Review Article

A systemic review of association between UDP glucuronosyltransferase family 1 member A1 (UGT1A1) polymorphisms in Gilbert's syndrome in Sickle Cell Disease

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Abstract

Gilbert's syndrome (GS) is a benign hereditary disorder of bilirubin metabolism due to a mutation in the UDP glucuronosyltransferase family 1 member A1 *(UGT1A1)* gene which results in hyperbilirubinaemia and related complications mainly cholelithiasis. It can be co-inherited along with sickle cell anaemia, thalassaemias and other haemoglobinopathies including glucose-6-phosphate dehydrogenase deficiency, hereditary spherocytosis and cystic fibrosis. More than 100 mutations have been reported in *UGT1A1* gene and the most common as insertion of extra (TA) nucleotides in the promoter region of TATA box. The more the number of TA repeats, the higher is the bilirubin levels. These mutations result in a 10%–35% reduction in the *UGT1A1* enzyme activity resulting in mild to moderate unconjugated hyperbilirubinaemia and related complications. For diagnosis the mode of inheritance is more important than testing in the patients. However; the inheritance pattern of GS differs in ethnicities. For early diagnosis to prevent worsening of the symptoms and for timely management one should be aware of the inheritance pattern in patient. In this systemic analysis we studied the association between complications in GS with the genotypes and complications. It was found that TA7/7 is more significant in GS with sickle cell disease (SCD) group when compared to healthy controls with 2.2% chances of having this genotype in GS with SCD than healthy controls. The significance of having TA7/7 genotype is similar in GS with SCD and α-thalasaemia group. However, there is a high recommendation to carry out multicentre studies and conduct meta-analyses for establishing universal recommendations.

Keywords: Gilbert's syndrome, haemoglobinopathies, *UGT1A1* polymorphisms

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INTRODUCTION

Sickle cell disease (SCD) an autosomal recessive (AR) genetic disorder has become a public health issue in most of countries. Its genetic defect is a single nucleotide mutation (GAG codon changing to GTG) of the β-globin gene, which results in glutamate (E/Glu) being substituted by valine (V/Val) at position 6 (E6V substitution). The disorder manifests as a multi-system disorder and patients show varying degrees of anemia due to sickling of red

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cells leading to hypoxia. These sickle shaped Red cells get stuck in the blood vessels and block the blood flow that causes acute pain crisis and may lead to target organ damage as chronic complication. There are various genotypes observed in patients viz. HbSS, HbSC, HbSD, HbSE, HbSO, HbSβ_0 thalassaemia and HbSβ^+ thalassaemia.[1]

Gilbert's syndrome (GS) is a benign hereditary disorder of bilirubin metabolism due to a mutation in the UDP glucuronosyltransferase family 1 member A1 *(UGT1A1)* gene which results in decreased activity of the bilirubin uridine diphosphate glucuronosyltransferase enzyme.[1,2] It is typically inherited as autosomal recessive (AR) and occasionally as autosomal dominant pattern depending on the type of mutation. It can be co-inherited along with SCD, thalassaemias, glucose-6-phosphate dehydrogenase (G6PD) deficiency, cystic fibrosis and hereditary spherocytosis (HS). Bilirubin is a toxic product, formed by the breakdown of haem moiety from red cells and haemoproteins. Free bilirubin is conjugated with glucuronic acid, converted into soluble form and eliminated from the body by UDP-glucuronosyltransferase 1A1 (UGT1A1), a liver enzyme responsible for glucuronidation of bilirubin. The normal range of serum bilirubin is 0.3–1.2 mg/dL.[2,3-6]

More than 100 mutations have been reported in *UGT1A1* gene which includes different replacement, deletion and insertion of amino acids, but the most common mutation is the insertion of extra (TA) nucleotides in the promoter region of the TATA box. The wild type genotype of the *UGT1A1* promoter TATA box contains six repetitions, A (TA) 6TAA, however, the (TA) repeats representing GS can vary between 5 and 8. The more the number of TA repeats, the higher is the bilirubin levels. These mutations result in a 10%–35% reduction in the UGT1A1 enzyme activity, resulting in mild/moderate unconjugated hyperbilirubinaemia (bilirubin levels vary between normal ranges to 6 mg/dL), jaundice and risk of gallstones.[3,5,7] Most patients with this syndrome are asymptomatic except for fatigue, abdominal pain, fat intolerance, tiredness, headaches, dizziness or depression and episodes of jaundice.[2,3,5,7,8]

Usually diagnosis of GS is done by testing unconjugated bilirubin on fasting and bilirubin levels are normal. Alternatively, testing of unconjugated bilirubin levels after the administration of 50 mg nicotinic acid by overnight fasting or calorie restriction of 400-kcal can be done. Later, unconjugated bilirubin is measured after 30–60 min for 5 h after the administration of nicotinic acid (or rifampicin/phenobarbital). Bilirubin levels are increased in patients with GS. At present, these classical tests are being replaced by molecular testing of *UGT1A1* through polymerase chain reaction to find the number of repeats and further predicting the manifestations.[4,9]

The mode of inheritance is more important in the patients. The inheritance pattern of GS differs depending on the ethnicities. It is important to find the co-inheritance pattern of GS in SCD patients and its most common genotypes to sketch a road of early diagnosis to prevent worsening of the symptoms and for timely management.

