The invention provides a method for improving the efficacy of drug trials, the method comprising the step of screening samples from potential participants for the genetic basis of Gilbert’s Syndrome and eliminating or including potential participants in a drug trial in the knowledge of them possessing or not possessing the genetic basis of Gilbert’s Syndrome.
### Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

<table>
<thead>
<tr>
<th>AM</th>
<th>Armenia</th>
<th>GB</th>
<th>United Kingdom</th>
<th>MW</th>
<th>Malawi</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT</td>
<td>Austria</td>
<td>GE</td>
<td>Georgia</td>
<td>MX</td>
<td>Mexico</td>
</tr>
<tr>
<td>AU</td>
<td>Australia</td>
<td>GN</td>
<td>Guinea</td>
<td>NE</td>
<td>Niger</td>
</tr>
<tr>
<td>BB</td>
<td>Barbados</td>
<td>GR</td>
<td>Greece</td>
<td>NL</td>
<td>Netherlands</td>
</tr>
<tr>
<td>BE</td>
<td>Belgium</td>
<td>HU</td>
<td>Hungary</td>
<td>NO</td>
<td>Norway</td>
</tr>
<tr>
<td>BF</td>
<td>Burkina Faso</td>
<td>IE</td>
<td>Ireland</td>
<td>NZ</td>
<td>New Zealand</td>
</tr>
<tr>
<td>BG</td>
<td>Bulgaria</td>
<td>IT</td>
<td>Italy</td>
<td>PL</td>
<td>Poland</td>
</tr>
<tr>
<td>BJ</td>
<td>Benin</td>
<td>JP</td>
<td>Japan</td>
<td>PT</td>
<td>Portugal</td>
</tr>
<tr>
<td>BR</td>
<td>Brazil</td>
<td>KE</td>
<td>Kenya</td>
<td>RO</td>
<td>Romania</td>
</tr>
<tr>
<td>BY</td>
<td>Belarus</td>
<td>KG</td>
<td>Kyrgyzstan</td>
<td>RU</td>
<td>Russian Federation</td>
</tr>
<tr>
<td>CA</td>
<td>Canada</td>
<td>KP</td>
<td>Democratic People's Republic of Korea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF</td>
<td>Central African Republic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>Congo</td>
<td>KR</td>
<td>Republic of Korea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CH</td>
<td>Switzerland</td>
<td>KZ</td>
<td>Kazakhstan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>Côte d'Ivoire</td>
<td>LI</td>
<td>Liechtenstein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CM</td>
<td>Cameroon</td>
<td>LK</td>
<td>Sri Lanka</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CN</td>
<td>China</td>
<td>LR</td>
<td>Liberia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>Czechoslovakia</td>
<td>LT</td>
<td>Lithuania</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CZ</td>
<td>Czech Republic</td>
<td>LU</td>
<td>Luxembourg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DE</td>
<td>Germany</td>
<td>LV</td>
<td>Latvia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DK</td>
<td>Denmark</td>
<td>MC</td>
<td>Monaco</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EE</td>
<td>Estonia</td>
<td>MD</td>
<td>Republic of Moldova</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>Spain</td>
<td>MG</td>
<td>Madagascar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FI</td>
<td>Finland</td>
<td>ML</td>
<td>Mali</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FR</td>
<td>France</td>
<td>MN</td>
<td>Mongolia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>Gabon</td>
<td>MR</td>
<td>Mauritania</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Notes
- Codes are used for identification purposes.
- States party to the PCT are listed as of the last update.
- Codes are international and may vary by publication.

---

**FOR THE PURPOSES OF INFORMATION ONLY**

- States and countries listed may change over time.
- Updates and corrections are possible.
- Always refer to the official sources for the most current information.
"Drug Trial Assay System"

The present invention relates to drug trials, usually carried out for or on behalf of pharmaceutical companies. More particularly the invention relates to a method for improving the efficacy of drug trials.

In the different stages of drug trials, regulatory authorities in different European countries and the FDA in the USA require extensive data to be provided in order to approve use of the drugs.

It is important that as much information as possible is available in relation to all participants who take part in drug trials, from volunteers who take part in phase 1 trials to patients involved in stage 3 clinical trials.

In particular, if certain individuals or groups of individuals have severe or abnormal reactions to drug administration, further studies involving that drug will be in jeopardy unless the reason for the reaction is realised.

