

Supplementary Information

Relevance of Human Aldoketoreductases and Microbial β -Glucuronidases in

Testosterone Disposition

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Running title: AKRs and GUSs in Testosterone Disposition

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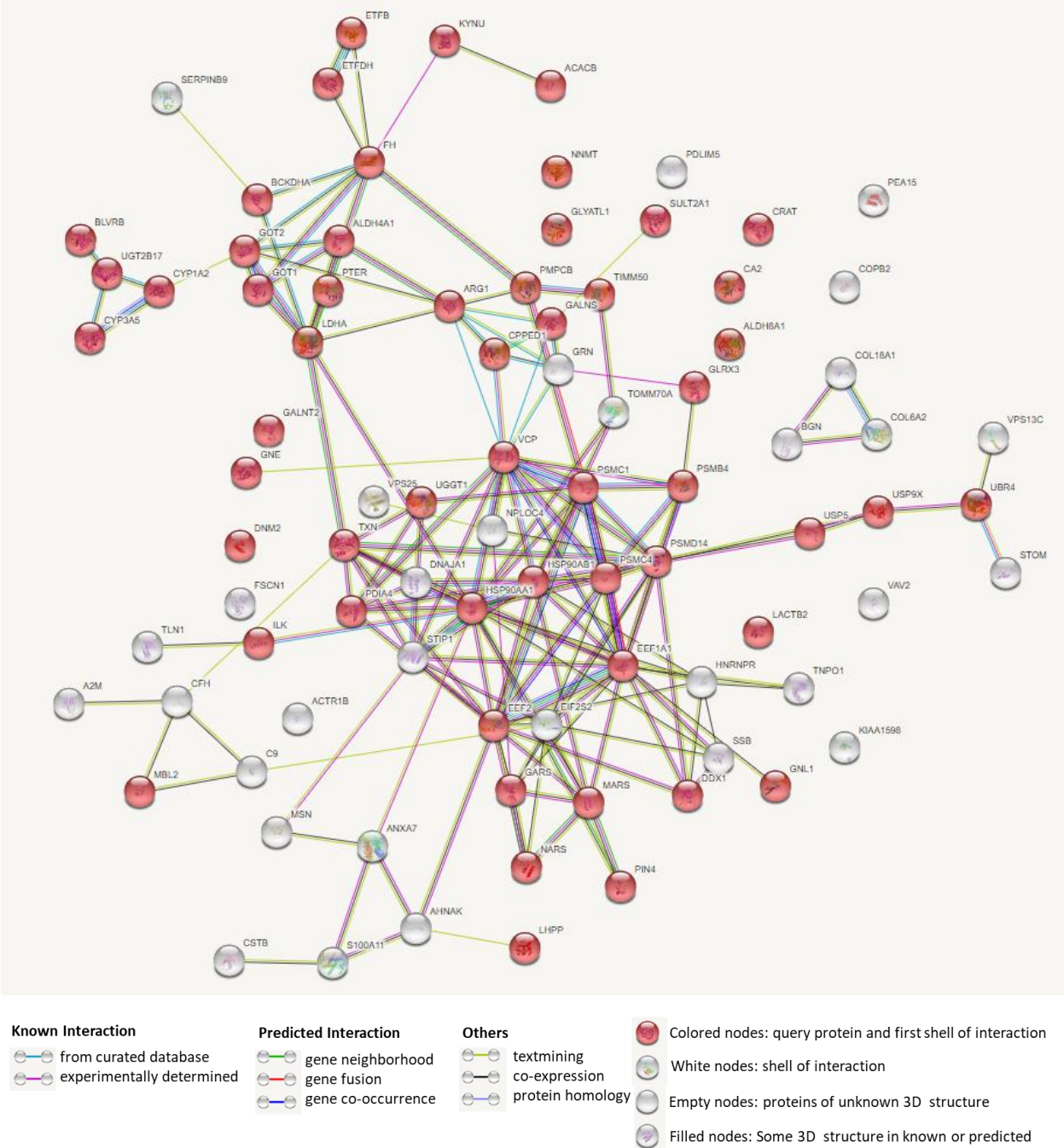


Fig. S1: Protein interactome analysis by STRING from downregulated proteins in UGT2B17 deletion. Significantly downregulated proteins include UGT2B17, CYP3A5, CYP1A2, and SULT2A1