We, therefore, attempted a systemic review of previously published case–control studies to compare the genotypes of *UGT1A1* in SCD patients, thalassaemia compare with healthy controls to find the most common genotypes. Furthermore, we studied the association between complications in GS with the genotype to find the detection through clinical results.

MATERIAL AND METHODS

We conducted a systemic analysis of previously published different studies on GS in SCD associated with thalassaemia. For our literature search, we used keywords such as *UGT1A1*, Sickle Cell Disease and Gilbert's Syndrome. Eligible studies were retrieved from PubMed, Science Direct and Google Scholar databases. All the references related to GS, gallstone and hyperbilirubinaemia were included. Searching was limited to publications in English language with no restriction of publication period. The reference lists of all potentially eligible articles were searched through PubMed and Google scholar.

Published case–control and observational studies were included provided they met the following criteria: (i) contained the number of patients for the genotypes: TA6/6, TA6/7 and TA7/7; (ii) Included either GS patients with SCD or GS patients with SCD and α or β-thalassaemia; and (iii) reported complications and biological parameters related to GS including gallstones, total bilirubin levels, HbF and Hb levels.

The studies were excluded if the number of patients were not clearly differentiated according to genotype or haemoglobinopathies. Studies those included GS with any other mutations were also excluded.

Data extraction

For all shortlisted studies, the following data was extracted from the original publications: the place of research, year of publication, haemoglobinopathies, genotypes and the number of patients for each genotype, number of patients having gallstones according to genotype, total bilirubin

level (mg/dL), HbF (%) and Hb levels (g/dL). The process adopted for retrieval and selection of papers in this analysis is shown in Figure 1.

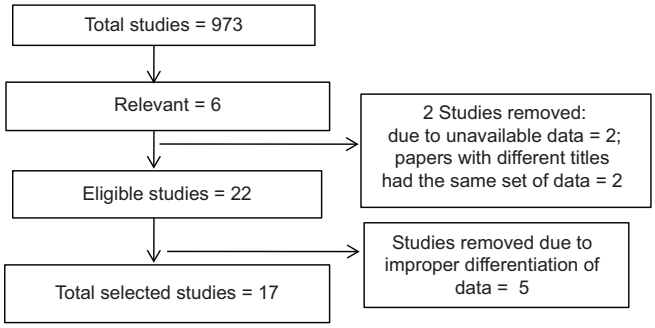

Figure 1: Flow diagram for literature searching

Statistical analysis

The significance of each genotype in GS with SCD was evaluated. The pooled estimate and corresponding 95% confidence interval (CI) limits were calculated based on random effect model to find the association between *UGT1A1* polymorphism with SCD and complications related to GS including gallstones, total bilirubin levels, HbF and Hb levels as per the genotypes.

To compare the significance of each genotype in different haemoglobinopathies, high-resolution forest plots were prepared to depict both odds ratio (OR) and 95% CI limits. By forest plot, we evaluated heterogeneity among studies using Q statistic and I^2 index, assuming that I^2 values of 25%, 50% and 75% represented as low, medium and high heterogeneity, respectively. We considered an I^2 value of >50% as indicative of substantial heterogeneity. Pooled estimate was calculated based on the fixed-effects model which was reported using the Woolf's (inverse variance) method. Publication bias was evaluated using Begg's funnel plot and Egger's test. $P < 0.05$ is considered to be statistically significant.

The analysis was performed using 'R' version 1.0.136 (RStudio Team [2019]). RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL (http://www.rstudio.com) and 'Meta' R package for (version 4.9-7).

OBSERVATIONS AND RESULTS

In our analysis, based on the inclusion criteria, number of patients and their complications were extracted from seventeen studies.[10-26] A total of 2387 GS phenotypes were included in this analysis, 1897 patients having GS with SCD, 95 patients having GS with SCD and α-thalassaemia, 97 patients having GS with SCD and β-thalassaemia and 350 healthy controls. Thus, the above-mentioned studies were divided into four arms for the analysis.

The numbers of patients extracted from each study are documented in Table 1[10-26] according to the genotype and haemoglobinopathies. For complications GS with SCD, out of 17 studies, 11 studies 533 patients having gallstones, 9 studies reported bilirubin levels, 6 studies reported HbF and Hb levels in different genotypes. The extracted data for same has been documented in Table 2.