The knowledge of pharmacogenetics can play an important
role in understanding the impact of drug metabolism on
pharmacokinetics, role of receptor variants in drug
response and in the selection of patient populations
for clinical studies.

Considerable effort has been expended in attempting to
identify the pharmacogenetic basis of idiosyncratic
adverse drug reactions, particularly hypersensitivity
reactions. While there is clear evidence for
pharmacogenetic influence on susceptibility to
hypersensitivity reactions, necessary and sufficient
pharmacogenetic defects have not been identified.

The clinical implications of genetic polymorphism in
drug metabolism have been studied extensively (See
424).

Gilbert's Syndrome (GS) is a benign unconjugated
hyperbilirubinaemia occurring in the absence of
structural liver disease and overt haemolysis and
characterized by episodes of mild intermittent
jaundice. It is part of a spectrum of familial
unconjugated hyperbilirubinaemias including the more
severe Crigler-Najjar (CN) syndromes (types 1 and 2).
GS is the most common inherited disorder of hepatic
bilirubin metabolism occurring in 2-12% of the
population and is often detected in adulthood through
routine screening blood tests or the fasting associated
with surgery/intercurrent illness which unmasks the
hyperbilirubinaemia\(^1\). The most consistent feature in
GS is a deficiency in bilirubin glucuronidation but
altered metabolism of drugs has also been reported\(^5\).
Altered rates of bilirubin production, hepatic haem
production and altered hepatic uptake of bilirubin have
been reported in some GS patients\(^2\).
Due to the benign nature of the syndrome and its prevalence in the population it may be more appropriate to consider GS as a normal genetic variant exhibiting a reduced bilirubin glucuronidation capacity (which in certain situations such as fasting, illness or administration of drugs) could precipitate jaundice.

In drug trials where high levels of serum total bilirubin is detected for certain individuals, it is not clear whether this is because the individuals have Gilbert’s Syndrome or if it because of an effect of the drug. Whereas presently, results are explained merely by saying that the individuals have Gilbert’s Syndrome, it is suspected that in the future, it will be necessary to prove this fact.

Where a jaundiced phenotype is apparent after volunteers have been accepted for a trial and have been subjected to five days of a strict diet, no alcohol and no smoking, the jaundiced appearance giving an indication that the individuals have Gilbert’s Syndrome, may cause them to be ruled out of the trials. Therefore, where approximately 250 individuals would be required for phase 1 trials and about 6000 patients for phase 3 trials, unnecessary time and effort would have been spent during the first 5 days of these trials and individuals having Gilbert’s Syndrome may be ill effected.

The present invention aims to provide a method of improving the efficacy of drug trials in view of the problems mentioned above.

According to the present invention there is provided a method for improving the efficacy of drug trials, the method comprising the step of screening samples from
individuals for the genetic basis of Gilbert's Syndrome.

In a preferred embodiment of the invention the method comprises the steps taking a sample from each potential participant in a drug trial, screening the samples for the genetic basis of Gilbert's Syndrome, identifying participants having the genetic basis of Gilbert's Syndrome.

The sample may comprise blood, a buccal smear or any other sample containing DNA from the individual to be tested.

In one embodiment the method comprises the further step of eliminating participants having the genetic basis of Gilbert's Syndrome from the drug trial.

In an alternative embodiment, the method can comprise the further step of selecting participants having the genetic basis of Gilbert's syndrome and eliminating others from the drug trial.

In a further alternative the results of the drug trials can be interpreted in the knowledge that certain participants have Gilbert's Syndrome.

Preferably the method comprises the steps of isolating DNA from each sample, amplifying the DNA in a region indicating the genetic basis of Gilbert's Syndrome, isolating amplified DNA fragments by gel electrophoresis and identifying individuals having the genetic basis of Gilbert's disease.

Preferably the DNA is amplified using the polymerase chain reaction (PCR) using a radioactively labelled
pair of nucleotide primers.

The primers are designed to prime the amplification reaction at either side of an area of the genome known to be associated with Gilbert’s Syndrome.

Preferably the DNA region indicating the genetic basis of Gilbert’s Syndrome is the gene encoding UDP-glucuronosyltransferase (UGT).

By gene is meant, the non coding and coding regions and the upstream and downstream noncoding regions.

In a preferred embodiment the DNA to be amplified is in an upstream promoter region of the UGT1*1 exon1.

