

**Epigenetic alterations of a novel antioxidant gene *SLC22A3* predispose susceptible individuals to increased risk of esophageal cancer**

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**Supplementary Tables**

**Table S1.** Summary of age and sex of ESCC high- and low-risk cohorts.

Cohort	Sex		Age (yrs)	
	Male	Female	Range	Mean±SD
<b>High-risk region</b>				
Cases	n=90	n=43	33-82	58.5±8.1
Controls	n=75	n=40	27-77	54.0±11.5
<b>Low-risk region</b>				
Cases	n=81	n=31	40-78	58.2±8.6
Controls	n=70	n=30	30-79	57.0±9.8

**Table S2.** List of PCR primers for cloning, expression, methylation, sequencing and mutagenesis analyses.

<b>PCR</b>	<b>Primer</b>	<b>Sequence (5'-3')</b>
<b>qPCR analysis</b>	SLC22A3-QF	TTTCTGCTCTTTTCGGCTAGCAG
	SLC22A3-QR	ATACCCTTGGTTTCAGGCAAAG
	GAPDH-QF	CATGAGAAGTATGACAACAGCCT
	GAPDH-QR	AGTCCTTCCACGATACCAAAGT
<b>Bisulfite sequencing</b>	SLCBGS-F1 (BGS Region 1)	GTAGGTTGTTGGTAGGGATT
	SLCBGS-R1 (BGS Region 1)	TCAAACACAACAACAAAACAC
<b>Methylight analysis</b>	SLCMF	AAAGGAGTTTCGCGTTAGGTC
	SLCMR	GCACCCGACCGAAATAAACG
	SLCPRO	6-FAM-ATACGACCTAACTACGCGCCAAAA-ZEN/3'IBFQ
	ACTBF	TGGTGATGGAGGAGGTTTAGTAAGT
	ACTBR	AACCAATAAAACCTACTCCTCCCTTAA
	ACTBPRO	VIC-ACCACCACCCAACACACAATAACAAACACA-MGB