Significance of *UGT1A1* polymorphism in Gilbert's syndrome with sickle cell disease and their complications

Incidence of the pooled estimate for TA6/6 in patients having GS with SCD had the pooled estimate of 16%

Table 1: Description of various studies and cases considered for comparison in different arms

Study[reference]	Year	Country	Number of Patients											
			Diseased Group: GS with SCD			Diseased Group: GS with SCD and α-thalassemia			Diseased Group: GS with SCD and β- thalassemia			Healthy Controls		
			TA6/TA6	TA6/TA7	TA7/TA7	TA6/TA6	TA6/TA7	TA7/TA7	TA6/TA6	TA6/TA7	TA7/TA7	TA6/TA6	TA6/TA7	TA7/TA7
Passon[10]	2001	USA	14	36	16	-	-	-	-	-	-	-	-	-
Carpenter[11]	2008	USA	81	121	41	-	-	-	-	-	-	-	-	-
Heeney[12]	2003	USA	17	24	18	-	-	-	-	-	-	-	-	-
Olatunya[13]	2019	Nigeria	25	31	22	-	-	-	-	-	-	5	21	4
Chaouch[14]	2013	Tunisia	40	31	21	-	-	-	-	-	-	30	31	11
Chaouch[15]	2015	Tunisia	26	35	9	-	-	-	-	-	-	-	-	-
Martins[16]	2008	African Portugal	19	36	14	18	24	15	-	-	-	-	-	-
Chaar[17]	2005	France	79	115	48	-	-	-	-	-	-	-	-	-
de Azevedo[18]	2017	Brazil	17	22	10	5	10	2	-	-	-	-	-	-
Pandey[18]	2012	India	9	15	26	-	-	-	11	21	38	32	53	10
Farheen[19]	2006	India	4	15	76	-	-	-	-	-	-	-	-	-
Italia[21]	2010	India	31	13	12	13	5	3	-	-	-	-	-	-
Haverfield[22]	2005	Jamica	136	178	106	-	-	-	-	-	-	-	-	-
AlFadhli[23]	2013	Kuwait	30	68	6	-	-	-	-	-	-	26	34	2
Kalotychou[24]	2013	Greece	-	-	-	-	-	-	12	9	6	18	12	7
Vasavda[25]	2007	UK	34	64	39	-	-	-	-	-	-	10	26	18
Adekile[26]	2005	USA	10	35	22	-	-	-	-	-	-	-	-	-

GS = Gilbert's syndrome; SCD = sickle cell disease

Table 2: No of patients with gallstones, total bilirubin levels, HbF and Hb levels in patients having GS with SCD

Study[reference]	No of patients: GS with SCD			No of patients: GS			Total bilirubin Levels (mg/dL)			HbF(%)			Hb (g/dL)		
	TA6/TA6	TA6/TA7	TA7/TA7	TA6/TA6	TA6/TA7	TA7/TA7	TA6/TA6	TA6/TA7	TA7/TA7	TA6/TA6	TA6/TA7	TA7/TA7	TA6/TA6	TA6/TA7	TA7/TA7
Passon[10]	14	36	16	5	13	14	24±0.8	3.0±1.1	5.8±3.1				8±0.8	8±0.6	7.9±1.0
Carpenter[11]	81	121	41	12	40	20	2.36±1.13	2.90±1.54	4.24±2.11						
Heeney[12]	17	24	18	7	12	16	2.5±1.0	3.2±1.5	6.1±4.1	7.0±4.4	8.8±5.3	8.5±4.9	7.8±1.1	8.3±0.9	7.6±0.9
Olatunya[13]	25	31	22	0	1	4	1.4 (0.4–3.8)	1.8 (0.8–4.6)	2.8 (1.2–8.1)	10.7 (2.5-32)	8.2 (1.7-24.4)	9.7 (1.3-20.6)	7.2 (6.2-10)	7.5 (6.3-9.7)	7.3 (6.3-10)
Chaouch[14]	40	31	21	10	15	20	0.88-1.99	0.88-5.85	2.05-5.85						
Chaouch[15]	26	35	9	10	26	8	0.88-1.99	0.88-5.26	2.05-5.26						
Martins[16]	19	36	14	11	23	13	2.55±1.78	3.20±3.14	4.67±3.67	8.33±5.27	10.03±6.39	8.46±5.19	7.88±0.92	7.93±1.37	8.40±1.11
Chaar[17]	79	115	48				1.77±0.76 and 2.63±0.99	3.27±1.99 and 4.03±2.04	5.90±2.57 and 6.54±2.28						
de Azevedo[18]	17	22	10	16	18	8	1.2–2.4	(1.6–3.1)	2.6–8.1	15.8 (±7.9)	14.8 (±7.6)	17.1 (±9.0)			
Pandey[18]	9	15	26	0	1	7	2.97±0.34	3.17±0.59	3.21±0.45						
Haverfield[22]	136	178	106	31	53	49									
AlFadhli[23]	30	68	6	12	52	6	1.76±0.77	3.39±1.54	10.45±0.2						
Adekile[26]	10	35	22				3.7±1.5	3.8±2.3	5.6±0.4	8.4±6.4	8.4±6.4	6.6±5.4	7.9±1.2	8.7±1.3	8.3±1.2
Italia[21]	31	13	12				2.3±1.2	3.4±2.3	4.2±1.7						

