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Genetic risk factors of decreased bone mineral accretion in children with asthma receiving multiple oral corticosteroid bursts

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Table E1. Relationship between variables and BMA in white CAMP subjects

Variables	Beta	P value
Cumulative dose of prednisone	-3.04×10^{-6}	7.96×10^{-5}
Covariates measured at baseline		
Age	-7.19×10^{-4}	0.149
Gender (female *vs*. male)	0.00384	0.002
BMD	0.148	$< 2 \times 10^{-16}$
Height	-3.45×10^{-5}	0.535
BMI	-1.94×10^{-4}	0.357
Vitamin D	-1.55×10^{-5}	0.669
Tanner stage 2 (*vs*. 1)	0.00136	0.379
Tanner stage 3 (*vs*. 1)	-0.00137	0.631
Tanner stage 4 (*vs*. 1)	-0.0271	4.32×10^{-11}
Tanner stage 5 (*vs*. 1)	-0.0470	1.52×10^{-4}

* Multiple linear regression analysis

Table E2. Beta coefficients and *P* values of associations between variables and BMA*

Variables	Beta	*P* value
CumOCS	-1.074×10^{-6}	0.3428
Age	9.954×10^{-4}	0.0397
Female (*vs.* male)	3.389×10^{-3}	0.0004
BMD (*z* score)	0.1497	$<2 \times 10^{-16}$
Height	-3.635×10^{-5}	0.5004
BMI	-1.224×10^{-4}	0.5492
Serum concentration of vitamin D	2.392×10^{-5}	0.4941
Tanner stage 2 (*vs.* Tanner stage 1)	1.698×10^{-3}	0.2585
Tanner stage 3 (*vs.* Tanner stage 1)	-9.935×10^{-4}	0.7180
Tanner stage 4 (*vs.* Tanner stage 1)	-2.251×10^{-2}	3.570×10^{-10}
Tanner stage 5 (*vs.* Tanner stage 1)	-4.541×10^{-2}	1.420×10^{-4}
rs2074439HE (*vs.* rs2074439MA)	-5.411×10^{-4}	0.7193
rs2074439MI (*vs.* rs2074439MA)	-1657×10^{-3}	0.4655
rs9896933HE (*vs.* rs9896933MA)	-6.734×10^{-3}	0.0803
rs9896933MI (*vs.* rs9896933MA)	-8.235×10^{-3}	1.880×10^{-5}
CumOCS:rs2074439HE (*vs.* CumOCS:rs2074439MA)	-2.147×10^{-6}	0.01754
CumOCS:rs2074439MI (*vs.* CumOCS:rs2074439MA)	-8.666×10^{-6}	1.480×10^{-4}
CumOCS:rs9896933HE	-1.260×10^{-5}	8.077×10^{-3}

(*vs.* CumOCS:rs9896933MA)		
CumOCS:rs9896933MI	-7.525×10^{-6}	2.790×10^{-5}
(*vs.* CumOCS:rs9896933MA)		

[*]A regression model: BMA ~ cumulative dose of OCS + age + sex + BMD + height + body mass index + serum concentration of vitamin D + Tanner stage + rs2074439 genotype (additive model) + rs9896933 genotype (additive model) + cumulative dose of OCS*rs2074439 genotype + cumulative dose of OCS*rs9896933. CumOCS; cumulative oral corticosteroid, BMA; bone mineral density, BMI: body mass index, MA; major allele homozygote, HE; heterozygote, MI; minor allele homozygote

Table E3. Beta coefficients and *P* values of associations between cumulative dose of OCS and BMA stratified by the number of mutant alleles of rs9896933 and rs2074439

Group	Beta	P value*
Whole population	-3.036×10^{-6}	7.96×10^{-5}
Subjects without mutant allele	-1.183×10^{-6}	0.3504
Subjects with one mutant allele	-2.587×10^{-6}	0.0298
Subjects with two mutant alleles	-7.178×10^{-6}	5.38×10^{-5}
Subjects with three mutant alleles	-2.871×10^{-5}	1.57×10^{-5}

*Adjusted by age, sex, BMD, height, body mass index, serum concentration of vitamin D, and Tanner stage measured at baseline