**Table S3.** Summary of association results for 6q25 SNPs selected for post hoc genome-wide association study.

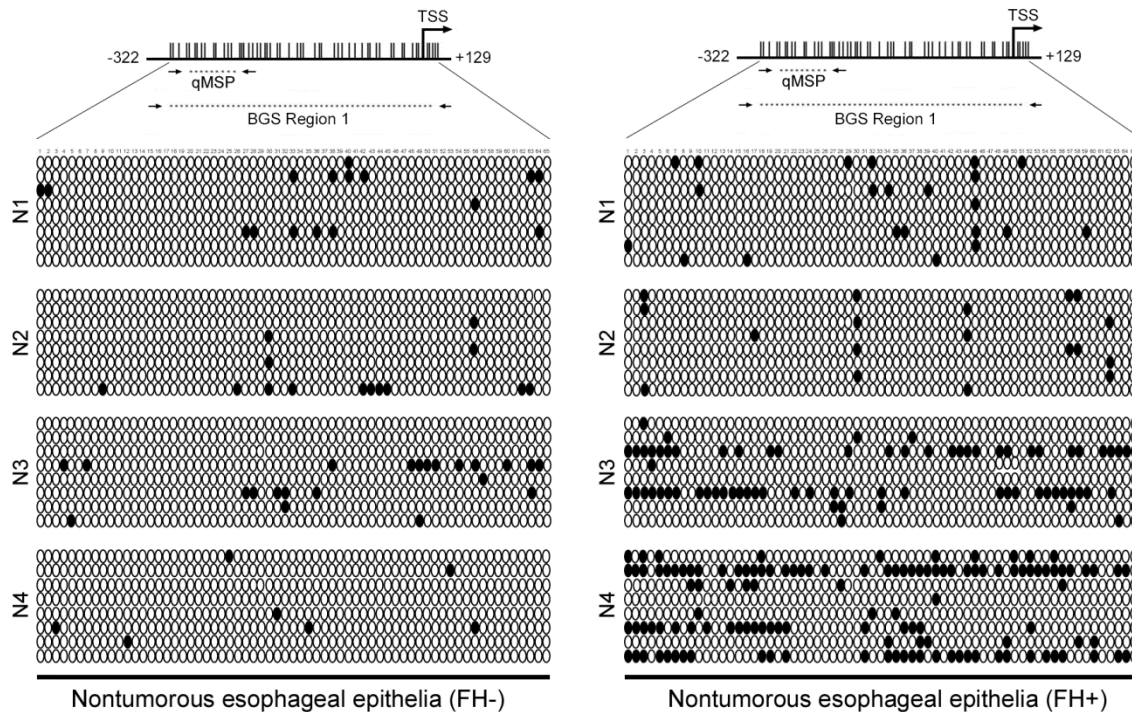
CHR	SNP	BP	Allele <sup>a</sup>	MAF <sup>b</sup>		$\chi^2$	P value	OR	95%CI
				F_A <sup>c</sup>	F_U <sup>d</sup>				
6q	<b>rs9457930<sup>e</sup></b>	160841556	A	0.15	0.26	34.32	4.68E-09	0.508	0.504-0.512
6q	rs651164	160501364	G	0.44	0.40	2.58	1.09E-01	1.140	1.134-1.146
6q	rs316009	160595754	A	0.05	0.04	2.58	1.09E-01	1.365	1.348-1.382
6q	rs486359	160694431	G	0.36	0.33	2.53	1.11E-01	1.146	1.140-1.152
6q	rs3119311	160613097	G	0.14	0.16	2.32	1.28E-01	0.838	0.832-0.844
6q	rs2665355	160757358	C	0.36	0.39	2.03	1.54E-01	0.887	0.883-0.892
6q	rs539298	160690350	G	0.36	0.34	1.81	1.79E-01	1.120	1.114-1.126
6q	rs2450973	160558745	A	0.11	0.09	1.80	1.80E-01	1.195	1.185-1.205
6q	rs596881	160557229	A	0.11	0.09	1.74	1.88E-01	1.189	1.179-1.199
6q	rs3127575	160564002	A	0.04	0.05	1.49	2.23E-01	0.782	0.772-0.792
6q	rs3103353	160558066	A	0.04	0.05	1.44	2.30E-01	0.785	0.775-0.795
6q	rs6455682	160501901	A	0.41	0.38	1.39	2.38E-01	1.102	1.096-1.108
6q	rs474617	160620431	G	0.42	0.40	1.37	2.42E-01	1.100	1.095-1.106
6q	rs3127594	160558094	T	0.04	0.05	1.33	2.48E-01	0.792	0.782-0.802
6q	rs3103352	160558715	A	0.04	0.05	1.33	2.49E-01	0.793	0.783-0.803
6q	rs366920	160657940	G	0.34	0.31	1.32	2.50E-01	1.106	1.100-1.112
6q	rs569919	160687173	A	0.36	0.34	1.26	2.62E-01	1.099	1.093-1.105
6q	rs677985	160539896	G	0.11	0.13	1.18	2.77E-01	0.872	0.865-0.879
6q	rs7761031	160615732	G	0.22	0.20	1.17	2.80E-01	1.112	1.105-1.119
6q	rs2450974	160558735	G	0.11	0.09	1.09	2.97E-01	1.148	1.139-1.158
6q	rs3127583	160647121	A	0.12	0.13	1.07	3.00E-01	0.879	0.873-0.886
6q	rs2481031	160676719	C	0.12	0.13	1.00	3.18E-01	0.885	0.878-0.891
6q	rs446291	160661096	A	0.13	0.12	0.98	3.22E-01	1.127	1.118-1.135
6q	rs4646283	160501492	G	0.05	0.06	0.81	3.69E-01	0.848	0.839-0.858
6q	rs2634658	160620759	G	0.22	0.20	0.72	3.95E-01	1.087	1.080-1.094
6q	rs1018234	160716048	A	0.42	0.40	0.69	4.08E-01	1.070	1.065-1.076
6q	rs316003	160565822	G	0.19	0.18	0.67	4.14E-01	1.088	1.081-1.095
6q	rs315990	160582133	C	0.19	0.18	0.67	4.14E-01	1.088	1.081-1.095
6q	rs315995	160585451	G	0.19	0.18	0.62	4.29E-01	1.085	1.078-1.092
6q	rs316007	160596398	G	0.14	0.13	0.62	4.32E-01	1.096	1.088-1.104
6q	rs2774230	160597604	C	0.14	0.13	0.62	4.32E-01	1.096	1.088-1.104
6q	rs479078	160566602	G	0.19	0.18	0.57	4.52E-01	1.080	1.073-1.087
6q	rs1685986	160626651	A	0.21	0.20	0.56	4.55E-01	1.077	1.070-1.084
6q	rs316019	160590272	A	0.12	0.11	0.55	4.57E-01	1.098	1.089-1.107
6q	rs388170	160773276	A	0.37	0.38	0.53	4.65E-01	0.941	0.936-0.946
6q	rs9365168	160860110	G	0.49	0.48	0.52	4.69E-01	1.060	1.055-1.066
6q	rs7761377	160952471	G	0.46	0.48	0.52	4.72E-01	0.941	0.937-0.946
6q	rs638360	160598483	C	0.14	0.13	0.50	4.79E-01	1.086	1.079-1.094
6q	rs617217	160586346	G	0.18	0.17	0.50	4.82E-01	1.077	1.070-1.085
6q	rs316030	160607856	C	0.42	0.43	0.49	4.86E-01	0.945	0.940-0.949
6q	rs3125056	160655271	G	0.02	0.03	0.48	4.89E-01	0.834	0.821-0.848