HbF = Foetal haemoglobin; Hb = Haemoglobin; GS = Gilbert's syndrome; SCD = sickle cell disease

(12%–21%) and complication of cholelithiasis had the pooled estimate of 28% (13%–50%) as shown in Figures 2a and 2b, respectively. The pooled mean of total bilirubin levels (mg/dL), HbF (%) and Hb (g/dL) at 95% CI was 3.68 (3.58–3.78), 10.50 (9.93–11.06) and 7.74 (7.33–8.54), respectively, in TA6/TA6 (Figures 2c, 2d and 2e.)

Incidence of the pooled estimate for TA6/7 in patients having GS with SCD had a pooled estimate of 25% (19%–31%) (Figure 3a). Incidence for cholelithiasis had the pooled estimate of 44% (26%–63%) (Figure 3b). The pooled mean of total bilirubin levels (mg/dL), HbF (%) and Hb (g/dL) was 3.28 (3.14–3.42), 10.27 (7.93–12.60) and 8.21 (7.90–8.53), respectively, in TA6/TA7 (Figures 3c, 3d and 3e).

Incidence of the pooled estimate for TA7/7 was 14% (10%–19%) in patients having GS with SCD (Figure 4a). The pooled estimate was 76% (53%–90%) for incidence of cholelithiasis (Figure 4b). For TA7/TA7 genotype, the pooled mean total bilirubin levels (mg/dL), HbF (%) and Hb (g/dL) were 6.50 (6.41–6.59), 9.23 (6.39–12.07), 8.02 (7.65–8.39), respectively (Figures 4c, 4d and 4e)

The analysis results for pooled estimated showed that TA6/7 was more significant in patients having GS with SCD (with OR 25% and range of 19%–31% at 95% CI). However, patients with TA7/7 had increased risk of cholelithiasis and so raised bilirubin levels whereas HbF levels were high in patients with TA6/6.

Comparison of different arms

Gilbert's syndrome with sickle cell disease versus healthy controls

The comparative analysis of five studies results indicated an overall pooled OR of 0.96 ($P = 0.850$) for subgroup analysis of TA6/TA6 (Table 3). The results concluded that there was no significant association between GS with SCD and healthy control in TA6/TA6, with heterogeneity ($I^2 = 51.4\%$, $P = 0.083$; Figure 5a). Whereas, in TA6/TA7, the overall pooled OR was 1.63 ($P = 0.067$). These results concluded that, there was no any significant association between GS with

