Metabolic Abnormalities in Williams-Beuren Syndrome

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Supplementary Materials

Supplementary Materials and Methods

Table S1. Crude values of biochemical parameters studied in 154 WBS individuals.

Table S2. Primer sequences for genotyping and qRT-PCR.

Table S3. Alterations in biochemical parameters in children and adults with WBS.

Table S4. Candidate SNPs genotyped in WBS individuals.

Table S5. UGT1A1 genotype frequency and bilirubin concentrations in healthy controls and WBS individuals.

Table S6. Metabolic parameters analyzed in WBS mice models at 4.5 weeks of age.

Table S7. Anthropometric parameters analyzed in WBS mice models at 25 weeks.

Figure S1. Schematic representation of WBS deletion and the WBS murine models used in this study (Modified from[36]). (PD: proximal deletion, DD: distal deletion, CD: complete deletion)

Figure S2. A. Histogram of TSH plasma levels in percentiles in WBS individuals. **B.** Histogram of the distribution of TSH z-score values.

Figure S3. A. Histogram of glucose plasma levels in percentiles in WBS individuals. **B.** Histogram of the distribution of glucose z-score values.

Figure S4. A. Histogram of total cholesterol plasma levels in percentiles in WBS individuals. **B.** Histogram of the distribution of total cholesterol z-score values.

Figure S5. Relative expression of *Ugt1a1* studied in liver of WBS mouse models (Mean ± SD). Four animals per genotype were studied.

Supplementary Materials and Methods

Metabolic analyses in mice

All mice were male and were housed under basal conditions. Biochemical evaluations were performed at two time points: 4.5±0.2 and 26±1.1 weeks old. After fasting for 8 hours, blood was extracted from the mandibular vein; plasma was collected after centrifugation and stored at -80ºC until use. Triglycerides, total cholesterol and high-density lipoprotein levels were determined with an enzymatic kit, while low-density lipoprotein, total and direct bilirubin levels were determined with a colorimetric kit, following manufacturer's instructions (Gernon). Total protein plasma levels were determined by Bradford`s method (Pierce) following manufacturer's instructions. Biochemical parameters were determined in duplicates, except in samples with small plasma quantity. TSH was determined by an ELISA sandwich kit (Gentaur) following manufacturer`s instructions.

An intraperitoneal glucose tolerance test (IPGTT) was done in 11 weeks old mice of all four models (n=8-16), using different animals from those used for the other metabolic analyses. Mice were fasted for 6 hours before starting the procedure. A drop of blood was taken from the tail and analyzed for basal glucose levels (t=0) using the Accu-Check Aviva glucometer (Roche). A dose of 2 g D-glucose/kg was injected into the intraperitoneum of mice and blood was extracted from the tail at time points 15, 30, 45, 60, 90 and 120 minutes post-injection for glucose measurement.

Histological and anthropometric analyses

Mice were sacrificed after concluding the biochemical studies for anthropometric and histological characterization. Total body, liver, perirenal and gonadal fat weight were measured. To compare between genotypes, we calculated the percentage of the weight of the liver and fat with respect to total body weight.

Langerhans islets were analyzed in a separate group of 53 weeks old mice, 3 WT and 6 CD animals. The pancreatic glands were obtained after 4% paraformaldehyde perfusion, embedded and sectioned at 6 µm. Hematoxilin and eosin staining was performed for morphological examination under light microscopy. The number of Langerhans islets was counted for each animal and the total area was analyzed. For statistical analysis, the islet size was divided in five groups.

Supplementary Tables

Table S1. Crude values of biochemical parameters studied in 154 WBS individuals.

Table S2. Primer sequences for genotyping and qRT-PCR.