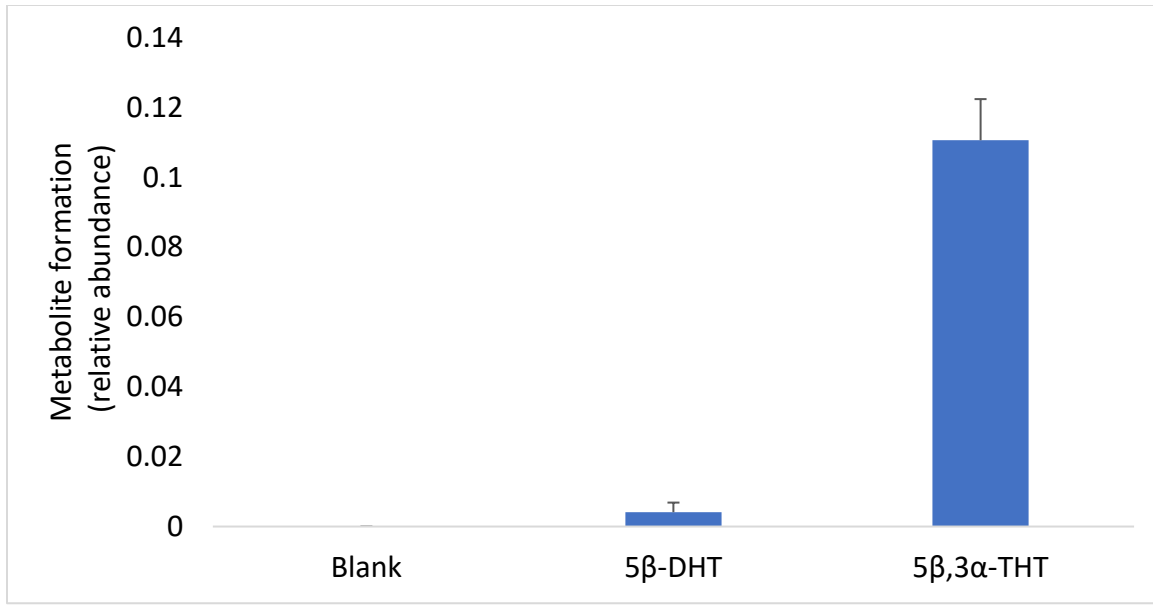


Fig. S2: In vitro formation of testosterone metabolites by AKR pathway when incubated at 1 μ M for 30 min with human liver cytosol from UGT2B17 gene deletion subjects.

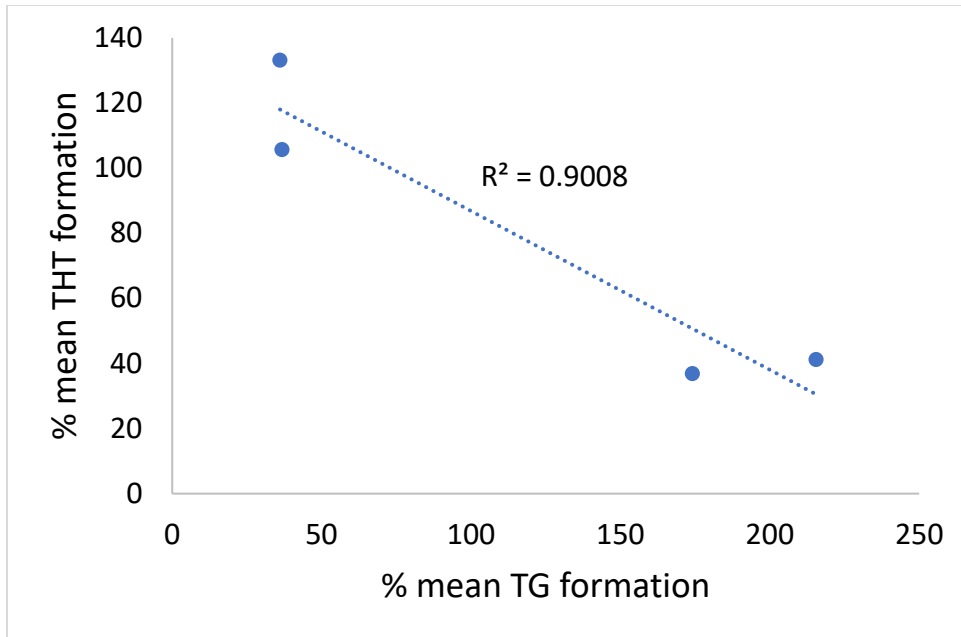


Fig. S3: Negative correlation between TG and THT formation from testosterone incubation in human hepatocytes with high and low UGT2B17 expression.