6q	rs2450975	160557965	A	0.16	0.15	0.47	4.92E-01	1.080	1.072-1.087
6q	rs2457571	160754818	A	0.32	0.34	0.45	5.00E-01	0.944	0.939-0.949
6q	rs2665357	160768157	A	0.49	0.47	0.42	5.15E-01	1.054	1.049-1.059
6q	rs511215	160624933	A	0.13	0.12	0.41	5.24E-01	1.080	1.072-1.089
6q	rs3123629	160826076	A	0.15	0.14	0.40	5.25E-01	1.075	1.067-1.083
6q	rs3119312	160620291	G	0.20	0.21	0.40	5.28E-01	0.939	0.933-0.945
6q	rs316167	160674742	A	0.49	0.48	0.39	5.34E-01	1.052	1.047-1.057
6q	rs2619268	160545968	A	0.44	0.45	0.36	5.46E-01	0.952	0.947-0.957
6q	rs4708867	160762715	G	0.09	0.10	0.35	5.52E-01	0.920	0.912-0.928
6q	rs7774579	160851139	G	0.49	0.48	0.35	5.56E-01	1.050	1.044-1.055
6q	rs9364554	160753654	A	0.32	0.33	0.34	5.57E-01	0.951	0.946-0.956
6q	rs572149	160685019	G	0.41	0.39	0.32	5.71E-01	1.048	1.042-1.053
6q	rs316021	160588031	G	0.23	0.22	0.30	5.84E-01	1.054	1.048-1.060
6q	rs7449650	160877104	C	0.48	0.47	0.30	5.84E-01	1.045	1.040-1.051
6q	rs791195	160518476	A	0.20	0.19	0.30	5.86E-01	1.057	1.050-1.064
6q	rs9365164	160776833	A	0.36	0.37	0.29	5.88E-01	0.956	0.951-0.961
6q	rs6455688	160916317	G	0.44	0.43	0.28	5.94E-01	1.044	1.039-1.050
6q	rs393271	160662229	G	0.48	0.47	0.28	5.97E-01	1.044	1.038-1.049
6q	rs3798221	160918138	A	0.44	0.43	0.28	5.98E-01	1.044	1.039-1.049
6q	rs3912161	160589708	G	0.09	0.10	0.27	6.02E-01	0.929	0.921-0.937
6q	rs7740824	160728891	A	0.32	0.33	0.27	6.05E-01	0.956	0.951-0.962
6q	rs316016	160591490	A	0.21	0.21	0.25	6.20E-01	1.050	1.044-1.057
6q	rs316015	160592254	G	0.21	0.21	0.25	6.20E-01	1.050	1.044-1.057
6q	rs3127572	160602208	G	0.10	0.10	0.24	6.23E-01	0.936	0.928-0.944
6q	rs2221750	160728437	A	0.23	0.22	0.24	6.25E-01	1.048	1.042-1.055
6q	rs316013	160592615	G	0.21	0.21	0.24	6.26E-01	1.049	1.043-1.056
6q	rs7745775	160753458	C	0.23	0.22	0.24	6.27E-01	1.048	1.042-1.054
6q	rs316018	160590484	A	0.21	0.21	0.23	6.28E-01	1.049	1.043-1.056
6q	rs3127602	160812231	A	0.15	0.14	0.23	6.34E-01	1.056	1.049-1.064
6q	rs368123	160639583	G	0.46	0.45	0.22	6.43E-01	1.038	1.033-1.044
6q	rs2076828	160792776	G	0.43	0.44	0.20	6.52E-01	0.964	0.959-0.969
6q	rs13209686	160515223	G	0.16	0.16	0.20	6.55E-01	0.952	0.945-0.958
6q	rs2292334	160778178	A	0.43	0.44	0.19	6.62E-01	0.965	0.960-0.970
6q	rs6923877	160916787	G	0.44	0.43	0.18	6.73E-01	1.035	1.030-1.040
6q	rs568567	160625144	G	0.41	0.41	0.18	6.73E-01	0.966	0.961-0.971
6q	rs2504926	160796280	A	0.43	0.44	0.17	6.83E-01	0.967	0.962-0.972
6q	rs7761293	160890953	A	0.15	0.15	0.16	6.88E-01	1.046	1.039-1.054
6q	rs2048327	160783522	G	0.43	0.44	0.15	7.03E-01	0.969	0.964-0.974
6q	rs10945673	160830889	A	0.34	0.33	0.15	7.03E-01	1.034	1.028-1.039
6q	rs9355803	160823168	A	0.34	0.33	0.14	7.05E-01	1.033	1.027-1.038
6q	rs2457564	160797630	A	0.48	0.47	0.14	7.07E-01	1.031	1.026-1.036
6q	rs9355296	160937983	A	0.41	0.42	0.13	7.24E-01	0.971	0.966-0.976
6q	rs316014	160592496	T	0.21	0.21	0.12	7.24E-01	1.036	1.029-1.042
6q	rs316029	160606658	A	0.09	0.08	0.12	7.26E-01	1.051	1.042-1.061
6q	rs9346816	160702652	A	0.41	0.40	0.10	7.47E-01	1.027	1.022-1.032
6q	rs9355804	160823612	A	0.34	0.33	0.10	7.52E-01	1.027	1.022-1.033