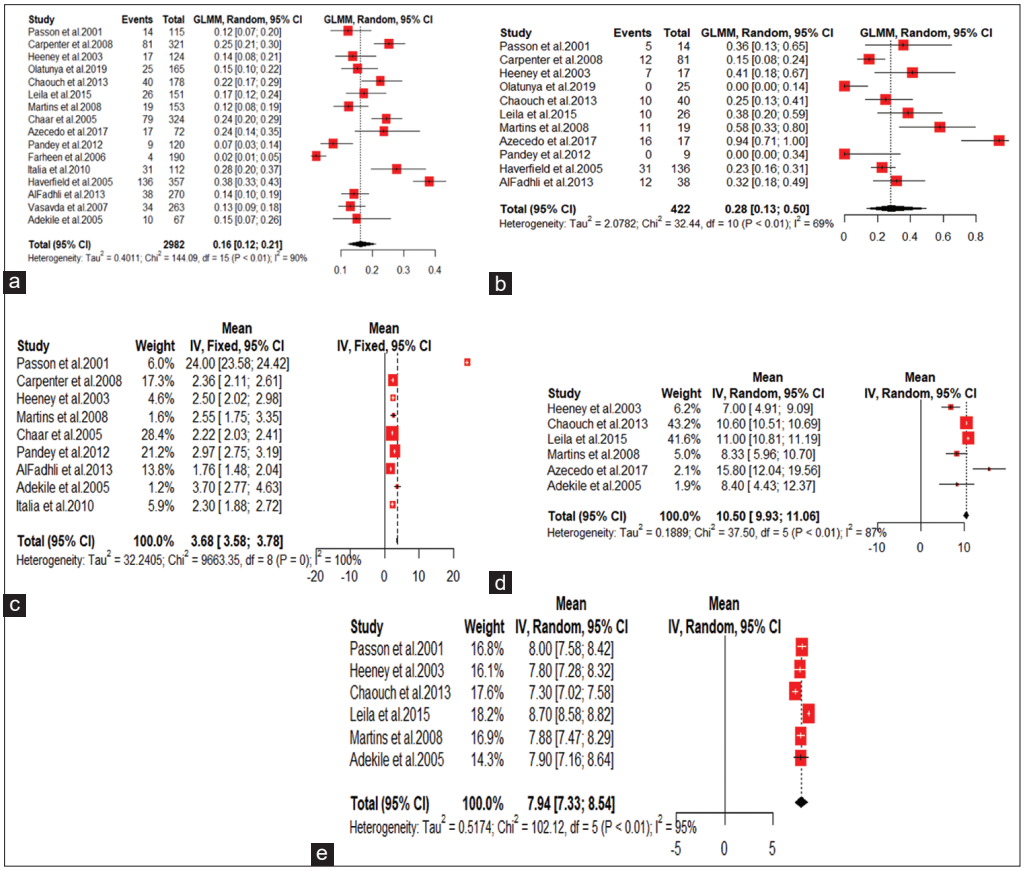

Figure 2: Forest plots for incidence of TA6/6 in Gilbert's syndrome with sickle cell disease (a), incidence of gallstone in TA6/6 (b), mean total bilirubin levels in TA6/6 (c), mean HbF levels in TA6/6 (d) and mean Hb levels in TA6/6 (e)
Hb = haemoglobin; CI = confidence intervals; GS = Gilbert's syndrome; SCD = Sickle cell disease; IV = Inverse-Variance weighting; GLMM = Generalised linear mixed model

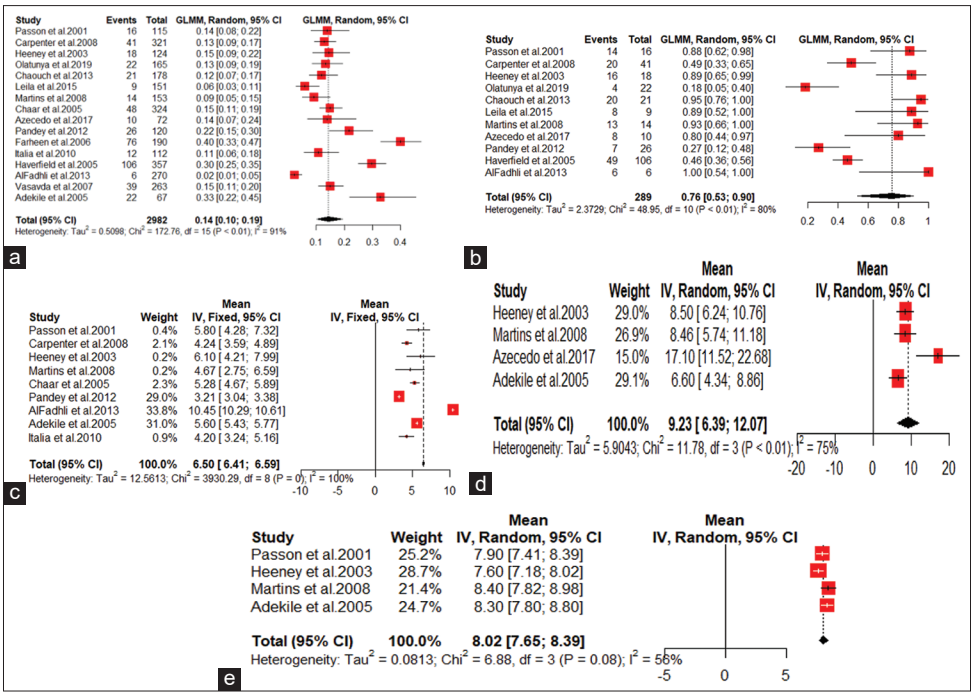

Figure 3: Forest plots for incidence of TA6/7 in GS with SCD (a), incidence of gallstone in TA6/7 (b), mean total bilirubin levels in TA6/7 (c), mean HbF levels in TA6/7 (d) and mean Hb levels in TA6/7 (e)
Hb = Haemoglobin; CI = Confidence intervals; GS = Gilbert's syndrome; SCD = Sickle cell disease; IV = Inverse Variance weighting; GLMM = Generalised linear mixed model