Most preferably the DNA to be amplified includes the region between -35 and -55 nucleotides at the 5’ end of UGT1*1 exon.

According to the invention there are provided suitable primers for use in a PCR reaction including primer pairs;

A/B (A, 5’-AAGTGAACCTCCCTGCTACCTT-3’,
B, 5’-CCACTGGGATCAACAGTATCT-3’) or
C/D (C, 5’-GTCACGTGACACAGTCAACAC-3’;
D 5’-TTTGCTCCTGCCAGGTT-3’)

The invention further comprises a kit for screening individuals for participation in drug trials, the kit comprising primers for amplifying DNA in a region of the genome indicating the genetic basis of Gilbert’s Syndrome.
Using primer sequences as described herein, DNA can be
amplified and analysed using among others any of the
following protocols;

Protocol 1 Radioactive method

1. Extract DNA from Buccal Cells or 3ml Blood.

2. Choose primers from either side of the "TATA" box
region of UGT1*1 exon1 regulatory sequence.
Freshly end label one primer with [γ $^{32}$α]-ATP (40
min).

3. Amplifying a small region up to 100 bp in length
by PCR (2h).

4. Apply to 6% PAG denaturing gel (preparation,
loading, run time, 4h).

5. Expose (-70°C) wet gel to autoradiographic film
(15 min).

This method takes about 7h to complete. Polymorphisms
only observed in TATA box non coding region to date.

Protocol 2
Alternative Radioactive Method: Solid Phase
Minisequencing

1. Extract DNA (as above)

2. Prepare primers biotinylating one

3. Amplify DNA by PCR using primers
4. Captive biotinylated PCR products on streptavidin coated support and deactive.

5. Carry out primer extension reaction sequencing.

Protocol 3
Non-Radioactive Methods:

(a) Analysis by Single Strand Conformational Polymorphism (SSCP)

1. Extract DNA (as above).

2. Choose primers either side of the TATA Box.

3. Amplify a small region up to 100 bp in length by PCR (2H).

4. Denature and place on ice (15 min).

5. Load onto a non-denaturing PAG gel, (preparation/load/run time, 4h).

6. Stain with Ethidium bromide or silver nitrate (30 mm).

This method still takes about 7h to complete, but is potentially slightly cheaper since there is no radioactivity or autoradiography.

This method could be done on an automated DNA sequencer from stage 5, if primers are tagged with chromophores in PCR stages 2 and 3. Result would then be read automatically.

(b) Oligonucleotide Assay Hybridization

1. Extract DNA (as above).
2. Choose primers and amplify DNA by PCR up to 100 bp in length.

3. Apply DNA to plastic grids.

4. Screen bound DNA samples with specific DNA probes for TA₅, TA₆, TA₇ tagged with different coloured/fluorescent chromophores.

5. Read output automatically for experimental protocols.

References


The basis of the invention is illustrated in the following example with reference to the accompanying figures wherein:

Figure 1 illustrates genotypes at the TATA box sequence upstream of the UGT1*1 exon 1 determined by direct sequencing and radioactive PCR.

Figure 2 illustrates serum total bilirubin (μmol/l) plotted against UGT1*1 exon 1 genotype.

Figure 3 illustrates segregation of the 7/7 genotype with elevated serum total bilirubin concentration in a family with GS.

Figure 4 illustrates the 5′ sequence of the UGT1*1 exon 1 and the position of the primers with respect to the UGT gene.

Example

We have examined the variation in the serum total bilirubin (STB) concentration in a representative group of the Eastern Scottish population (drug-free, alcohol-free non-smokers) in relation to genotype at the UDP-glucuronosyltransferase subfamily 1 (UGT1) locus. Subjects with the 77/7 genotype in this population have a significantly higher STB than those with 6/7 or 6/6 genotypes. Of 14 control subjects who underwent a 24 hour fast to establish whether they had Gilbert Syndrome (GS), only 7/77 subjects had GS. In addition, one confirmed GS patient, two recurrent jaundice patients and 9 clinically diagnosed GS patients had the 7/7 genotype. Segregation of the 7/7 genotype with elevated STB concentration has also been demonstrated in a family of 4 Gilbert members. This incidence of
the 7/7 genotype in the population is 10-13%. Here, we
demonstrate a correlation between variation in the
human STB concentration and genotype at a TATA sequence
upstream of the UGT1*1 exon 1 and that the 7/7 genotype
is diagnostic for GS.

