

Supplemental data

Evidence of a Causal Effect of Estradiol on Fracture Risk in Men

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Supplemental Table 1. Associations of the SNPs Used as Instrumental Variables with Potential Confounders

Trait	SNPs used as instrumental variables			
	rs727479	rs5934505	rs10822186	rs12150660
Age	0.34	0.35	0.05	0.34
BMI	0.26	0.30	0.04	0.54
Smoking (current smoking yes/No)	0.50	0.18	0.05	0.27
Vigorous physical activity (Yes/No)	0.08	0.89	0.86	0.22

The associations between the different SNPs used as instrumental variables and possible confounding factors were evaluated in unrelated men of European origin in the UK Biobank (n=175,583). After Bonferroni correction, none of these passed the significance threshold ($0.05/16=0.003$).

Supplemental Table 2. Associations of E2-Associated SNPs with eBMD and Fractures

E2 SNPs	Chr	Position	EA	OA	Freq EA	Gene	E2					eBMD				All fractures					Non-vertebral major osteoporotic fractures					Wrist fractures					
							Beta	SE	P	N	F-Statistics	Variance explained (%)	Beta	SE	P	N	Beta	SE	P	N	N cases	Beta	SE	P	N	N cases	Beta	SE	P	N	N cases
rs5934505	23	8913826	T	C	0.73	FAM9B	-0.069	0.013	4.2E-08	9023	12.9	0.14	-0.036	0.003	2.0E-38	168216	0.020	0.009	2.4E-02	175205	17618	0.060	0.018	7.6E-04	175205	4369	0.073	0.023	1.5E-03	175205	2633
rs727479	15	51534547	C	A	0.35	CYP19	-0.145	0.013	8.2E-30	11165	102.6	0.91	-0.048	0.004	2.5E-40	168569	0.044	0.012	1.6E-04	175583	17650	0.065	0.022	4.0E-03	175583	4379	0.106	0.029	2.1E-04	175583	2637

eBMD = estimated BMD in the heel using ultrasound.

Beta for E2 is in SD per EA and is from Eriksson et al. (J Clin Endocrinol Metab 2018;103(3):991-1004) (assuming SD = 9.6 pg/ml from Framingham Heart Study the by far largest cohort included)

Beta for eBMD is per EA from linear regression in the current evaluation in UK Biobank

Beta for fractures is per EA from logistic regression of fracture status in the current evaluation in UK Biobank

Non-vertebral major osteoporosis fractures are defined as wrist, arm, or hip fractures

EA = Effect allele

OA = Other allele

Supplemental Table 3. Associations of T-Associated SNPs with eBMD and Fractures

T SNPs	Chr	Position	EA	OA	Freq EA	Gene	Testosterone					eBMD				All fractures					Non-vertebral major osteoporotic fractures					Wrist fractures					
							Beta	SE	P	F-Statistics	Variance explained (%)	N	Beta	SE	P	N	Beta	SE	P	N	N cases	Beta	SE	P	N	N cases	Beta	SE	P	N	N cases
rs5934505	23	8913826	T	C	0.73	FAM9B	-0.097	0.016	1.7E-09	53.6	0.6	8883	-0.036	0.003	2.0E-38	168216	0.020	0.009	2.4E-02	175205	17618	0.060	0.018	7.6E-04	175205	4369	0.073	0.023	1.5E-03	175205	2633
rs10822186	10	65350383	A	G	0.51	JMJD1C	-0.043	0.014	3.1E-03	99.4	1.1	8936	-0.002	0.003	6.4E-01	168569	0.019	0.011	9.8E-02	175583	17650	0.016	0.022	4.7E-01	175583	4379	0.017	0.028	5.5E-01	175583	2637
rs12150660	17	7521915	G	T	0.76	SHBG	-0.180	0.013	1.2E-41	338.2	2.3	14367	-0.001	0.004	7.5E-01	168569	-0.018	0.013	1.6E-01	175583	17650	-0.010	0.025	6.9E-01	175583	4379	-0.032	0.032	3.1E-01	175583	2637

eBMD = estimated BMD in the heel using ultrasound.
 Beta for testosterone is in SD per EA and is from Ohlsson et al. (PLoS Genet 2011;7(10):e1002313) (assuming SD = 176 ng/dL from MrOS Sweden)
 Beta for eBMD is per EA from linear regression in the current evaluation in UK Biobank
 Beta for fractures is per EA from logistic regression of fracture status in the current evaluation in UK Biobank
 Non-vertebral major osteoporotic fractures are defined as wrist, arm, or hip fractures
 EA = Effect allele
 OA = Other allele

Supplemental Table 4. Interactions Between E2-GRS and Age, BMI, eBMD, Smoking, Physical Activity, and ERα SNP rs4869742