Figure E1. The QQ-plot and Manhattan plot for the interaction results

A. QQ-plot

Red line; genome-wide significance

B. Manhattan plot

Figure E2. Correlations between cumulative dose of OCS and BMA stratified by genotype of rs9896933

A. Whole population, reference allele homozygotes, heterozygotes, and mutant allele homozygotes

B. Merge of individual regression lines

A slope obtained from simple linear regression analysis became steeper as the number of mutant alleles of two SNPs increased. P values were obtained from Pearson's correlation analysis, *r*; correlation coefficient. PD; Prednisone

Figure E3. Correlations between B cumulative dose of OCS and MA stratified by genotype of rs2074439

A. Whole population, reference allele homozygotes, heterozygotes, and mutant allele homozygotes

B. Merge of individual regression lines

A slope obtained from simple linear regression analysis became steeper as the number of mutant alleles of two SNPs increased. P values were obtained from Pearson's correlation analysis, *r*; correlation coefficient. PD; Prednisone

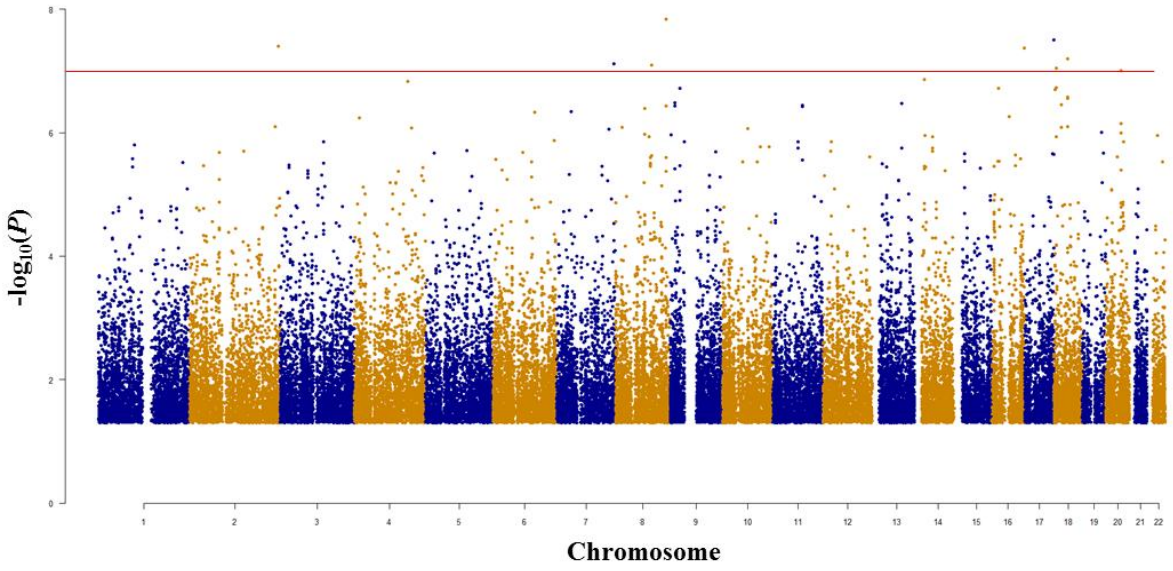
Figure E4. Correlations between cumulative dose of OCS and BMA stratified by the

number of mutant alleles of rs9896933 and rs2074439 (Merge of individual regression lines)
A slope obtained from simple linear regression analysis became steeper as the number of mutant alleles of two SNPs increased. P values were obtained from Pearson's correlation analysis, r; correlation coefficient

Figure E5. Comparison of BMA stratified by rs9896933 in asthmatic children without OCS bursts between those randomized to ICS and those randomized to placebo
Differences according to the treatment groups or to the genotypes of rs9896933 were not statistically significant.

Figure E1

A.



B.