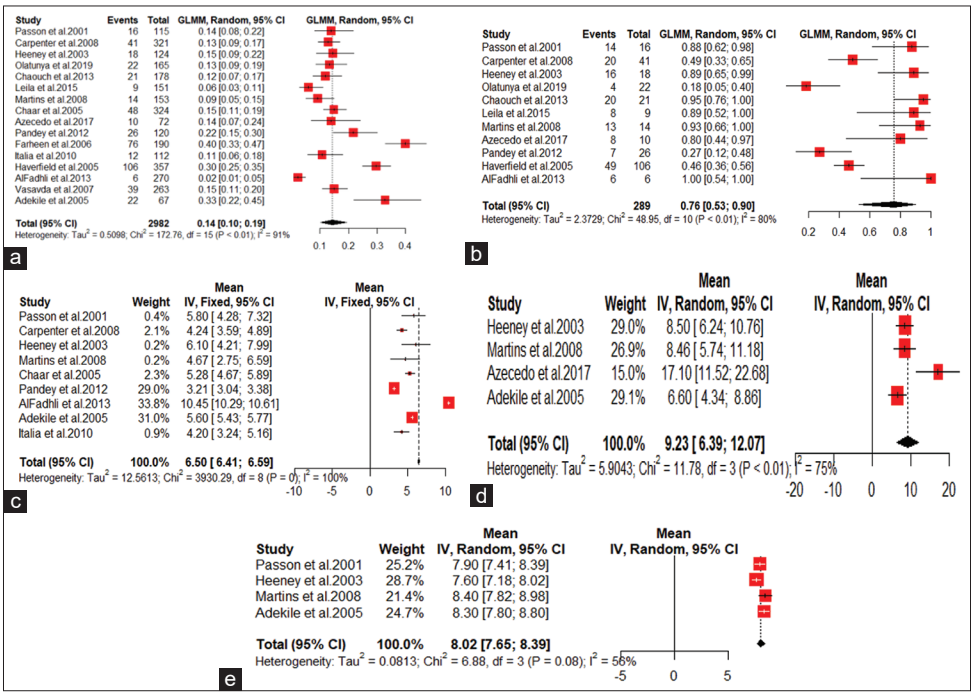

Figure 4: Forest plots for incidence of TA7/7 in GS with SCD (a), incidence of gallstones in TA7/7 (b), mean total bilirubin levels in TA7/7 (c), mean HbF levels in TA7/7 (d) and (e) mean Hb levels in TA7/7
Hb = haemoglobin; GS = Gilbert's syndrome; SCD = Sickle cell disease; IV = Inverse variance; GLMM = Generalised linear mixed model

SCD and healthy control in TA6/TA7, with significant heterogeneity (I^2 = 66.7%, P = 0.017; Figure 5b). No considerable publication bias was detected by Egger's tests and Begg's correlation test.

However, in TA7/TA7 group, the overall pooled OR was 2.18 (95% CI; 1.44–3.41, P = 0.006). These results concluded that, there was a significant association between GS with SCD and healthy control in TA7/TA7, with

Table 3: Statistical Analysis of Studies to compare genotypes between different arms

Comparisons	Group	Model Fitted	Pooled Odds ratio; 95% CI	*Z*	Fixed effect *P*	I^2	Test of heterogeneity	Linear regression test of funnel plot asymmetry	
								Egger's	Begg's
GS with SCD v/s Healty control	TA6/TA6	Random effect model	0.96 [0.63; 1.45]	-0.32	0.850	51.4% [0.0%; 81.1%]	0.083	0.624	0.547
	TA6/TA7	Random effect model	1.63 [0.39;1.03]	-2.47	0.067	66.7% [13.0%; 87.1%]	0.017	0.05	0.078
	TA7/TA7	Random effect model	2.18 [1.44; 3.41]	-2.47	0.001	79.1% [52%; 91.4%]	0.006	0.372	0.646
GS with SCD vs GS with SCD and αThal	TA6/TA6	Fixed effect model	0.88 [0.51; 1.52]	-0.45	0.655	0.0% [0.0%; 55.8%]	0.790	0.602	0.539
	TA6/TA7	Fixed effect model	1.10 [0.64; 1.87]	0.35	0.723	0.0% [0.0%; 90.1%]	0.348	0.601	0.348
	TA7/TA7	Fixed effect model	1.01 [0.52; 1.93]	0.02	0.984	0.0% [0.0%; 88.1%]	0.418	0.117	0.085

