Supplementary data for *"Longer telomeres in chronic, moderate, unconjugated hyperbilirubinaemia: insights from a human study on Gilbert's Syndrome"*

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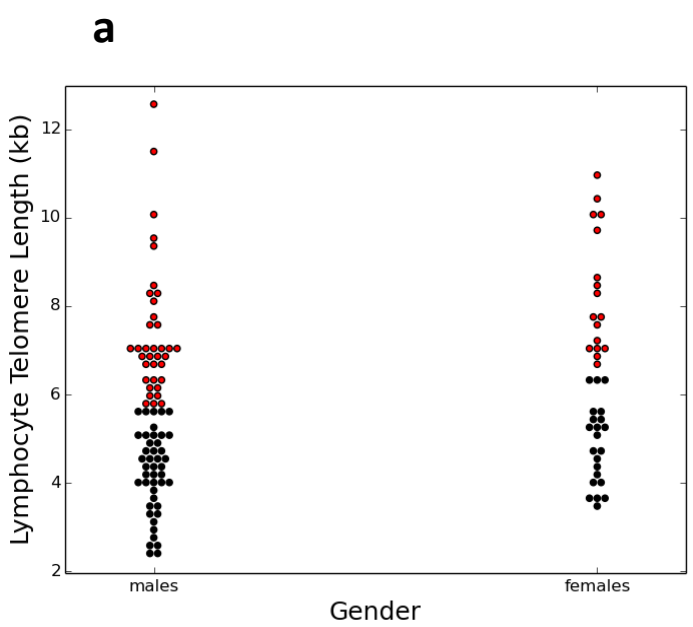
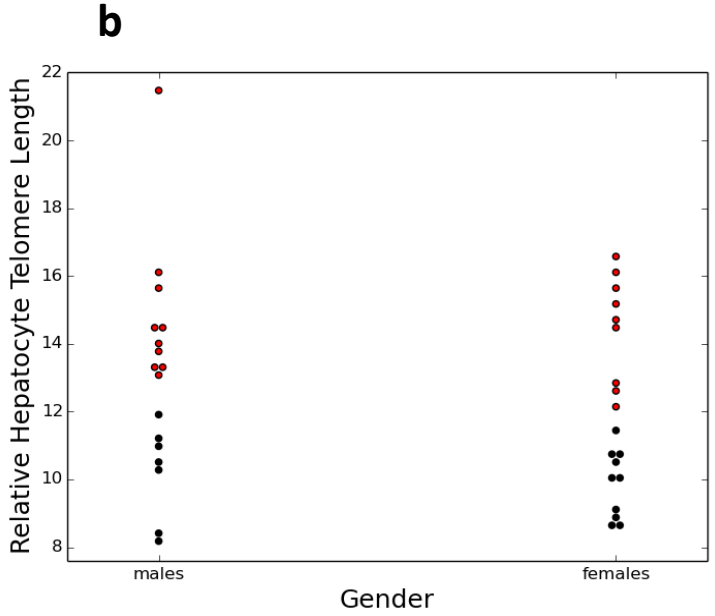
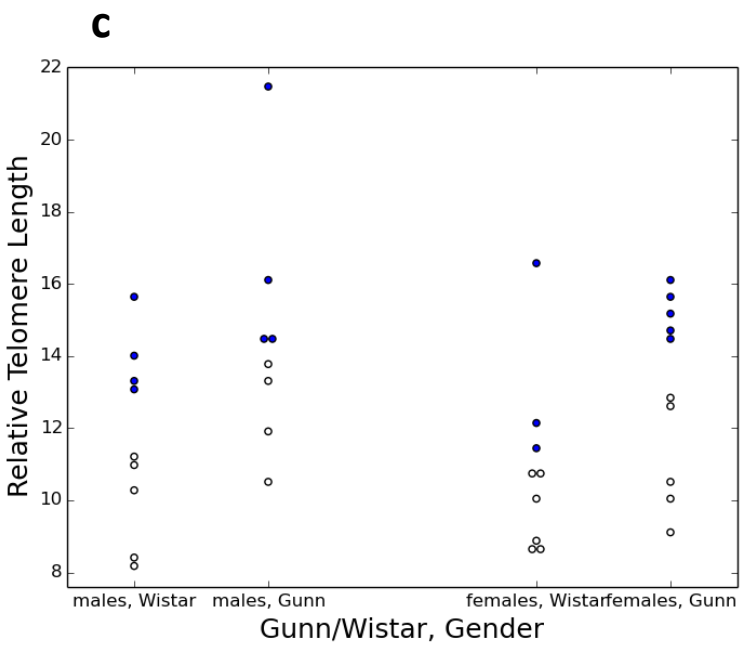
List of abbreviations:

BMI = Body mass index
BP = Blood pressure
CO-Hb = Carboxyhaemoglobin
CRP = C-reactive protein
FRAP = Ferric reducing ability of plasma
GSH/GSSG = Reduced/oxidized glutathione
HCY = Homocysteine
HOMA-IR = Homeostatic model assessment – insulin resistance
IL = Interleukin
MAP = Mean arterial pressure
MDA = Malondialdehyde
PLA2 = Phospholipase A2
SAA = Serum amyloid alpha
TL = Telomere Length
TNF = Tumor necrosis factor

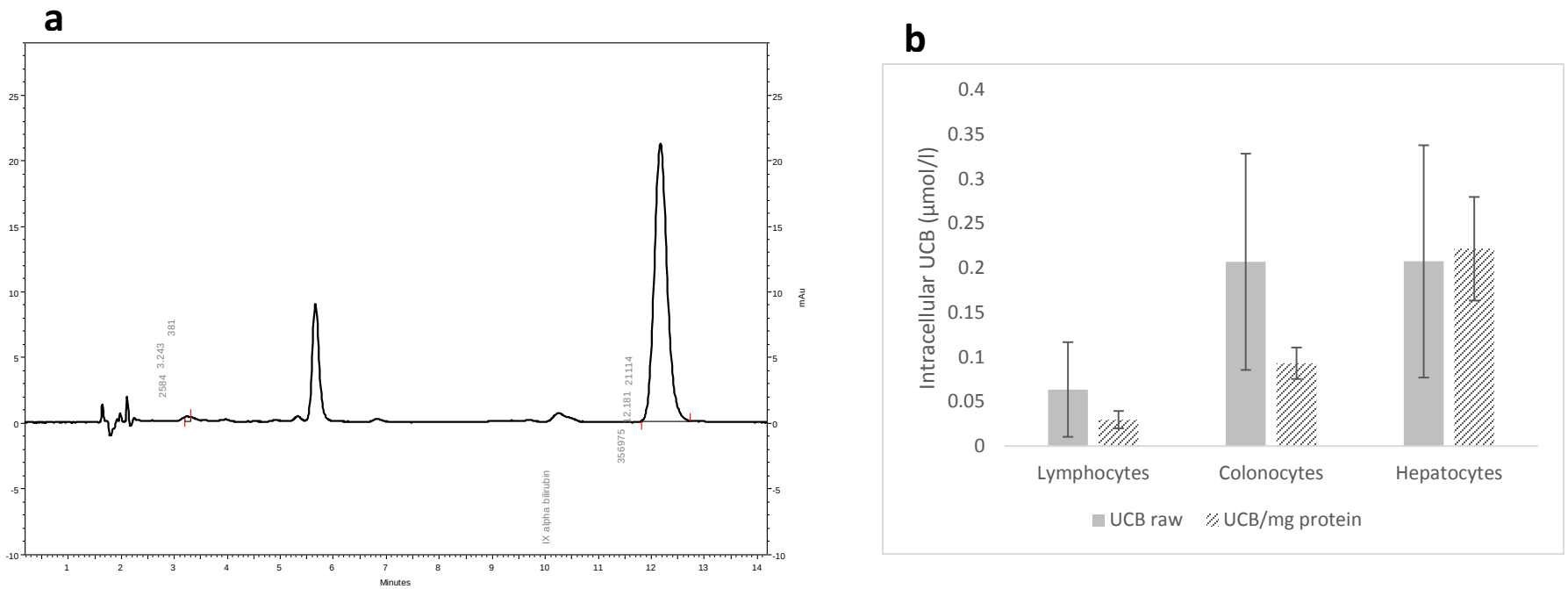






Supplementary figure S1. Gender differences in mean telomere length in a) Human lymphocytes ($P < 0.1$). b) Gunn and Wistar rats' hepatocytes (n.s.) c) Differences between males and females in Gunn and Wistar ruts separately.

Different colors represent values below and above the group mean.