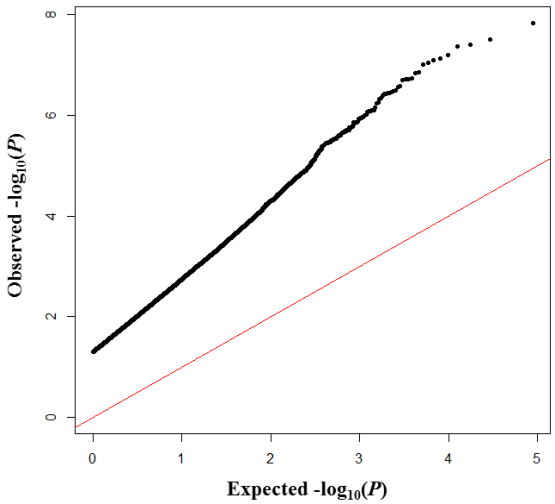
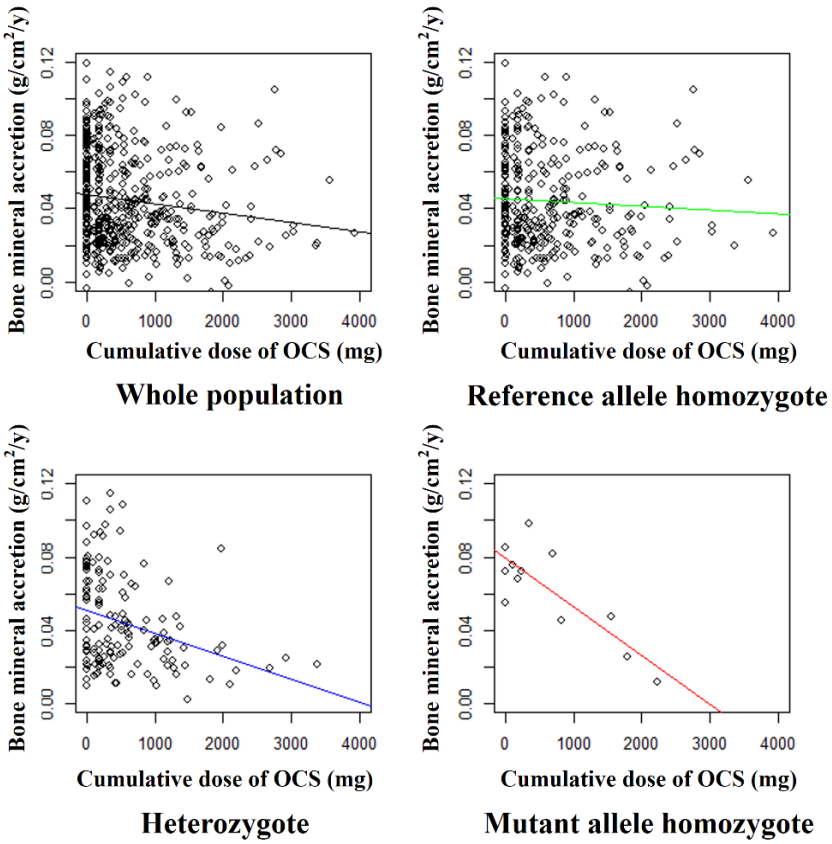


Figure E2.

A.


Bone mineral accretion (g/cm²/y)
0.00
0.04
0.08
0.12
0
1000
2000
3000
4000
Cumulative dose of OCS (mg)
Whole population
Reference allele homozygote
Heterozygote
Mutant allele homozygote

B.