GS = Gilbert's syndrome; SCD = sickle cell disease

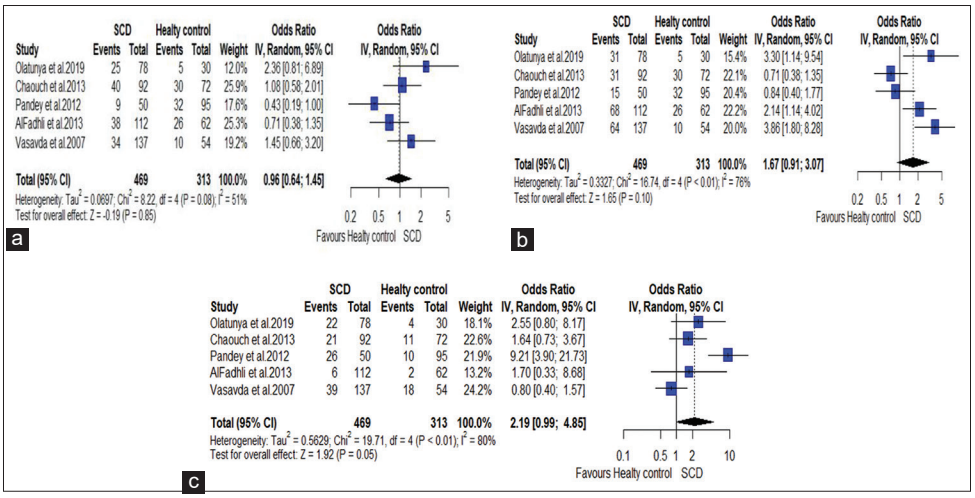

Figure 5: Comparison of studies between GS with SCD and Healthy control. (Forest plot using fixed effect model. Horizontal bars indicate the amount of variation (95% confidence intervals of the parameter estimates). Sizes of square indicate weight in the pooled effect size, (a) for TA6/6, (b) TA6/7 and (c) TA7/7)
SCD = Sickle cell disease; CI = Confidence intervals; IV = Inverse-variance weighting

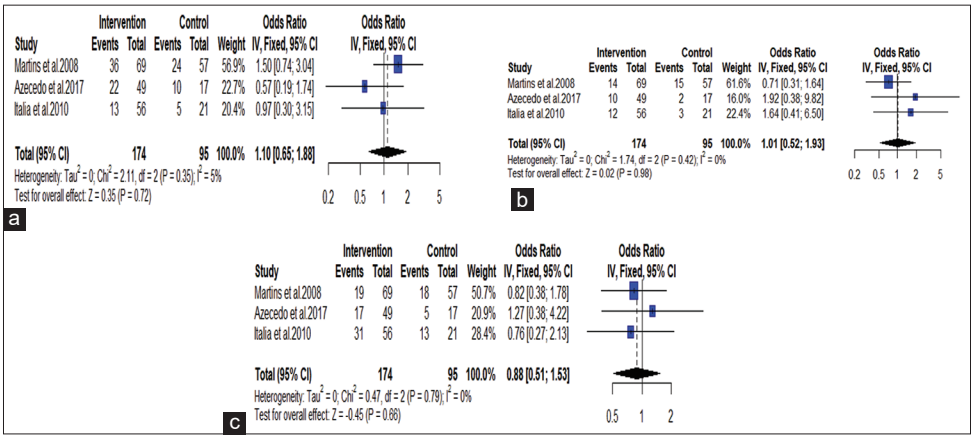

Figure 6: comparison of studies between GS with SCD and GS with SCD and α-thalassemia.
(Forest plot using fixed effect model. Horizontal bars indicate the amount of variation (95% confidence intervals of the parameter estimates). Sizes of square indicate weight in the pooled effect size, (a) for TA6/6, (b) TA6/7 and (c) TA7/7)
; IV = Inverse-Variance weighting

significant heterogeneity (I^2 = 79.1%, P = 0.006). No considerable publication bias detected by Egger's tests and Begg's correlation test (Figure 5c). Thus TA7/7 is more significant in GS with SCD group when compared to healthy controls. There are 2.18% chances of having this genotype in GS with SCD than healthy controls.

Gilbert's syndrome with sickle cell disease versus Gilbert's syndrome with sickle cell disease and α-thalassaemia

The analysis of three studies indicated an overall pooled OR 0.88 (P = 0.655) for TA6/TA6 with no significant difference between GS with SCD and GS with SCD and α-thalassaemia with less heterogeneity (I^2 = 0.0%, P = 0.790; Figure 6a).

In patients with TA6/TA7, the overall pooled OR 1.10 ($P = 0.723$) with no significant association between GS with SCD and GS with SCD and α-thalassaemia in TA6/TA7, with less significant heterogeneity ($I^2 = 0\%$, $P = 0.017$). No considerable publication bias was detected by Egger's tests and Begg's correlation test (Figure 6b).

However, in TA7/TA7 group, there was an overall pooled OR 1.01 ($P = 0.984$) with no significant association between GS with SCD and GS with SCD and α-thalassaemia in TA7/TA7, with significant heterogeneity ($I^2 = 0\%$, $P = 0.418$; Figure 6c). The significance of having TA7/7 genotype is similar in GS with SCD and GS with SCD and α-thalasaemia group.

DISCUSSION

Our systemic analysis on the *UGT1A1* genetic association with various complications of GS in SCD in 2387 patients having GS with SCD retrieved from 17 studies revealed that TA6/7 is more significant in patients having GS with SCD. However, patients having TA7/7 have increased risk of having high bilirubin levels and cholelithiasis.