Supplementary figure S2. UCB measurements in human serum and rat lymphocytes, hepatocytes and colonocytes. a) A representative chromatogram of unconjugated bilirubin measurement in serum. Only IX-α Bilirubin isomer is detectable in serum samples and tissue samples derived from rats. No peak present in human lymphocytes. b) Intracellular content of UCB in Gunn rat lymphocytes, colonocytes and hepatocytes. Error bars represent standard deviation.

Supplementary Table S1. Gender differences in mean values between GS and non-GS individuals. Values are presented as mean ± standard deviation. The indices represent different levels of significance obtained using two-tailed independent samples T-test:

[a] $P < 0.05$, [aa] $P < 0.01$ when comparing females to males within the same condition (GS or control), *$P < 0.05$, **$P < 0.01$ when comparing GS to controls of the same gender, [b] $P < 0.1$ females compared to males within the same condition (GS or control), [#] $P < 0.1$ GS compared to controls of the same gender.

The variable sample sizes presented are due to missing values in some of the parameters.

	Control		*GS*	
	Males (n = 36-40)	Females (n = 19-20)	Males (n = 37-40)	Females (n = 18-20)
Age (years)	36 ± 14	41 ± 14	36 ± 14	41 ± 14
UCB (µmol/l)	9.65 ± 3.53	8.35 ± 3.05	35.21 ± 10.53**	28.94 ± 6.85[a]**
Lymph. TL (kb)	5.10 ± 2.00	6.19 ± 2.12[b]	6.20 ± 1.87*	6.52 ± 2.03
BMI (kg/m^2)	25.74 ± 5.15	24.66 ± 4.42	23.34 ± 3.07*	21.75 ± 2.71[a]*
Body fat (%)	21.82 ± 7.37	30.76 ± 7.77[aa]	20.90 ± 6.05	24.42 ± 6.96 [b]*
CO-Hb (%)	1.42 ± 0.85	1.13 ± 0.30[b]	1.23 ± 0.30	1.18 ± 0.44
Heme (µmol/l)	0.77 ± 0.12	0.73 ± 0.09	0.74 ± 0.13	0.78 ± 0.18
Iron (µmol/l)	23.27 ± 8.98	22.79 ± 10.71	32.14 ± 10.73**	26.43 ± 7.32[a]
Albumin (g/l)	47.00 ± 3.18	45.75 ± 1.77[b]	48.06 ± 3.42	45.17 ± 2.15[aa]
Estradiol (pg/ml)	31.75 ± 9.91	117 ± 104.38[aa]	31.87 ± 11.71	70.20 ± 74.47[aa]
Testosterone (ng/ml)	5.60 ± 1.54	0.31 ± 0.14[aa]	6.12 ± 1.90	0.34 ± 0.13[aa]
HCY (µmol/l)	11.28 ± 3.87	8.77 ± 2.02[aa]	12.24 ± 5.20	9.28 ± 1.88[aa]
FRAP (mmol Fe^{2+}/l)	561 ± 91	518 ± 167	827 ± 170**	691 ± 169[aa]**
GSH/GSSG	1.15 ± 0.46	1.47 ± 0.32[aa]	1.11 ± 0.36	1.37 ± 0.42[a]
MDA (nmol/ml)	1.65 ± 0.55	1.95 ± 0.52[b]	1.75 ± 0.50	1.68 ± 0.47
IL6 (rfu)	2.58 ± 0.26	2.48 ± 0.31	2.35 ± 0.25**	2.40 ± 0.32
IL1β (rfu)	1.59 ± 0.28	1.41 ± 0.21[aa]	1.41 ± 0.23**	1.43 ± 0.33
TNF (rfu)	26.17 ± 5.24	25.13 ± 4.85	25.38 ± 5.92	26.37 ± 4.74

CRP (mg/dl)	0.17 ± 0.29	0.15 ± 0.18	0.10 ± 0.12	0.05 ± 0.05*
SAA (mg/dl)	4.62 ± 1.88	5.73 ± 2.78[b]	4.42 ± 1.34	4.31 ± 1.18*
Uric acid (mg/dl)	52.30 ± 18.36	38.85 ± 14.39[aa]	56.55 ± 14.52	40.15 ± 15.32[aa]
PLA2 (mg/dl)	32.60 ± 47.10	30.15 ± 42.34	43.65 ± 51.48	27.75 ± 34.20
BP systolic (mmHg)	136 ± 15	126 ± 15[a]	133 ± 11	123 ± 13[aa]
BP diastolic (mmHg)	71 ± 11	64.79 ± 11[b]	70.25 ± 10	60.94 ± 12[aa]
MAP (mmHg)	114 ± 12	105 ± 13[a]	111 ± 10	101 ± 11[aa]
HOMA_IR	1.84 ± 1.62	1.43 ± 0.90	1.11 ± 0.87*	1.00 ± 0.66[#]

