice.

The risk of a particular disease in people who have symptoms of that disease is higher than the risk in the general population. The probability in the patient group can be calculated for cases where there are several possible causes and when the possible causes are mutually exclusive or co-exist. In the simplest case, a symptom is caused by only one disease, and the probability of symptom given disease is 1. In that case, the risk of having the disease, given the symptom, increases as the inverse of the prevalence of the disease in the general population, so as always to have the value 1.

The probabilities are less straightforward when a benign genetic polymorphism can mimic a symptom of disease, as is the case of Gilbert's syndrome causing jaundice. The lower the post- to pretest probability ratio for liver disease of the genetic test for Gilbert's, the more efficient is the test. The ratio depends on the penetrance of the Gilbert genotype and on the prevalence of liver/hemolytic disease. When the penetrance is low, near zero, the postinvestigation probability is similar to the pretest patient-group probability: little additional information is obtained by testing for the genotype. When the penetrance approaches 1, on the other hand, the post- to pretest probability ratio decreases towards a value that is the inverse of the increase in disease risk in those with jaundice compared with the general population. This means that if the Gilbert genotype were completely penetrant (\( p = 1 \)), its presence would reduce the increased risk of liver or hemolytic disease found in someone with a high serum bilirubin back to the risk of someone in the general, unselected population.

We assume that Gilbert's is recessive for the alteration in the promoter sequence for UDPGT. Our findings would be equally relevant if the abnormal genotype were dominant, with the heterozygous state able to cause symptoms.

There has been debate about what the genetic defect is in Gilbert's syndrome. Koiwai et al. (5) suggest that Gilbert's syndrome is caused by heterozygosity for the mutation that causes Crigler-Najjar in the homozygous state. The frequency of these mutations is likely to be less than that of the promoter mutations, although the genetic loci could interact to give heterogeneous phenotypes. Only 1 of the 77 people Monaghan et al. (2) studied had jaundice without signs of liver or hemolytic disorders or the promoter mutation, and may have been heterozygous for the Crigler-Najjar mutation. If such a mutation were included as part of the Gilbert genotype, the calculations remain as above. Otherwise, the predictive value of testing for the promoter mutation alone decreases as the prevalence of other genotypes that are not tested for increases.

Evidence suggests that the prevalence of the homozygous UDPGT promoter mutation genotype is \( \sim 12\% \) (2, 4). It is evident that far less than this proportion of the population present with unexplained jaundice, that is, the penetrance seems to be well below 100%. A low penetrance does, however, call into question the role of the polymorphism in the mechanism of Gilbert's syndrome. We chose an arbitrary value for penetrance of 10%. It is known that the bilirubin concentration may rise above reference values only when the individual fasts. The prevalence of liver or hemolytic disease is difficult to estimate, and is certainly higher in the selected population whose blood samples arrive for testing in the clinical chemistry laboratory than in the population at large.

For a positive Gilbert genotype to produce a fivefold reduction of risk of harmful disease being the cause of
jaundice, D, the prevalence of harmful disease needs to be <1.5%, with values of 12% for G, frequency of the Gilbert genotype, and 10% for p, penetrance being assumed (Fig. 4). The usefulness of a positive Gilbert genotype result therefore highly depends on the prevalence of the benign and pathological conditions and on the penetrance of the benign genotype in the cohort studied. Unless these values can be ascertained, even approximately, it is impossible to deduce how much reassurance can be provided by the presence of the Gilbert genotype, and hence, how safe it is not to carry out the usual clinical investigations for liver or hemolytic disease.

One particular area that may benefit from this type of analysis is transfusion medicine. A recent report suggests that a considerable amount of icteric plasma, which is most likely to be the product of Gilbert’s syndrome, is rejected under suspicion of undetected hepatitis virus contamination (6). All of the donors of icteric plasma in this study had strong biochemical evidence of Gilbert’s syndrome. The data we present here suggest that only if the Gilbert gene was 100% penetrant would a genetic test reduce the risk of icteric plasma to that of non-icteric plasma. If, as appears to be the case here, the genotype is not always expressed, then the risk of liver disease is still higher in someone with icteric plasma than in someone without icteric plasma, even if that individual is shown to carry the Gilbert genotype. The efficiency of this test would also be absolutely dependent on the prevalence of occult viral contamination in all donors.

The results here are applicable to any other disease where symptoms may result from a benign variant of normal. If the benign and the disease condition cannot co-exist, then the presence of the benign cause excludes disease, with a likelihood ratio for disease of a positive test being zero. Such a result would be modified if the test were a genetic one, where penetrance is <100%, although in this case, the benign cause and the harmful disease are less likely to be mutually exclusive. If the benign variant and the disease can co-exist, as is the case for liver/hemolytic disease and Gilbert’s syndrome, then a positive test for the benign cause is less effective in excluding the disease as a cause of symptoms. This is an important consideration, given an increasing reliance on genetic testing and given the increasing number of polymorphisms being identified.

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References