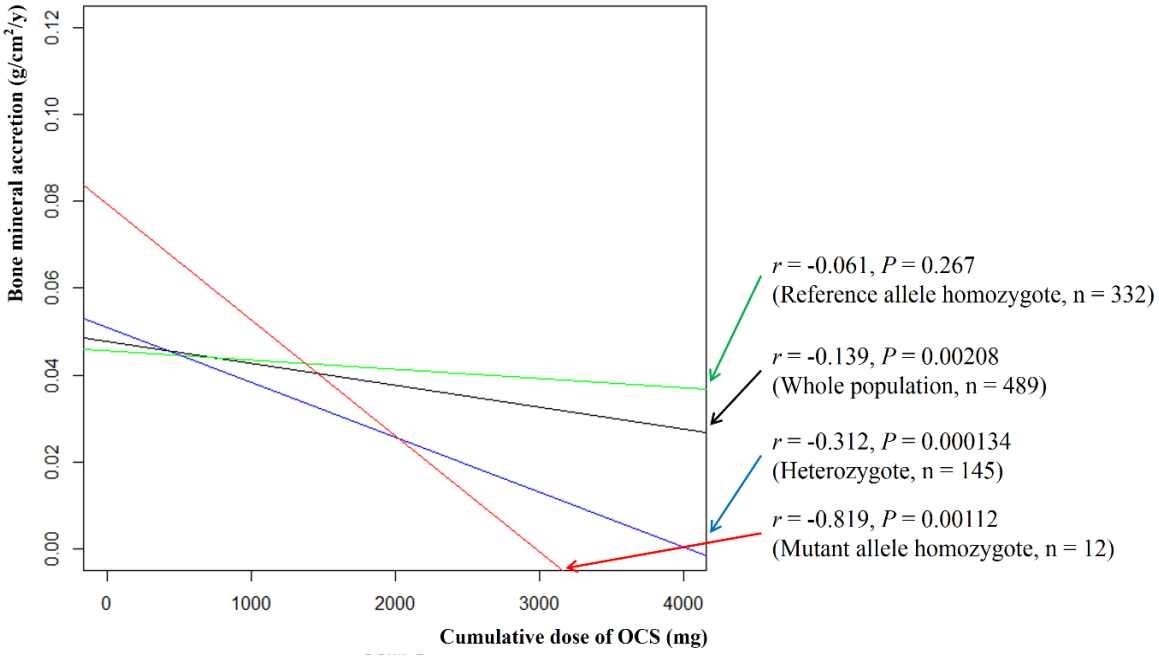
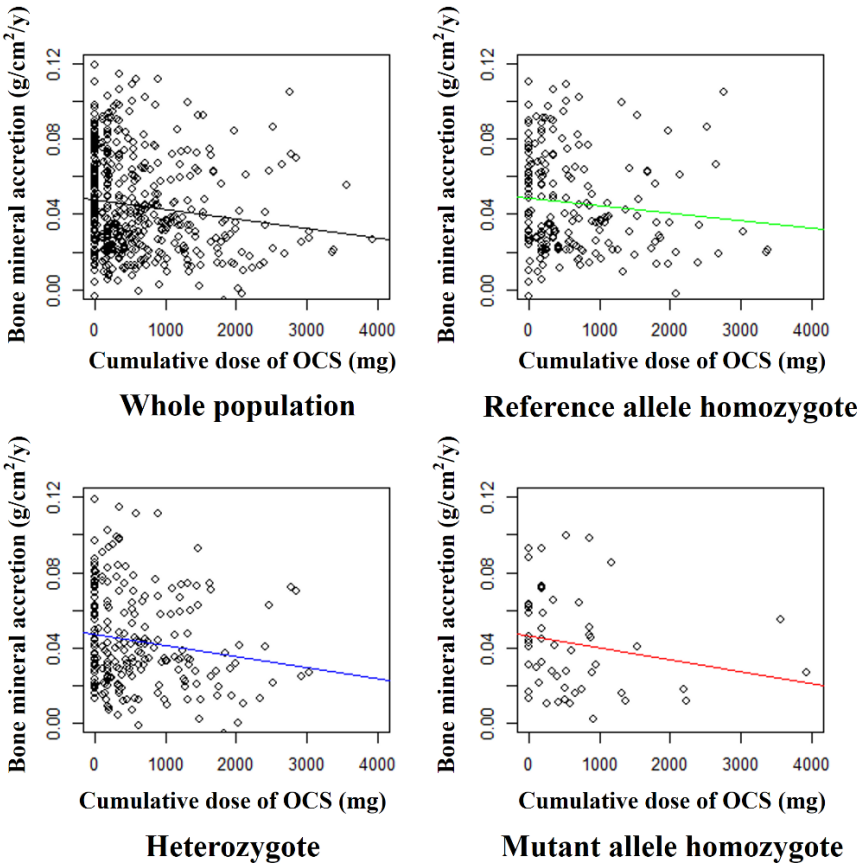

Bone mineral accretion (g/cm²/y)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0
1000
2000
3000
4000
Cumulative dose of OCS (mg)
r = -0.061, P = 0.267
(Reference allele homozygote, n = 332)
r = -0.139, P = 0.00208
(Whole population, n = 489)
r = -0.312, P = 0.000134
(Heterozygote, n = 145)
r = -0.819, P = 0.00112
(Mutant allele homozygote, n = 12)

Figure E3.