Use	Gene		Sequence	Amplicon Size	Location	Tmº
MLPA	*Gtf2i*	L	gggttccctaagggttgga//gaatcatggcccaagtagtgatgtctgcct	101	Exon 2	87.08ºC
		R	tgcctgccgaagatgaagagtcttcagag//tctagattggatcttgctggcac		Exon 2	85.64ºC
MLPA	*Cyln*	L	gggttccctaagggttgga//ctaatccacaaggtcatccgaattggcttccca	111	Exon 5	88.22ºC
		R	tccaccagtccagccaaggccaagaagaccaaacgc//tctagattggatcttgctggcac		Exon 5	90.46ºC
MLPA	*Rfc2*	L	gggttccctaagggttgga//gccctcaggagaaccatggaaatctactcgaagacg	116	Exon 6	88.96ºC
		R	cttcgcccttgcttgtaatgcttcggataagatcatcg//tctagattggatcttgctggcac		Exon 6	86.83ºC
MLPA	*Baz1b*	L	gggttccctaagggttgga//gctgaggaaagatacagtgaacgcatttggacgtgtaagag	114	Exon 2	87.47ºC
		R	ggaagcagtcagctaacacacaaggagctgac//tctagattggatcttgctggca		Exon 2	86.19ºC
MLPA	*Limk1*	L	gggttccctaagggttgga//cgagtgcatgaggttgacgctactttgttgcacc	104	Exon 1	88.98ºC
		R	tggagggaagaacgtatgggagaggaag//tctagattggatcttgctggcac		Exon 1	85.61ºC
MLPA	*Fkbp6*	L	gggttccctaagggttgga//ccggcgttatatcaaggccctcttcaggtgtggaca	113	Exon 6	90.59ºC
		R	ggtgagttgagggtagtggggagatcgcgactgacg//tctagcttccatcttgctggca		Intron 6	91.11ºC
MLPA	*Cutl1*	L	gggttccctaagggttgga//caaaggaccattaagcccaatctatgtc	99	Exon 24	83.84ºC
		R	gtgttttcaaggaagaaaacggaaatgtg//tctacattggatcttgctggcac		Exon 24	83.07ºC
MLPA	*Wbscr17*	L	gggttccctaagggttgga//gatcggggtgttgtttgatggcttcactgaggagagtcaaa	118	Exon 1	89.88ºC
		R	tgttggtgttgaacttgatcgcggttgctgactgtac//tctagattggatcttgctggc		Exon 1	86.67ºC
Expression	*Ugt1a1*	L	atcatgcccaacatggtttt	158	Exon 1	63.9 ºC
		R	atggctttcttctccggaat		Exon 2	63.7 ºC
Expression	*Rps28*	L	tagggtaaccaaagtgctgggc	103	Exon 1-2	64.49ºC
		R	gacatttcggatgatagagcgg		Exon 3	63.02ºC

Ugt1a1 primers were designed with Primer3.[21]

Table S3. Alterations in biochemical parameters in children and adults with WBS.

	TSH >97,5th	Glucose >97,5th	Cholesterol >95th	Triglyceride <5th	TB >97,5th	BD >97,5th	BI >97,5th	Iron >97,5th	Transferrin >97,5th	TP >97,5th	Albumin >97,5th
Children	37.7% (23/61)	8% (9/113)	4,2% (4/95)	15.8% (13/82)	13.6% (9/66)	8,1% (3/37)	21.6% (8/37)	26 % (13/50)	0% (0/49)	27% (17/63)	9,6% (5/52)
Adults	13.6%* (3/22)	5,3% (2/38)	2.9% (1/34)	24.2% (8/33)	39.1% *(9/23)	6,25% (1/16)	62,5%* (10/16)	10% (2/20)	15% (3/20)	28% (7/25)	14.3% (3/21)

Frequency of biochemical alterations separated by age. Children were considered younger than 18 years. Significant differences were found in TSH (p 0.037), TB (p 0.0088) and IB (p 0.0039).

Table S4. Candidate SNPs genotyped in WBS individuals and their association to triglyceride and total bilirubin levels.

Gene (SNP)	Function	Alleles	HapMap CEU (Freq)	Literature			WBS & Parents (Freq)	WBS Cohort			
				Study Freq.	Effect	p-value		Controls Freq (n)	Cases Freq (n)	OR (95CI)	p-value
MLXIPL **(rs799160)**	Transcription factor that regulates triglyceride synthesis and storage	A	0.469	0.50	Effect (SE) A: 2.8 (0.9) %	$4x10^{-2}$	0.58	0.625 (15)	0.476 (10)	0.545 (0.166-1.793)	0.318
		G	0.531	0.50			0.42	0.375 (9)	0.524 (11)		
SLCO1B1 **(rs4149056)**	Liver-specific member of the organic anion transporter family	T	0.85	0.84	One or two copies of the C-allele increases 4.9-9.5% TB	$6,7x10^{-13}$	0.83	0.79 (41)	0.85 (29)	0.643 (0.202-2.048)	0.455
		C	0.15	0.16			0.17	0.21 (11)	0.15 (5)		

Literature frequencies and odds ratio (OR) were obtained from[17 18]. In the final columns, allelic frequencies in controls versus cases, as well as the OR obtained are shown.

Table S5. *UGT1A1* genotype frequency and bilirubin concentrations in healthy controls and WBS individuals.