Supplementary table S2. Gender differences in mean values between Gunn and Wistar rats. Values are presented as mean ± standard deviation. The indices represent different levels of significance obtained using two-tailed independent samples T-test:

[a] P < 0.05, [aa] P < 0.01comparing females to males within the same rodent model (Gunn/ Wistar), *P < 0.05, **P < 0.01 comparing Gunn to Wistar rats of the same gender, [b] P < 0.1 females to males within the same rodent model (Gunn/Wistar), [#] P < 0.1 comparing Gunn to Wistar rats of the same gender.

The variable sample sizes presented are due to missing values in some of the parameters.

	Wistar		*Gunn*	
	Males (n = 9)	Females (n = 9-11)	Males (n = 8-9)	Females (n = 10-11)
UCB (µmol/l)	0.58 ± 0.18	0.55 ± 0.20	97.88 ± 19.77**	112.38 ± 29.64**
Relative TL	11.66 ± 2.54	10.91 ± 2.47	14.46 ± 3.27[#]	13.12 ± 2.55[#]

Supplementary Table S3. Age differences in mean values in GS and non-GS individuals. Values are presented as mean ± standard deviation. The indices represent different levels of significance obtained using two-tailed independent samples T-test:

[a] $P < 0.05$, [aa] $P < 0.01$ when comparing individuals ≥ 35 years old to < 35 y.o. within the same condition (GS/control), *$P < 0.05$ comparing GS and control of the same age group, [b] $P < 0.1$ comparing individuals ≥ 35 years old to < 35 y.o. within the same condition (GS/control), [#] $P < 0.1$ comparing GS and control of the same age group.

The variable sample sizes presented are due to missing values in some of the parameters.

	Control		*GS*	
	< 35 y.o. (n = 30-33)	≥ 35 y.o. (n = 25-27)	< 35 y.o. (n = 30-33)	≥ 35 y.o. (n = 23-27)
Age (years)	27 ± 4	50 ± 11[aa]	27 ± 4	50 ± 11[aa]
UCB (µmol/l)	10.03 ± 3.63	8.22 ± 2.87[a]	33.57 ± 10.12**	32.57 ± 9.69**
Lymph. TL (kb)	5.83 ± 2.10	5.02 ± 2.01	6.57 ± 2.17	5.99 ± 1.52[#]
BMI (kg/m^2)	23.43 ± 3.74	27.77 ± 5.15[aa]	22.61 ± 3.01	23.06 ± 3.09**
Body fat (%)	21.30 ± 5.58	29.33 ± 9.61[aa]	22.23 ± 6.06	21.79 ± 7.12**
CO Hb (%)	1.15 ± 0.37	1.53 ± 0.97[a]	1.26 ± 0.43	1.14 ± 0.18*
Heme (µmol/l)	0.75 ± 0.11	0.77 ± 0.11	0.74 ± 0.13	0.77 ± 0.16
Iron (µmol/l)	25.47 ± 9.53	20.20 ± 8.75[a]	31.31 ± 10.45*	29.06 ± 9.63*
Albumin (g/l)	47.15 ± 3.07	45.88 ± 2.39[b]	47.91 ± 3.68	46.11 ± 2.59[a]
Estradiol (pg/ml)	62.00 ± 71.49	57.78 ± 74.29	38.58 ± 25.87[#]	52.85 ± 65.00
Testosterone (ng/ml)	4.51 ± 2.80	2.91 ± 2.62	4.86 ± 3.14	3.27 ± 3.00
HCY (µmol/l)	10.38 ± 3.95	10.56 ± 3.04	12.22 ± 5.68	10.11 ± 2.41[a]
FRAP (mmol Fe^{2+}/l)	568 ± 106	521 ± 139	806 ± 190**	748 ± 166**
GSH/GSSG	1.17 ± 0.45	1.37 ± 0.41[b]	1.17 ± 0.41	1.22 ± 0.40
MDA (nmol/ml)	1.68 ± 0.58	1.83 ± 0.52	1.77 ± 0.50	1.68 ± 0.46
IL-6 (rfu)	2.56 ± 0.30	2.54 ± 0.26	2.35 ± 0.26**	2.38 ± 0.29*
IL1-β (rfu)	1.53 ± 0.24	1.53 ± 0.31	1.43 ± 0.23	1.40 ± 0.30