The inheritance of GS has been described as autosomal
dominant or autosomal dominant with incomplete
penetrance based on biochemical analysis. More recent
reports have suggested that the mildly affected
(Gilbert) members of families in which CN type 2 (CN-2)
occurs are heterozygous for mutations in the UDP-
glucuronosyltransferase subfamily 1 (UGT1) gene which
cause CN-2 in the homozygous state. The inheritance of
GS in these families is autosomal dominant while CN-2
is autosomal recessive. However, the incidence of
CN-2 in the population is very rare and the frequency
of alleles causing CN-2 would not be sufficient to
explain the population incidence of GS.

An abstract by Bosma et al suggested a correlation
between homozygosity for a 2bp insertion in the TATA
box upstream of UGT1*1 exon 1 and GS (no mutations were
found in the coding sequence of the UGT1*1 gene). In
this report we demonstrate that the primary genetic
factor contributing to the variation in the serum total
bilirubin (STB) concentration in the Eastern Scottish
population is the sequence variation reported by Bosma
et al. In addition, we show that the 7/77 genotype is
associated with GS and occurs in 10-13% of the
population.

Methods
Patients and Controls
Whole blood (3ml) was collected into EDTA(K3)
Vacutainer tubes (Becton Dickinson) from one confirmed
male Gilbert patient (diagnosed following a 48 hour restricted diet\textsuperscript{13}), two female patients with recurrent jaundice/associated elevated STB (29-42 \(\mu\text{mol/l}\)) and 9 (1 female, 8 male) clinically diagnosed GS subjects (persistent elevation of the STB amidst normal liver function tests.) The patients were aged 22-45 years.

77 non-smoking residents selected at random from the Tayside/Pife region of Scotland (39 females aged 19-58 years, mean 32.41\(\pm\) 10.94; 38 males aged 23-57, means 35.58 \(\pm\) 9.04) participated in this study. Whole blood (9ml) was collected 8-10am into EDTA(K3) Vacutainer tubes (Becton Dickinson) for DNA extraction and SST Vacutainer tubes (Becton Dickinson) for biochemical investigations. The subjects had not taken any medication or alcohol in the previous 5-7 days and had fasted overnight (12 hours). 14 controls subsequently underwent further biochemical tests (following a 3 day abstinence from alcohol) before and after a 24 hour 400-calorie diet\textsuperscript{14} to determine if they had GS. All patients/controls were fully informed of the study and gave consent for their blood to be used in this study.

Biochemistry and DNA Extraction

The following biochemical tests were performed on control blood samples; alanine aminotransferase, albumin, alkaline phosphatase, amylase, STB, cholesterol, creatinine, creatine kinase, free thyroxine, gamma-glutamyl-transferase, glucose, HDL-cholesterol, HDL-cholesterol/total cholesterol, iron, lactate dehydrogenase, percentage of saturated transferrin (PSAT), proteins, serum angiotensin converting enzyme, thyroid stimulating hormone, transferrin, triglycerides, urate, urea. 14 controls also had pre- and post-fasting (24 hour) alanine
aminotransferase, albumin, alkaline phosphatase, STB and urate measured. DNA was prepared using the Nucleon II Genomic DNA Extraction Kit (Scotlab) according to manufacturer's instructions.

Genotyping

Polymerase Chain Reaction

Primer pairs A/B (A, 5'-AAGTGAACTCCCTGCTACCTT-3'; B, 5'-CCACTGGGATCAACAGTATCT-3') or C/D (C, 5'-GTCACGTGACACAGTCAAAC-3'; D, 5'-TTTGTCTCGTCCAGAGGTT-3') flanking the TATA box sequence upstream of the UGT1*1 exon 1 were used to amplify fragments of 253-255bp and 98-100bp, respectively. Amplifications (50μl) were performed in 0.2mM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP), 50mM KCl, 10mM Tris.HCl (pH 9.0 at 25°C), 0.1% Triton X-100, 1.5mM MgCl2, 0.25μM of each primer, 1 Unit of Taq Polymerase (Promega) and human DNA (0.25-0.5μg). The polymerase chain reaction (PCR) conditions using the Perkin-Elmer Cetus DNA Thermal Cycler were: 95°C 5 min followed by 30 cycles of 95° 30 sec, 58°C 40 sec, 72°C40 sec.