E2-GRS Interaction terms	eBMD				All fractures				
	Beta	SE	P	N	OR	95% CI	P	N	N cases
Base model									
E2-GRS (per SD Increase)	0.041	0.002	7.1E-63	168569	0.97	(0.96, 0.99)	6.6E-04	175583	17650
Age (Per SD Increase)	-0.040	0.002	2.0E-58	168569	0.77	(0.76, 0.78)	2.7E-245	175583	17650
<i>Interaction: Age:E2-GRS</i>	0.002	0.002	3.8E-01	168569	0.99	(0.98, 1.01)	2.4E-01	175583	17650
BMI (Per SD Increase)*	0.079	0.003	5.8E-177	168569	0.98	(0.97, 1)	8.8E-02	175583	17650
<i>Interaction: BMI:E2-GRS</i>	0.004	0.003	1.3E-01	168569	1.00	(0.98, 1.02)	9.6E-01	175583	17650
eBMD (Per SD Increase)	ND	ND	ND	ND	0.78	(0.76, 0.79)	9.0E-188	167588	16570
<i>Interaction: eBMD:E2-GRS</i>	ND	ND	ND	ND	0.99	(0.98, 1.01)	5.0E-01	167588	16570
Current Smoker (Yes/No)	-0.259	0.007	2.9E-263	167952	1.40	(1.34, 1.46)	9.5E-54	174954	17578
<i>Interaction: Smoking:E2-GRS</i>	-0.001	0.007	8.7E-01	167952	1.00	(0.95, 1.04)	8.2E-01	174954	17578
Vigorous physical activity (Yes/No)	0.218	0.005	<2.2E-308	153398	1.20	(1.16, 1.24)	5.0E-24	159692	15958
<i>Interaction: Physical activity:E2-GRS</i>	0.005	0.005	3.4E-01	153398	1.00	(0.96, 1.03)	9.6E-01	159692	15958
ERα SNP rs4869742 (per C allele)	0.088	0.004	2.3E-120	168569	0.95	(0.93, 0.97)	3.3E-05	175583	17650
<i>Interaction: rs4869742:E2-GRS</i>	0.002	0.004	6.6E-01	168569	1.01	(0.98, 1.03)	6.3E-01	175583	17650
Fully adjusted model									
E2-GRS (per SD Increase)	0.042	0.003	1.4E-61	152966	0.97	(0.96, 0.99)	1.0E-03	159248	15908
Age (Per SD Increase)	-0.036	0.003	4.8E-44	152966	0.78	(0.77, 0.8)	7.1E-185	159248	15908
<i>Interaction: Age:E2-GRS</i>	0.003	0.003	2.5E-01	152966	0.99	(0.97, 1)	1.5E-01	159248	15908
BMI (Per SD Increase)*	0.086	0.003	8.5E-188	152966	1.00	(0.98, 1.02)	7.7E-01	159248	15908
<i>Interaction: BMI:E2-GRS</i>	0.004	0.003	1.5E-01	152966	cd ..	(0.98, 1.02)	7.0E-01	159248	15908
eBMD (Per SD Increase)	ND	ND	ND	ND	0.77	(0.76, 0.79)	1.7E-169	152176	14953
<i>Interaction: eBMD:E2-GRS</i>	ND	ND	ND	ND	0.99	(0.98, 1.01)	5.7E-01	152176	14953
Current Smoker (Yes/No)	-0.229	0.008	1.7E-182	152966	1.43	(1.37, 1.50)	1.5E-52	159248	15908
<i>Interaction: Smoking:E2-GRS</i>	-0.002	0.008	8.4E-01	152966	1.01	(0.96, 1.05)	7.9E-01	159248	15908
Vigorous physical activity (Yes/No)	0.206	0.005	<2.2E-308	152966	1.23	(1.18, 1.27)	1.1E-29	159248	15908
<i>Interaction: Physical activity:E2-GRS</i>	0.006	0.005	2.9E-01	152966	1.00	(0.96, 1.03)	9.1E-01	159248	15908
ERα SNP rs4869742 (per C allele)	0.087	0.004	3.9E-108	152966	0.95	(0.92, 0.97)	1.9E-05	159248	15908
<i>Interaction: rs4869742:E2-GRS</i>	0.003	0.004	4.7E-01	152966	1.00	(0.98, 1.03)	7.3E-01	159248	15908

eBMD = estimated BMD in the heel using ultrasound

Beta for eBMD in SD is given per SD in E2-GRS, SD in Age, Smoker Yes/No, Vigorous physical activity Yes/No, per C allele in ERα SNP rs4869742

OR for fractures is given per SD in E2-GRS, SD in Age, Smoker Yes/No, Vigorous physical activity Yes/No, per C allele in ERα SNP rs4869742

ND = Not determined

Base model : adjusted for age, height, and weight

Fully adjusted model : adjusted for age, height, weight, smoking, and physical activity

*When BMI was included, the model was not adjusted for height and weight

Supplemental Table 5. Estimated Causal Effect of T on eBMD and Fractures

Genetic instrument	eBMD			All fractures			Non-vertebral major osteoporotic fractures			Wrist fractures		
	Beta	SE	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
rs10822186	-0.04	0.08	6.4E-01	1.55	(0.92, 2.61)	9.8E-02	1.45	(0.53, 3.95)	4.7E-01	1.48	(0.41, 5.34)	5.5E-01
rs12150660	-0.01	0.02	7.5E-01	0.90	(0.79, 1.04)	1.6E-01	0.95	(0.72, 1.24)	6.9E-01	0.84	(0.59, 1.18)	3.1E-01
rs5934505	-0.37	0.03	1.9E-38	1.23	(1.03, 1.48)	2.4E-02	1.86	(1.30, 2.66)	7.6E-04	2.12	(1.33, 3.38)	1.5E-03
IVW MR Effect using all 3 SNPs	-0.14	0.02	4.6E-16	1.03	(0.93, 1.15)	5.5E-01	1.22	(0.99, 1.51)	6.8E-02	1.18	(0.90, 1.55)	2.4E-01
IVW MR Effect excl rs5934505	-0.01	0.02	6.7E-01	0.94	(0.82, 1.08)	3.6E-01	0.97	(0.75, 1.27)	8.5E-01	0.87	(0.62, 1.21)	4.1E-01

eBMD = estimated BMD in the heel using ultrasound

Beta for eBMD is given as SD eBMD per SD decrease in testosterone

OR for fractures is given per SD decrease in testosterone

Non-vertebral major osteoporotic fractures are defined as wrist, arm, or hip fractures

IVW = inverse variance weighted