Genotype	Genotype frequency population	TB >17.5 µmol/L population	Genotype Frequency WBS % (Nº)	TB >97.5th percentile % (Nº)
$(TA)_6$ / $(TA)_6$	39.8%	1%	48.5% (50/103)	13.2% (5/38)
$(TA)_6$ / $(TA)_7$	50.8%	4%	39.8% (41/103)	13.3% (4/30)
$(TA)_7$ / $(TA)_7$	9.3%	67%	11.7% (12/103)	72.7% (8/11)

Frequency of control individuals and WBS individuals, with bilirubin levels available, with the different *UGT1A1* genotypes and high bilirubin serum concentration (above 17.5 µmol/L).[45] TB: total bilirubin.

Table S6. Metabolic parameters analyzed in WBS mice models at 4.5 weeks of age.

	WT	DD	PD
Age (weeks)	4.4 ± 0.17	4.3 ± 0.26	4.7 ± 0.12
Triglycerides (mg/dL) (nº mice)	93 ± 15.79 (8)	60 ± 7.38* (5)	66.5 ± 9.397* (8)
Total Cholesterol (mg/dL) (nº mice)	106 ± 12.31 (8)	120.4 ± 12.78 (5)	125.2 ± 9.34 (5)
HDL (mg/dL) (nº mice)	61.6 ± 11.76 (8)	65.95 ± 18.04 (4)	70.26 ± 11.75 (5)
LDL (mg/dL) (nº mice)	41.14 ± 8.22 (5)	51.17 ± 7.4 (3)	48.74 ± 8.88 (5)
Total Bilirubin (mg/dL) (nº mice)	0.616 ± 0.185 (8)	0.694 ± 0.288 (5)	0.33 ± 0.107 (5)
Direct Bilirubin (mg/dL) (nº mice)	0.238 ± 0.044 (8)	0.198 ± 0.052 (5)	0.136 ± 0.025* (5)
Indirect Bilirubin (mg/dL) (nº mice)	0.379 ± 0.169 (8)	0.496 ± 0.244 (5)	0.194 ± 0.108 (5)

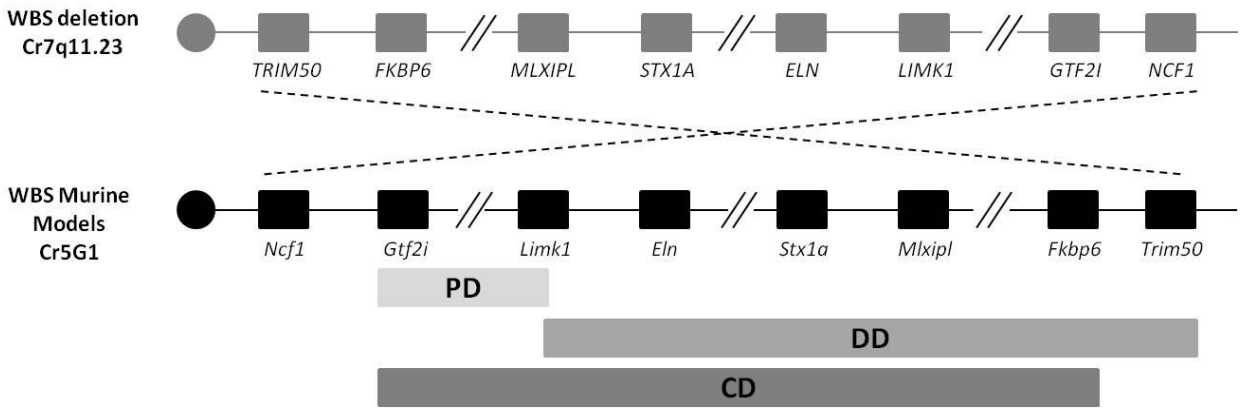
All values are the mean ± SD. Asterisks represent significant differences of at least p <0.007 (after Bonferroni´s correction) compared to wild type (WT) mice.

Table S7. Anthropometric parameters analyzed in WBS mice models at 25 weeks.

	WT (n=15)	CD (n=9)	DD (n=11)	PD (n=10)
Total Body Weight (gr.)	31.9 ± 3.1	31.06 ± 3.5	31.8 ± 3.4	35.6 ± 4.1*
Liver Weight (%)	4.25 ± 0.59	4.39 ± 0.49	4.46 ± 0.38	4.7 ± 0.12*
Perirenal Fat Weight (%)	3.2 ± 1.1	3.37 ± 1.0	3.67 ± 1.22	4.2 ± 0.87*
Gonadal Fat Weight (%)	1.25 ± 0.48	1.37 ± 0.52	1.45 ± 0.44	2.03 ± 0.62*
Total Fat (%)	4.45 ± 1.54	4.74 ± 1.48	5.12 ± 1.61	6.25 ± 1.45*

All values are the mean ± SD. Asterisks represent significant differences of at least p <0.05 compared to wild type (WT) mice.