Direct Sequencing

Amplification was confirmed prior to direct sequencing by agarose gel electrophoresis. Sequencing was performed using [γ-32P]-dATP (NEN Dupont) with the USB Sequenase™ PCR Product Sequencing Kit according to manufacturer's instructions. Sequenced products were resolved on 6% denaturing polyacrylamide gels. The dried gels were exposed overnight to autoradiographic film prior to developing.

Radioactive PCR
Amplification was performed as above using primer pair C/D except that 2.5 pmol of primer C was radioactively 5' end-labelled with 2.5μCi of [γ-³²P]-ATP (NEN Dupont) prior to amplification. Products were resolved on 6% denaturing polyacrylamide gels and the wet gels exposed to autoradiographic film (-70°C 15 min) and the autoradiographs developed.

Statistics

A t-test was used to determine if there was a significant age difference between males and females. χ² analysis was used to assess any difference in the distribution of the 6/6, 6/7 and 7/7 genotypes in males and females and also to determine if the 7/7 subjects from the 24 hour fasted group had STB elevated into the range diagnostic for GS⁴. An analysis of variance was performed to compare mean STB in males and females within each genotype group. A non-parametric test, the Mann-Whitney U-Wilcoxon Rank Sum W Test was used to determine whether there was a significant difference in mean STB between males and females (irrespective of genotype). Correlations and significance tests were performed for STB versus PSAT and STB versus iron. A probability (p) of ( 0.05 was accepted as significant.

Results

In Figure 1 a photographic representation of the sense DNA sequences obtained by PCR/direct sequencing of DNA samples having the genotypes 6/6, 6/7 and 7/7 is shown. The common allele, (TA)₆TAA, is denoted by "6" while the rarer allele, (TA)₇TAA, is denoted by "7". Below each sequence is an overexposed photographic representation of the 98 to 100bp resolved fragments amplified using primer pair C/D which flank the TATA sequence upstream
of the UGT1*1 exon 1. The additional fragments of 99
and 101 bases are thought to be artifacts of the PCR
process where there is non specified addition of an
extra nucleotide to the 3' end of the amplified
product\textsuperscript{21}. Figures 1b illustrates results after testing
a range of unknown individuals.

In Figure 2 males (M) and females (F) are plotted
separately. Each circle/square represents the result
of a single control subject. The squares indicate the
14 controls who also underwent the 24 hour restricted
diet (see Methods). The filled circles/squares
represent those who had a lower than normal PSAT (≤
22%) while the half-tone circles represent those who
had a higher than normal PSAT (≥ 55%). The mean STB
concentrations (indicated by the horizontal lines) for
males were 13.24 ± 3.88 (6/6), 13.94 ± 6.1 (6/7)
including control h or 12.69 ± 3.34 excluding control
h, 29 ± 14.45 (7/7) and for females were 9 ± 3.62
(6/6), 12.2 ± 3.53 (6/7), 21.6 ± 7.8 7/7). The
encircled result is from control h (discussed in the
text).

In Figure 3 males and females are represented by
squares and circles, respectively. Filled and half-
filled circles/squares indicate the genotypes 7/7 and
6/7, respectively. The numbers in parentheses below
each member of the pedigree are the STB concentrations
measured after a 15 hour fast and 7 day abstinence from
alcohol. All family members were non smokers who were
not taking any medication when the biochemical tests
were performed. Elevated STB are underlined.
Individual members of each generation (I or II) are
denoted by the numbers 1-4 above each circle/square.
Generation III have not yet been tested.
There was no significant age difference between males and females (t = -1.38, p = 0.17). Genotypes were determined initially by amplification/sequencing and later by the radioactive PCR approach. Individuals homozygous for the common allele, heterozygous or homozygous for the rarer allele have the genotypes 6/6, 6/7 and 7/7, respective. 12 DNA samples (2 of 6/6, 3 of 6/7 and 4 of 7/7) were analysed by both methods and genotype results were identical (see Figure 1).