A.


Bone mineral accretion (g/cm²/y)
0.00
0.04
0.08
0.12
0
1000
2000
3000
4000
Cumulative dose of OCS (mg)
Whole population
Reference allele homozygote
Heterozygote
Mutant allele homozygote

B.

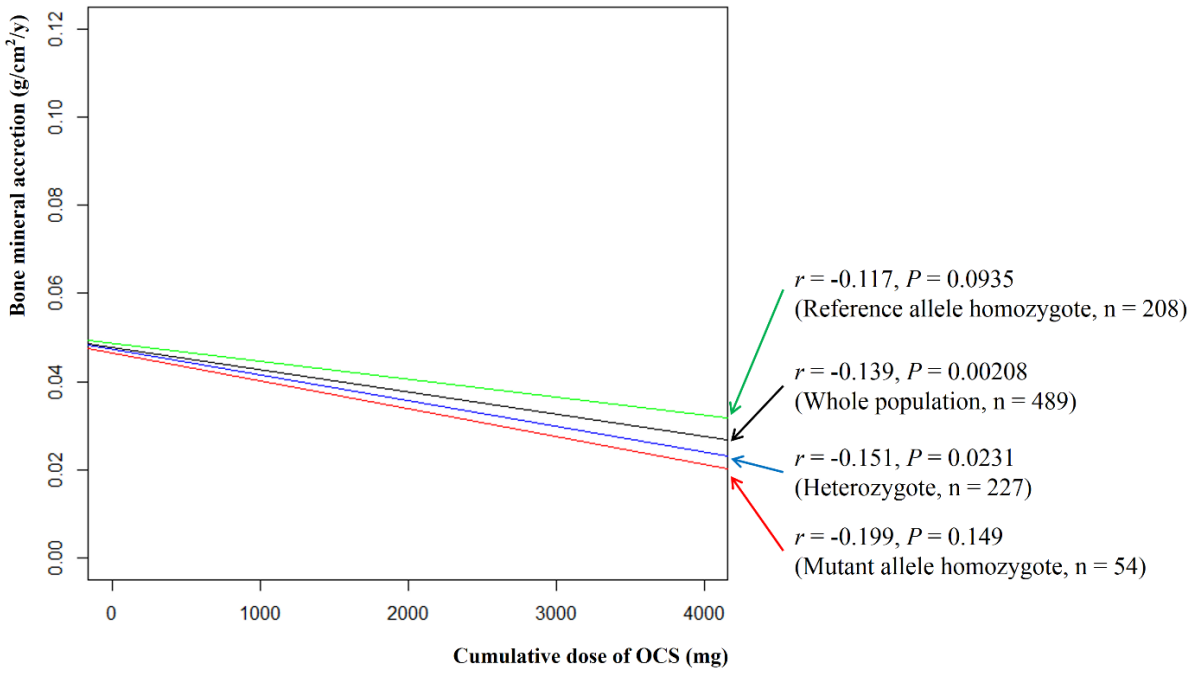

Bone mineral accretion (g/cm²/y)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0
1000
2000
3000
4000
Cumulative dose of OCS (mg)
r = -0.117, P = 0.0935
(Reference allele homozygote, n = 208)
r = -0.139, P = 0.00208
(Whole population, n = 489)
r = -0.151, P = 0.0231
(Heterozygote, n = 227)
r = -0.199, P = 0.149
(Mutant allele homozygote, n = 54)

Figure E4.