Supplementary Figures



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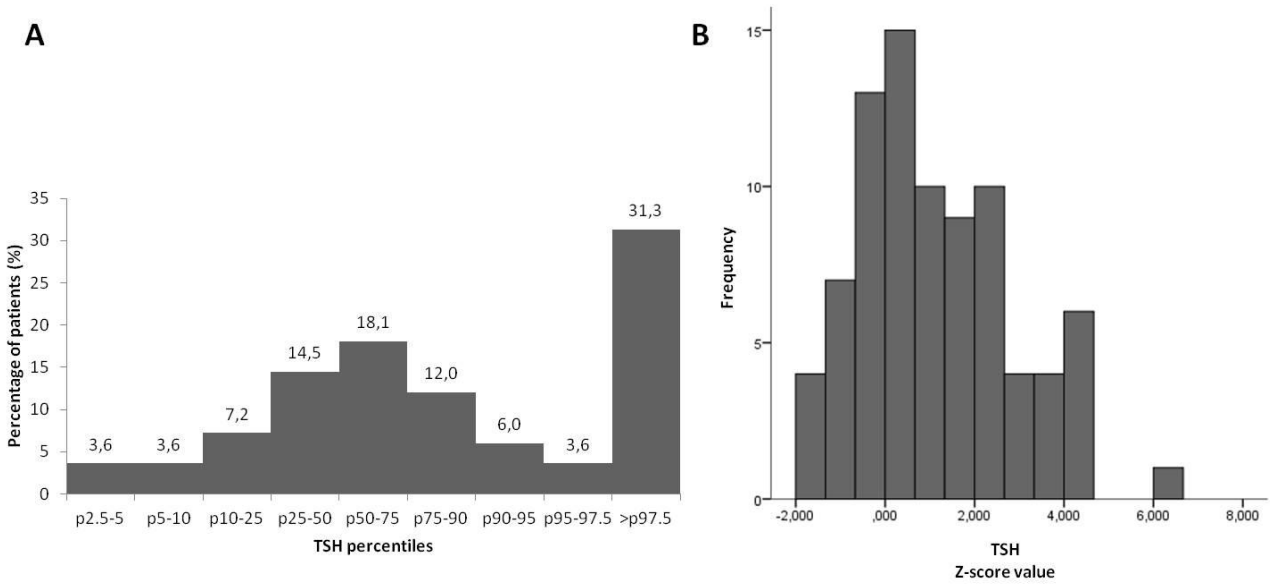