Genotype frequencies in male controls were 6/6 (44.74%), 6/7 (44.74%), 7/7 (10.52%) and in female controls were 6/6 (35.9%), 6/7 (51.3%), 7/7 (12.8%). There was no significant difference between the genotype proportions in the two groups ($\chi^2 = 0.6$ at 2 df, $p = 0.7$). Control h (encircled in Figure 2) had a STB which was 2.4 SD above the mean STB for that group (mean calculated including control h). The results for control h were repeatable and he is currently being investigated to exclude haemochromatosis. Comparison of mean STB in males and females revealed that females have a significantly lower concentration than males ($p = 0.031$ including control h; $p + 0.0458$ excluding control h).

There was a strong correlation between genotype and mean STB concentration within the control group ($p < 0.001$) irrespective of whether control h was included and there was a significant difference in mean STB between males and females of the same genotype ($p < 0.05$) irrespective of whether control h was included (see Figure 2). All patients studied had the 7/7 genotype.

Correlations between STB/PSAT ($r = 0.4113$, $p =$ 0.001)(see Figure 2) and STB/iron females ($p = 0.001$) than males ($p = 0.01$) but when control h is excluded there was no significant correlation in males.
The STB concentrations of control who underwent the 24 hour restricted diet (see Methods) are shown in Table 1. The normal fasting response is a small rise in the base-line STB (not exceeding a final concentration of 25μmol/l) most of which is unconjugated while GS patients have a lone biochemical feature a raised STB (>25μmol/l but <50μmol/l) most of which is unconjugated14. The 6/6 and 6/7 controls had post-fasting STB of ≤ 23μmol/l while all 7/7 controls were ≥ 31μmol/l. Other liver function tests were within acceptable ranges for the age and sex of the subjects. The 7/77 genotype correlates with a fasted STB (24 hour) within the range diagnostic for GS14 (p < 0.01)(see Table 1). In addition, the 7/7 genotype segregates with elevated STB concentration in a family with 4 GS members (Figures 3).

Table 1 shows a comparison of the UGT1*1 exon 1 genotype with elevation in the serum total bilirubin after a 24 hour 400-calorie restricted diet14.

An elevation of the fasting STB to a final concentration in the range 25-50μmol/l is considered to be diagnostic for GS14. The 7/7 subject denoted by * has a fasting and non-fasting STB of > 50μmol/l but this value is within a range considered by others to conform to a diagnosis of GS14.
Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Sex</th>
<th>Before</th>
<th>After</th>
<th>Fasting bilirubin &gt;25 &amp; &lt;50μmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/6</td>
<td>M</td>
<td>8</td>
<td>17</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>9</td>
<td>19</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>12</td>
<td>15</td>
<td>NO</td>
</tr>
<tr>
<td>6/7</td>
<td>F</td>
<td>8</td>
<td>17</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>9</td>
<td>13</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>11</td>
<td>12</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>17</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>8</td>
<td>10</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>15</td>
<td>23</td>
<td>NO</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>17</td>
<td>18</td>
<td>NO</td>
</tr>
<tr>
<td>7/7</td>
<td>F</td>
<td>9</td>
<td>34</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>12</td>
<td>34</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>19</td>
<td>31</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>62</td>
<td>96</td>
<td>NO*</td>
</tr>
</tbody>
</table>

Discussion

A few recent reports claim to have identified the genetic cause of GS\textsuperscript{10-12}. Clinical diagnosis of GS is often based on a consistent midly elevated non-fasting STB (>17 μmol/l) as the sole abnormal liver function test, intermittent jaundice or both. The diagnosis can be confirmed by elevation of the STB to 25-50μmol/l after a 24 hour 400-calorie diet\textsuperscript{14} or by elevation of the unconjugated bilirubin by > 90% within 48 hours of commencing a 400 calorie diet\textsuperscript{13}.

Sato's research group recently reported the occurrence of 7 different heterozygous missence mutations in unrelated Gilbert patients (most of the mutations have been found in the homozygous state in affected members of CN families), however, the non-fasted STB for these patients were > 52μmol/l (with the exception of one,
31\(\mu\)mol/l\(^{10,12}\). These non-fasted STB concentrations already exceed the diagnostic range for GS\(^{14}\), hence these patients have a more severe form of hyperbilirubinaemia than those studied in this report, while those in the Bosma et al\(^{12}\) abstract had STB concentrations similar to those studied here.