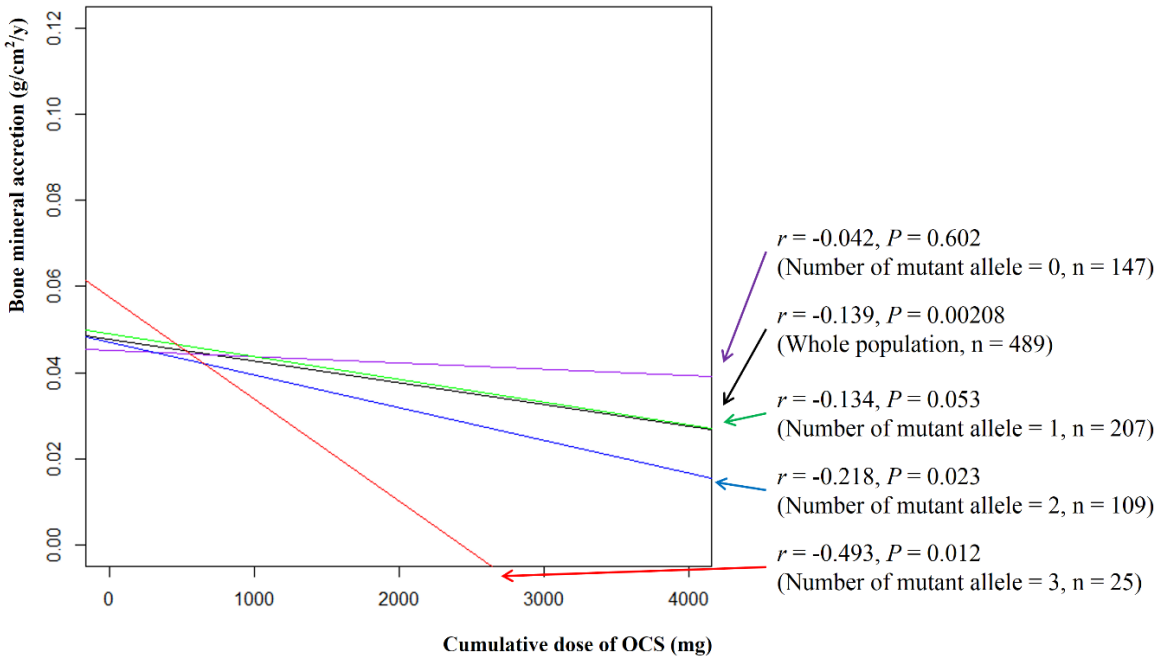
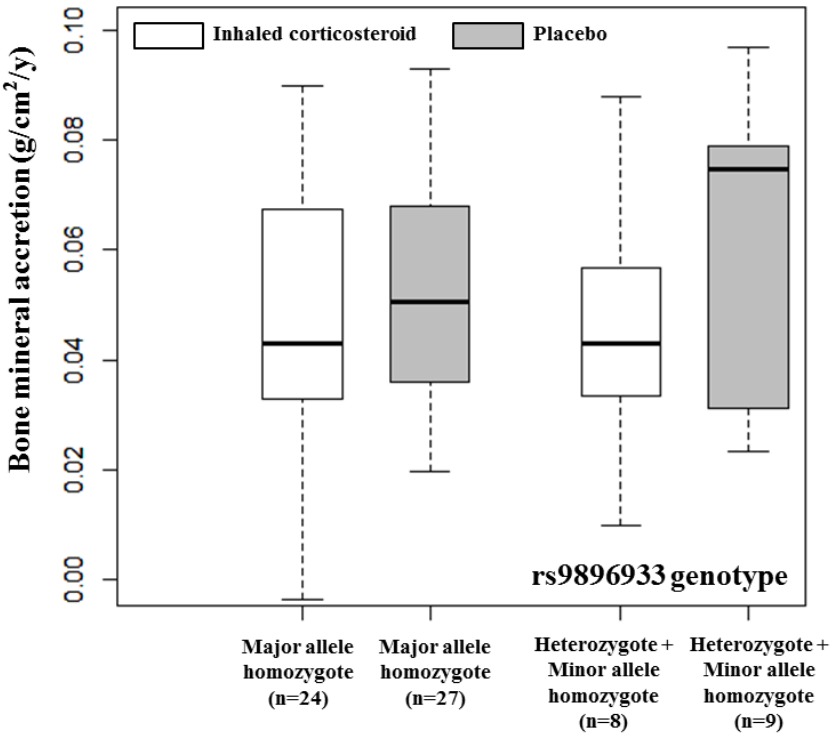

Bone mineral accretion (g/cm²/y)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0
1000
2000
3000
4000
Cumulative dose of OCS (mg)
r = -0.042, P = 0.602
(Number of mutant allele = 0, n = 147)
r = -0.139, P = 0.00208
(Whole population, n = 489)
r = -0.134, P = 0.053
(Number of mutant allele = 1, n = 207)
r = -0.218, P = 0.023
(Number of mutant allele = 2, n = 109)
r = -0.493, P = 0.012
(Number of mutant allele = 3, n = 25)

Figure E5.