From India, only three types of genotypes related DNA polymorphisms in the promoter region of *UGT1A1* have been reported: (TA) 6/(TA) 6, (TA) 6/(TA) 7 and (TA) 7/(TA) 7.[20,21] Other countries such as Africa and America have reported cases of five and eight TA repeats in the promoter region of *UGT1A1*.[27]

In the Slovenian population, no statistically significant difference was found in genotype frequencies between males and females ($P = 0.446$). A rare genotype (TA) 7/8 was observed in one Caucasian individual. On family analysis of this patient, it was seen that father had genotype (TA) 6/8 and was the carrier of the (TA) 8 allele. The sister had the genotype (TA) 6/8 and the two brothers had (TA) 7/8.[28]

In an article, three ethnic groups including Caucasians, Africans and Parkana Indians were studied. The homozygous and heterozygous genotypes were significantly different from the two former groups ($p = 0.0318$).[29] Another study[13] from Nigeria on a group of young SCA patients and healthy controls reported 10 genotypes including TA5/5, 5/6, 5/7, 5/8, 6/6, 6/7, 6/8, 7/7, 7/8 and 8/8. Genotype distributions of the patients and control group were not significantly different ($P = 0.09$ and $P = 0.22$, respectively) in them.[13]

The role of the TA repeats and the influence of some other relevant genetic modifiers, βS haplotype, α-thalassaemia and HbF levels were studied in patients with SCD. HbF had a significant negative linear correlation with serum bilirubin ($r = -0.304$, $P = 0.016$). There was a significant influence of the βS-globin haplotype and co-existing α-thalassaemia trait on serum bilirubin levels.[26]

TA insertion in the promoter of *UGT1A1* can be used as a molecular marker for GS is associated with hyperbilirubinaemia, β-thalassaemia, G6PD deficiency, HS, risk of gallbladder abnormalities and jaundice.

In a similar study[30] from Hyderabad, India the authors had scanned the incidence of gallbladder disease in Indian patients with GS. From 1191 GS patients, 106 patients had cholelithiasis, 18 patients had polyps and 17 had gallbladder wall thickening and were co-related with (TA) repeats and bilirubin levels. The risk of gallstone disease in males and females was 9.0% and 7.1%, respectively.[30]

A study[9] assessed the associated genotypes with the occurrence of cholelithiasis after adjustment of age, sex and body mass index (BMI, kg/m^2).[9] There was a significant difference observed in the distribution of the (TA) 7/(TA) 7 genotype between cases and controls ($p = 0.02$). Genotypes 6/7 and 7/7 show significant association (OR = 2.225, $P = 0.001$ and OR: 2.101, $P = 0.013$, respectively) with risk of cholelithiasis.[31] A Jamaican study[22] included the presence of (TA) 5/(TA) 8 repeats unlike in the Indian studies and co-related genotype with gallstone formation. Observed genotypes distributions of the three groups were not significantly different from the values expected under Hardy–Weinberg equilibrium. Patients with genotypes (TA) 7/(TA) 7 and (TA) 7/(TA) 8 were found to be at higher risk for gallstones ($P = 7.0 \times 10^{-4}$, $P = 0.005$ and $P = 0.03$).[22] A study[14] on children with SCD reported seven genotypes including (TA) 5/6, 6/6, 6/7, 7/7, 5/7, 7/8 and 8/8. In young children with SCD, (TA) 7/(TA) 7 and (TA) 7/(TA) 8 were significantly associated with gallstones ($P = 8.1 \times 10^{-8}$ and $P = 0.01$, respectively).

The impact of (TA) repeats on total bilirubin levels was proved in GS patients and healthy controls. In GS patients, the presence of (TA) repeats was associated with significantly increased bilirubin levels. In healthy controls, significant differences in bilirubin levels were obtained comparing individuals (TA) 6/(TA) 6 with (TA) 6/(TA) 7 ($P < 0.03$). Therefore, it was concluded that total bilirubin levels are dependent on the TA repeats in the TATA-box promoter region.[32]

A study[33] conducted in infants with genotypes (TA) 7/(TA) 7 showed a greater increase in jaundice index during the

first 2 days of life than (TA) 6/(TA) 6. No differences were observed between jaundice index increases of (TA) 6/(TA) 6 and (TA) 6/(TA) 7 subjects.[33] A case–control study[34] from Iran concluded no significant difference between TA repeats and jaundice and showed that (TA) 7/(TA) 7 mutations of GS were higher in infants with prolonged jaundice than infants without jaundice.

Our systemic analysis helps to predict complications in patients having GS with SCD. According to the genotype, patient's risk to gallstone and other complications can be predicted. However, for establishing uniform conclusions, there are high recommendations that many multicentre studies should be conducted to know the inheritance pattern of GS in SCD in different regions of world. Future studies concerning the impact of the (TA) repeats in *UGT1A1* on multifactor diseases such as gallbladder disorders, jaundice and cancer will help to value the individual risk and optimise therapy, leading to a decrease in the risk of side effects and improvement in the general condition of patients.

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Conflicts of interest

There are no conflicts of interest.

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