The example herein shows that the variation in the STB levels after an overnight fast (and in the absence of exposure to known inducers of the UGT1*1 isoform in GS, such as alcoholic\(^{15}\) and drugs\(^{16}\)) a representative group of the Eastern Scottish population is primarily due to (or associated with) the TATA box sequence variation reported by Bosma et al\(^{12}\). In agreement with previous work females have a significantly lower mean STB concentration than males\(^{17,18}\).

Individuals with the 7/7 genotype in the population have GS (see Table 1). One of the 7/7 controls indicated in Table 1 had a non-fasting STB similar to those reported for heterozygous carriers of CN-2 mutations\(^{7-11}\) which suggests that this subject may also be a carrier of a CN-2 mutation, alternatively, the very elevated bilirubin in this patient may be due to the coexistence of Reavon's Syndrome (characterized by a collection of abnormal biochemical results which are risk factors for coronary heart disease)\(^{19}\).

We have found that 10-13\% of the Eastern Scottish population have the genotype associated with mild GS. None of the Gilbert subjects from the control population were aware that they had an underlying metabolic defect in glucuronidation with testifies to its benign nature. Three 7/7 controls had STB concentrations comparable to mean levels observed in heterozygotes, however, they also had a lower than
normal PSAT (≤22%) (see Figure 2). The observed
correlation between STB and PSAT (p = 0.001) (Figure 2)
and STB and iron (females p = 0.001 and males p = 0.01
including control h) indicates that other genetic and
environmental factors affecting the serum PSAT and iron
values will in turn affect the STB concentration.

From the data presented here and previous reports it
seems clear that there are mild and more severe forms
of GS. The milder form (fasted STB 25-50μmol/l) is
either caused by (or is associated with) a homozygous
2bp insertion at the TATA sequence upstream of the
UGT1*1 exon 1 (autosomal recessive inheritance) while
the rarer more severe dominantly inherited forms
identified to date7-11 (non-fasted STB > 50μmol/l are due
to heterozygosity for a mutation in the coding region
of the UGT1*1 gene which in its homozygous state causes
CN-2. The particular genetic abnormality causing GS in
a patient will have implications for genetic
counselling as the dominantly inherited form of two GS
patients could result in offspring with CN-2, whereas
the recessive form in one or both GS patients would
have less serious implications. It is important to
discriminate between the two forms and provide suitable
genetic counselling for such couples. The rapid DNA
test presented here (less than 1 day for extracted DNA)
carried out in addition to biochemical tests following
a 12 hour overnight fast (without prior alcohol or drug
intake would permit such a diagnosis. The compliance
rate for the current 24 and 48 hour restricted diet
tests for GS13-14 is debatable and hence the overnight
fast has obvious advantages and only one blood sample
or a buccal smear is required (for genetic and
biochemical analysis) in contrast to the 2-3 blood
samplings required for the 24 and 48 hour tests. This
approach to GS testing would be cost effective in terms
of fewer patient return visits to clinics and in
identifying couples at risk of having children with
CN-2.

In addition, the recent finding of an increased
bioactivation of acetaminophen (a commonly used
analgesic which is eliminated primarily by
glucuronidation) in GS patients indicates the greater
potential for drug toxicity in these patients if
administered drugs which are also conjugated by UGT1
isoforms\(^{1}\). In fact, ethinylestradiol (EE2) has recently
been shown to be primarily glucuronidated by the UGT1*1
isoform in man\(^{20}\) and hence this could have implications
for female Gilbert patients taking the oral
contraceptive who are then more predisposed to
developing jaundice.

The tests outlined herein have obvious implications for
setting up drug trials in understanding unusual results
in ruling out individuals who may be adversely affected
by the drugs or in positively choosing these
individuals to determine the effects of particular
drugs on hyperbilirubinaemia.
References


14. Lascelles, P.T. and Donaldson, D. Calorie restriction test in "Diagnostic Function Tests in


CLAIMS

1. A method for improving the efficacy of drug trials, the method comprising the step of screening samples from potential participants for the genetic basis of Gilbert’s Syndrome and eliminating or including potential participants in a drug trial in the knowledge of them possessing or not possessing the genetic basis of Gilbert’s Syndrome.

2. A method as claimed in claim 1 comprising the steps of:

   a) taking a sample from each potential participant in a drug trial,
   
   b) screening the samples for the genetic basis of Gilbert’s Syndrome,
   
   c) identifying participants having the genetic basis of Gilbert’s Syndrome, and
   
   d) proceeding with drugs trials in the knowledge of participants possessing or not possessing the genetic basis of Gilbert’s Syndrome.