Inhaled corticosteroid
Placebo
Bone mineral accretion (g/cm²/y)
0.00
0.02
0.04
0.06
0.08
0.10
rs9896933 genotype
Major allele homozygote (n=24)
Major allele homozygote (n=27)
Heterozygote + Minor allele homozygote (n=8)
Heterozygote + Minor allele homozygote (n=9)

CHR	SNP	BP	A1	TEST	NMISS	BETA	STAT
8	rs7003550	135920080	1	ADDxCUMPD	484	-8.15E-06	-5.767
17	rs9896933	78491377	4	ADDxCUMPD	484	-8.56E-06	-5.627
2	rs7599706	237470426	3	ADDxCUMPD	484	-1.49E-05	-5.584
16	rs12447718	87097635	1	ADDxCUMPD	483	-1.31E-05	-5.571
18	rs4368243	36637481	4	ADDxCUMPD	480	-5.99E-06	-5.497
7	rs2316527	154253390	1	ADDxCUMPD	484	-1.13E-05	-5.462
8	rs4484658	96277902	4	ADDxCUMPD	484	-8.90E-06	-5.451
18	rs7506840	6512756	1	ADDxCUMPD	484	-1.11E-05	-5.432
20	rs10485681	39926486	3	ADDxCUMPD	484	-8.63E-06	-5.412
14	rs12432642	26304106	2	ADDxCUMPD	482	-1.39E-05	-5.348
4	rs336381	143218594	4	ADDxCUMPD	484	-2.92E-05	-5.337
18	rs11875727	6525878	1	ADDxCUMPD	484	-8.49E-06	-5.291
16	rs3743957	19397959	1	ADDxCUMPD	482	-2.70E-05	-5.287
9	rs10812520	27037246	4	ADDxCUMPD	484	-7.06E-06	-5.286
18	rs770236	2518528	3	ADDxCUMPD	484	-6.31E-06	-5.279
18	rs1367685	36645822	1	ADDxCUMPD	484	-5.75E-06	-5.223
18	rs1896950	36652154	3	ADDxCUMPD	483	-5.74E-06	-5.214
9	rs7873432	12241115	3	ADDxCUMPD	483	-1.88E-05	-5.181
13	rs2117906	79709010	3	ADDxCUMPD	484	-1.05E-05	-5.177
18	rs12608087	19650555	4	ADDxCUMPD	484	-1.21E-05	-5.169
11	rs567199	79353368	4	ADDxCUMPD	484	-1.29E-05	-5.161
9	rs10960540	12227630	2	ADDxCUMPD	482	-1.87E-05	-5.158
8	rs16905252	135882875	3	ADDxCUMPD	484	-8.12E-06	-5.156
11	rs563836	79343580	2	ADDxCUMPD	483	-1.29E-05	-5.153
8	rs7818862	78737832	2	ADDxCUMPD	484	-6.10E-06	-5.138
7	rs1608500	39923354	2	ADDxCUMPD	484	-1.37E-05	-5.116
6	rs9487754	112343990	2	ADDxCUMPD	484	-1.79E-05	-5.11
16	rs12149765	46589830	1	ADDxCUMPD	484	-6.97E-06	-5.076
4	rs1491262	13301398	4	ADDxCUMPD	484	-7.63E-06	-5.07
20	rs11697381	39923770	3	ADDxCUMPD	484	-7.85E-06	-5.026
2	rs11899696	229403044	4	ADDxCUMPD	484	-1.45E-05	-5.002
18	rs17678758	36676308	1	ADDxCUMPD	484	5.68E-06	5.001
18	rs10502442	19836827	4	ADDxCUMPD	483	-1.18E-05	-4.999
8	rs2293879	17545895	4	ADDxCUMPD	484	-1.32E-05	-4.996
4	rs4466023	153312942	1	ADDxCUMPD	484	-1.22E-05	-4.991
10	rs1903876	67436477	2	ADDxCUMPD	484	-8.15E-06	-4.988
7	rs17623382	140238696	3	ADDxCUMPD	484	-8.36E-06	-4.983
19	rs3745788	51686673	3	ADDxCUMPD	484	-1.11E-05	-4.959
20	rs6072515	39937195	1	ADDxCUMPD	484	-6.59E-06	-4.953
8	rs7822757	78770264	4	ADDxCUMPD	482	-5.91E-06	-4.945
9	rs1002393	2483381	3	ADDxCUMPD	484	-8.21E-06	-4.941
22	rs7285863	29321124	4	ADDxCUMPD	484	-9.14E-06	-4.936
14	rs12880735	26994025	1	ADDxCUMPD	483	-5.99E-06	-4.934
14	rs6572555	48727274	1	ADDxCUMPD	484	-6.12E-06	-4.928
18	rs9965173	7201755	3	ADDxCUMPD	484	-1.09E-05	-4.927
8	rs1353739	89842767	1	ADDxCUMPD	484	-7.66E-06	-4.924