TNF (rfu)	26.34 ± 4.97	25.19 ± 5.27	26.73 ± 5.34	24.56 ± 5.59
CRP (mg/dl)	0.10 ± 0.10	0.25 ± 0.35[a]	0.07 ± 0.07	0.10 ± 0.14*
SAA (mg/dl)	4.54 ± 1.55	5.54 ± 2.84[b]	4.18 ± 0.86	4.64 ± 1.65
Uric acid (mg/dl)	47.36 ± 17.31	48.37 ± 19.50	50.76 ± 20.38	51.48 ± 10.69
PLA2 (mg/dl)	33.61 ± 44.39	29.56 ± 46.97	38.12 ± 49.48	38.63 ± 44.11
BP systolic (mmHg)	131.10 ± 16.71	134.44 ± 14.58	128.97 ± 12.73	131.19 ± 13.56
BP diastolic (mmHg)	66.61 ± 13.10	72.07 ± 9.75[b]	66.06 ± 9.55	68.96 ± 14.38
MAP (mmHg)	109.17 ± 14.00	113.24 ± 11.32	107.58 ± 10.57	110.03 ± 12.19
HOMA_IR	1.22 ± 0.77	2.32 ± 1.79[aa]	1.00 ± 0.49	1.17 ± 1.05**

Supplementary table S4. Percentage of individuals carrying the GS-related (7/7) genotype among the 2 phenotypes: 6/6 - homozygotes with alleles containing 6 TA – repeats (short allele), 6/7 - heterozygotes with one short and one long allele, 7/7- homozygotes with two 7-TA long alleles.

		GS Phenotype		Total %
		no	yes	
UGT1A1 Genotype	6/6	43 %	2 %	26
	6/7	52 %	9 %	35
	7/7	5 %	89 %	54
Total N		58	57	115

Supplementary table S5. Descriptive values of UGT1A1 genotypes (6/6 wildtype homozygous, 6/7 heterozygous, 7/7 homozygous with reduced UGT1A1 activity). Values are expressed as mean ± standard deviation. *P < 0.05, **P < 0.01, #P < 0.1; Kruskal-Wallis ANOVA for nonparametric data.

	Genotype		
	6/6 (n = 25)	6/7 (n = 34)	7/7 (n = 52)
Age (years)	37 ± 14	36 ± 15	39 ± 14
Female gender (%)	31	31	35
UCB (µmol/l)**	9.27 ± 3.67	12.74 ± 9.07	31.93 ± 10.90
Lymphocyte telomere length (kb)*	5.22 ± 1.88	5.70 ± 2.27	6.33 ± 1.91
BMI (kg/m^2)#	25.32 ± 4.91	25.08 ± 4.87	23.12 ± 3.30
Iron (µmol/l)	23.94 ± 8.49	26.96 ± 12.44	27.84 ± 9.72

Supplementary table S6. Oligos used for qPCR-based telomere length measurement. Oligos a-e were designed according to (O'Callaghan and Fenech 2011).

Target/name	Species	Sequence (5'-3')	Amplicon size
Telomere standard[a]	Human/rodent	$(TTAGGG)_{14}$	84bp
36B4 standard[b]	Human	CAGCAAGTGGGAAGGTGTAATCCGTCTCCACAGACAAGGCCAGGAC TCGTTTGTACCCGTTGATGATAGAATGGG	75bp
Telomere F primer[c]	Human/rodent	CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGG TTTGGGTT	>76 bp
Telomere R primer	Human/rodent	GGCTTGCCTTACCCTTACCCTTACCC TTACCCTTACCCT	
36B4 F primer[d]	Human	CAGCAAGTGGGAAGGTGTAATCC	75bp
36B4 R primer[e]	Human	CCCATTCTATCATCAACGGGTACAA	
36B4 F primer	Rodent	AGGTCGAAGCAAAGGAAGAGTCG	84bp
36B4 R primer	Rodent	CTGACTTGGTGTGAGGGGCTT	

References

O'Callaghan, N. J. and M. Fenech (2011). "A quantitative PCR method for measuring absolute telomere length." Biol Proced Online **13**: 3.