3. A method as claimed in claim 1 or 2 wherein the sample is chosen from blood, buccal smear or any other sample containing DNA from the potential participants.

4. A method as claimed in any of the preceding claims further comprising the step of eliminating participants having the genetic basis of Gilbert’s Syndrome from a drug trial.
5. A method as claimed in any of claims 1 to 3 wherein the method comprises the further step of selecting only participants having genetic basis for Gilbert's Syndrome for a drugs trial.

6. A method as claimed in any of claims 1 to 3 further comprising the step of interpreting the results of the drugs trial in the knowledge that certain participants have Gilbert's Syndrome.

7. A method as claimed in any of the preceding claims wherein the method comprises the steps of:
   a) isolating DNA from each sample,
   b) amplifying the DNA inner region indicating the genetic basis for Gilbert's Syndrome,
   c) isolating amplified DNA fragments, and
   d) identifying individuals having the genetic basis of Gilbert's Syndrome.

8. A method as claimed in any of the preceding claims wherein the DNA is amplified using the polymerase chain reaction (PCR) using a radioactively labelled pair of nucleotide primers.

10. A method as claimed in any of claims 7 to 9 wherein the DNA region indicating the genetic basis of Gilbert's Syndrome is the gene encoding UDP-glucuronosyltransferase (UGT).

11. A method as claimed in any of claims 7 to 10 wherein the DNA to be amplified is in an upstream promoter region of the UGT 1*1 exon 1.
12. A method as claimed in any of claims 7 to 11 wherein the DNA to be amplified includes the regions between -35 and -55 nucleotides at the 5' end of UGT 1*1 exon.

13. A kit for screening individuals participation in drug trials, the kit comprising primers for amplifying DNA in the region of the genome indicating the genetic basis of Gilbert's Syndrome.

14. Primers for use in a method as claimed in any of the preceding claims including primer pairs, AB or CD as follows:

A/B (A, 5'-AAGTGAACCTCCTGCTACCTT-3',
B, 5'-CCACTGGGATCAACAGTATCT-3') or C/D (C, 5'-GTCACGTGACACAGTCAAAC-3';
D 5'-TTTGCTCCTGCCAGGTT-3').
Serum total bilirubin (μmol/l) vs Genotype

Genotype

6/6 6/7 7/7

M F M F M F

Fen2
Pedigree Showing Segregation of the Gilbert Phenotype with the \((TA)_7TAA/(TA)_7TAA\) Genotype.

I, II, III - generations in family  
* = genetic and biochemical data available

- [ ] male
- [ ] female
- [ ] homozygotes for the \((TA)_7TAA\) allele
- [ ] heterozygotes for the \((TA)_7TAA\) and \((TA)_6TAA\) alleles

(13) = total serum bilirubin  
(18-25) = elevated total serum bilirubin
-611 GTGAGTCGCTGCTTCCATGGCGTGCTGTTGGTGGGCTCTGCAGCCTC
-541 GACACCACTGTGTGCTGGACTTAAATAATGTTGGACGAAGGAATGAAACACATGATA
-491 CAAGTGAGCGCCAGCTACCGGGGAGCTTGAGTGCGCACCTTACAGGTTTCCATG6C
-431 GAAAAGCCGGGCGAAGCTTGGTCTTTTCTTCTTCTAAAGCCTTTCTGT
-371 TTAATTTCTGGAAAGAAGAGCTAACCTGGCTTCACTACATGCGTCTCTGCTCTG
-311 AACACCTTGTGTGGTGGAAATATCTAATTTAATGGATCCTGTGAGTTCTGGAAAGTACTT
-251 TGGCTGTCTCATTCAAGAATGTTATGATTAAGTAAATCCAGCGCACTTCAACTGTGTT
-191 GCCTATTAAGAAACCTATAAAAGCCTCCACTTTTTATCTCTGAAGATGAATCCCTGTGCT
-131 ACCTTTGTGGACTGACAGCTTTTTATGTCACCGTGAACACGTCGAAACATTTAACTTGCGTG
-71 ATCGATTGCTTTTGGCCATATATATATATATATAGGAAGAGGCGAAGCTCTG6GACGGA
-11 GCAAAGGCGCCATG6CTG

**FIG. 4**

SUBSTITUTE SHEET (RULE 26)