SNP	BETA_OS	P_OS	GENE
rs112369237	1.67846	9.73E-75	CD38
rs145016072	1.38126	2.63E-67	PRKCB1
rs10842534	-1.73043	2.73E-64	LRMP
rs112082439	2.37089	5.94E-60	MYH7
rs35076533	3.67106	1.57E-58	INDO
rs71429274	1.65205	2.44E-57	DES
rs187915687	3.00284	5.24E-55	GBP4
rs75380171	1.62739	3.56E-54	MYL4
rs186573288	1.16401	4.93E-54	HRC
rs201295780	0.813203	6.87E-53	UNQ5783
rs4149586	1.2784	5.82E-51	TNFRSF7
rs143643371	3.00608	5.43E-50	TNFRSF17
rs139506863	1.1875	1.75E-48	LAMP3
rs61828164	2.64194	2.28E-48	AIM2
rs142357617	0.798711	2.40E-48	CR2
rs71446273	0.960642	9.11E-48	FZD10
rs139094755	2.07021	6.60E-47	POU2AF1
rs11668714	1.09343	3.36E-46	HSH2D
rs72827441	1.27075	1.69E-45	IRF4
rs79286514	0.629891	3.56E-45	ZNF556
rs185822388	2.25339	1.26E-44	CD79A
rs111928500	0.95902	1.48E-44	CD79B
rs117563269	1.08162	1.65E-44	NCF1
rs201085497	0.790086	3.58E-44	AICDA
rs113604966	0.953991	4.29E-44	CACNG1
rs145670948	1.02995	4.68E-44	BLK
rs145495456	1.64687	5.54E-44	DHRS9
rs192431720	1.70851	1.18E-43	RSAD2
rs143202096	1.2059	1.36E-43	OASL
rs142950254	2.14217	1.47E-43	TNFSF7
rs33961580	2.54956	5.03E-43	SOX8
rs117551866	0.978061	7.10E-43	FCER2
rs111554017	-0.773563	1.64E-42	BCAS1
rs117644736	1.52226	3.07E-42	CKM
rs200421298	0.747791	3.07E-42	CYP2J2
rs141504323	1.49286	3.47E-42	TRIM55
rs117690530	2.00833	5.93E-42	OAS1
rs184707147	0.522112	1.75E-41	TCL1A
rs138349633	1.18714	6.30E-41	CLDN5
rs1762681	1.49729	8.50E-41	PRDM1
rs78758407	0.84547	1.27E-40	PTPRCAP
rs117461227	0.73743	1.43E-40	CBFA2T3
rs184821125	0.93691	5.50E-40	EEF1A2
rs199562883	-0.361184	6.69E-40	PTP4A3
rs189026316	0.784541	6.73E-40	COX6A2
rs4607407	0.528147	9.51E-40	CCR6