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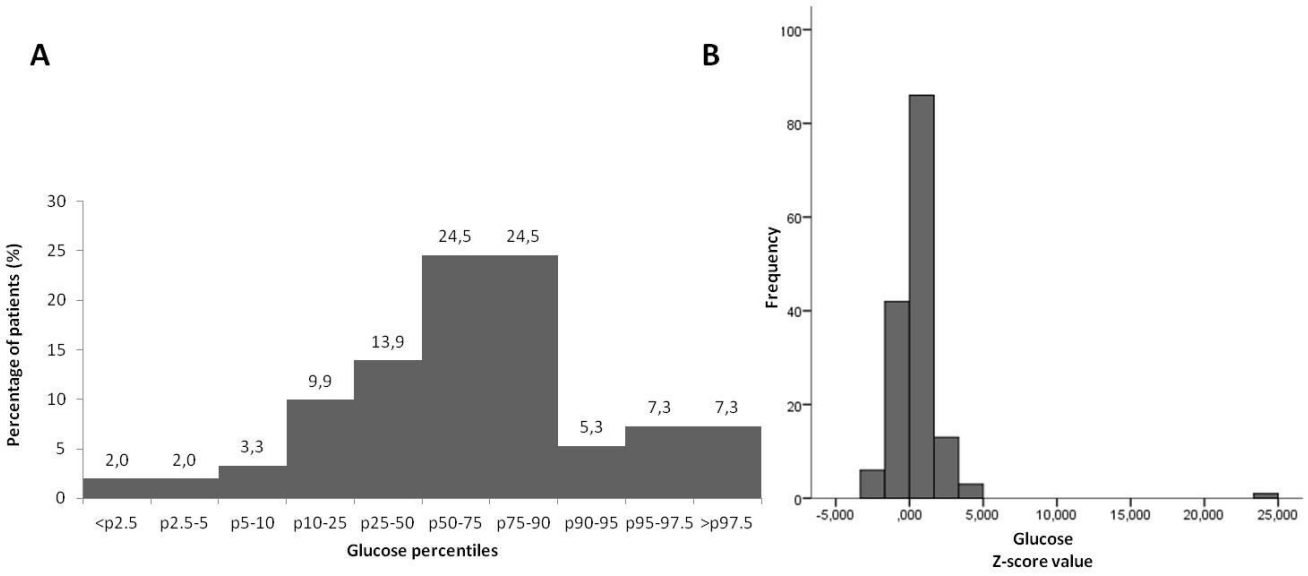
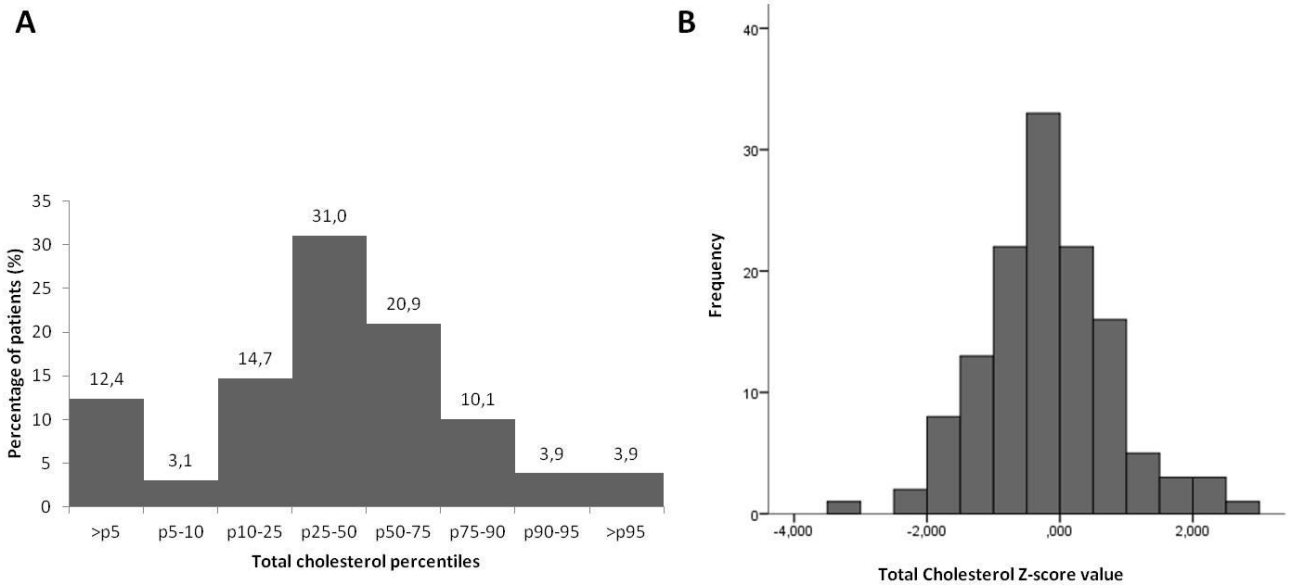


Figure S3. A. Histogram of glucose plasma levels in percentiles in WBS individuals. **B.** Histogram of the distribution of glucose z-score values.



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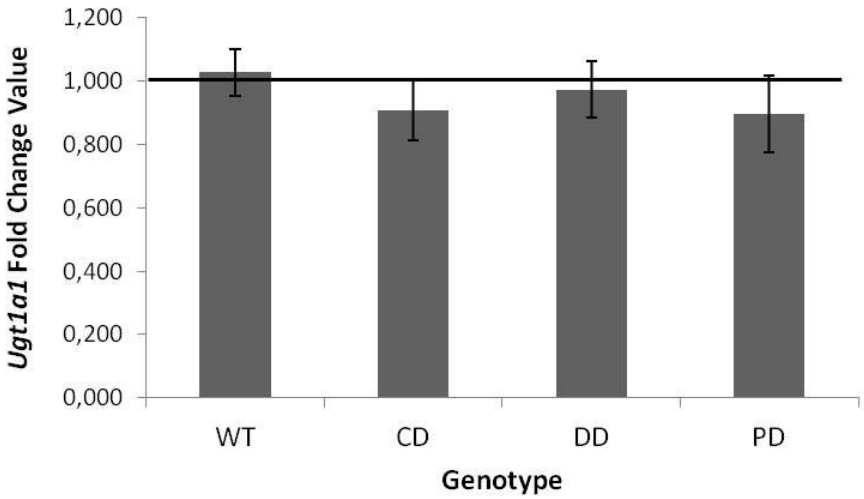

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