Table S1: MRM transitions used for quantification of various primary and secondary metabolites of testosterone					
Metabolite	Adduct	Precursor ion (m/z)	Daughter Ion (m/z)	CV (V)	CE (eV)
5 β -DHT	[M+H] ⁺	291.1	147.1	30	20
	[M+H] ⁺	291.1	159	30	20
	[M+H] ⁺	291.1	255	30	20
5 β , 3 α -THT	[M+H-H ₂ O] ⁺	275.1	161	30	20
	[M+H-H ₂ O] ⁺	275.1	174.8	30	15
	[M+H-H ₂ O] ⁺	275.1	257	30	15
5 β -DHT-Gluc	[M+H] ⁺	467.1	147.1	30	23
	[M+H] ⁺	467.1	159	30	23
	[M+H] ⁺	467.1	255	30	27
	[M+H] ⁺	467.1	273.1	30	27
5 β , 3 α -THT-Gluc	[M+H-H ₂ O] ⁺	451.1	161	30	25
	[M+H-H ₂ O] ⁺	451.1	174.8	30	20
	[M+H-H ₂ O] ⁺	451.1	257	30	20
	[M+H-H ₂ O] ⁺	451.1	275.1	30	20
AG-d4	[M+H-H ₂ O] ⁺	453.2	259.2	35	30
	[M+H-H ₂ O] ⁺	453.2	277.4	35	22
	[M+NH ₄] ⁺	488.3	259.3	35	30
T	[M+H] ⁺	289.1	109.1	35	30
	[M+H] ⁺	289.1	97.1	35	30
T-d3	[M+H] ⁺	292.2	97.1	35	30
	[M+H] ⁺	292.2	109.1	35	30
epiTG	[M+H] ⁺	465.2	289.2	35	30
	[M+H] ⁺	465.2	97.1	35	40
	[M+H] ⁺	465.2	109.1	35	40
TG	[M+H] ⁺	465.2	289.2	35	30
	[M+H] ⁺	465.2	97.1	35	40
	[M+H] ⁺	465.2	109.1	35	40
TG-d3	[M+H] ⁺	468.2	292.2	35	30
	[M+H] ⁺	468.2	97.1	35	40
	[M+H] ⁺	468.2	109.1	35	40

Table S2: Optimized enzyme concentration and pH used for TG deconjugation screening assay using the listed bacterial β -glucuronidases		
Bacterial species	Final enzyme concentration (nM)	Optimized pH
<i>Escherichia coli</i>	5	6.5
<i>Eubacterium eligens</i>	5	6.5
<i>Lactobacillus rhamnosus</i>	30	6.5
<i>Bacteroides uniformis</i>	30	6
<i>Roseburia hominis</i>	5	6
<i>Bacteroides fragilis</i>	25	5
<i>Bacteroides ovatus</i>	30	5
<i>Akkermansia muciniphila</i>	20	6.5
<i>Bacteroides coprocola</i>	50	4
<i>Bacteroides nordi</i>	50	5

Table S3: MRM transitions for testosterone and testosterone glucuronide using AB SCIEX 6500					
Metabolite	Adduct	Precursor ion (m/z)	Daughter Ion (m/z)	DP (V)	CE (eV)
T	[M+H] ⁺	289.1	109.1	80	30
	[M+H] ⁺	289.1	97.1	80	30
TG	[M+H] ⁺	465.2	289.2	70	25
	[M+H] ⁺	465.2	271.2	70	30
	[M+H] ⁺	465.2	109.1	70	35
TG-d3	[M+H] ⁺	468.2	292.2	70	25
	[M+H] ⁺	468.2	274.2	70	30
	[M+H] ⁺	468.2	256.2	70	35
T-d3	[M+H] ⁺	292.2	97.1	80	30
	[M+H] ⁺	292.2	109.1	80	30

Table S4: Differentially elevated metabolites in serum after oral testosterone dosing in men							
Metabolite ID	Fold-change	P value	Exact mass (m/z)	Predose		Testosterone treated	
				mean1	sd1	mean2	sd2
M486T45	135.8	9.45E-06	486.3061	564555	277163	76673454	14724405
M274T47_2	35.9	1.57E-05	274.2245	2215813	1009919	79627571	16457424
M451T54_1	108.1	7.99E-04	451.3248	448115	624399	48440130	20437127
M403T68_1	376.9	5.21E-07	403.2326	160100	117231	60347036	7121192
M449T47	103.2	7.36E-04	449.2533	695862	805690	71816597	29813267
M520T61_1	56.3	1.50E-03	519.8652	761412	544347	42854221	20225532
M432T47_1	137.3	9.99E-04	432.2461	678100	1070656	93102785	41053663
M234T38	63.2	8.76E-05	234.1699	2008252	369723	126976655	35564331
M484T47_1	105.7	3.08E-08	484.2904	2097666	4619778	221652247	19651543
M238T44_2	70.4	8.51E-07	238.1801	3508011	2393181	246926861	31785644
M376T52_2	103.6	8.55E-04	376.2594	2626951	2706553	272237792	116264603
M302T45_3	51.5	1.19E-03	302.1961	4193737	2531590	216117969	97276040
M450T54_2	42.1	8.69E-04	450.3213	4052959	4876921	170821561	72395557
M274T37_3	189.8	2.23E-06	274.2011	1772298	2057781	336402349	50803785
M465T39_2	846.6	5.71E-05	465.2482	429006	829933	363186969	95756105
M431T47	582.7	9.41E-04	431.2427	592296	293753	345146904	151224744