6q	rs791190	160533697	A	0.20	0.19	0.10	7.54E-01	1.032	1.026-1.039
6q	rs9364552	160702924	C	0.41	0.40	0.10	7.55E-01	1.026	1.021-1.031
6q	rs791183	160530114	G	0.43	0.43	0.08	7.73E-01	1.024	1.019-1.029
6q	rs554811	160614942	A	0.49	0.48	0.08	7.74E-01	1.024	1.018-1.029
6q	rs13202636	160949718	G	0.46	0.46	0.08	7.82E-01	1.023	1.018-1.028
6q	rs9365179	160944838	A	0.47	0.46	0.07	7.92E-01	1.022	1.016-1.027
6q	rs492315	160612684	A	0.18	0.18	0.06	8.08E-01	0.975	0.968-0.981
6q	rs10945682	160989931	A	0.46	0.46	0.05	8.18E-01	1.019	1.014-1.024
6q	rs512077	160694918	A	0.15	0.15	0.04	8.34E-01	0.977	0.970-0.984
6q	rs578560	160619688	G	0.10	0.10	0.04	8.47E-01	1.026	1.018-1.035
6q	rs2063345	160780144	G	0.42	0.42	0.03	8.56E-01	1.015	1.010-1.020
6q	rs1479353	160513864	C	0.36	0.36	0.02	8.94E-01	1.011	1.006-1.017
6q	rs3105748	160730710	G	0.45	0.45	0.02	8.98E-01	1.010	1.005-1.016
6q	rs10806731	160747825	A	0.45	0.45	0.02	8.98E-01	1.010	1.005-1.016
6q	rs9365171	160901726	A	0.37	0.37	0.01	9.34E-01	0.993	0.988-0.998
6q	rs10945657	160572919	C	0.08	0.08	0.01	9.34E-01	0.988	0.979-0.997
6q	rs2504921	160813996	A	0.34	0.34	0.01	9.40E-01	1.007	1.001-1.012
6q	rs7758229	160760242	A	0.24	0.24	0.01	9.42E-01	0.993	0.987-0.999
6q	rs4709432	160773919	G	0.23	0.23	0.00	9.55E-01	0.995	0.989-1.001
6q	rs3125050	160660826	C	0.08	0.08	0.00	9.56E-01	1.008	0.999-1.018
6q	rs2504920	160817862	C	0.04	0.04	0.00	9.60E-01	1.010	0.998-1.023
6q	rs3105749	160740968	G	0.45	0.45	0.00	9.60E-01	1.004	0.999-1.009
6q	rs1871389	160507511	A	0.36	0.36	0.00	9.67E-01	1.004	0.998-1.009
6q	rs2083867	160503038	G	0.36	0.36	0.00	9.70E-01	0.997	0.992-1.002
6q	rs3127574	160711360	G	0.45	0.45	0.00	9.86E-01	1.001	0.996-1.007
6q	rs884742	160727422	C	0.45	0.45	0.00	9.94E-01	1.001	0.996-1.006

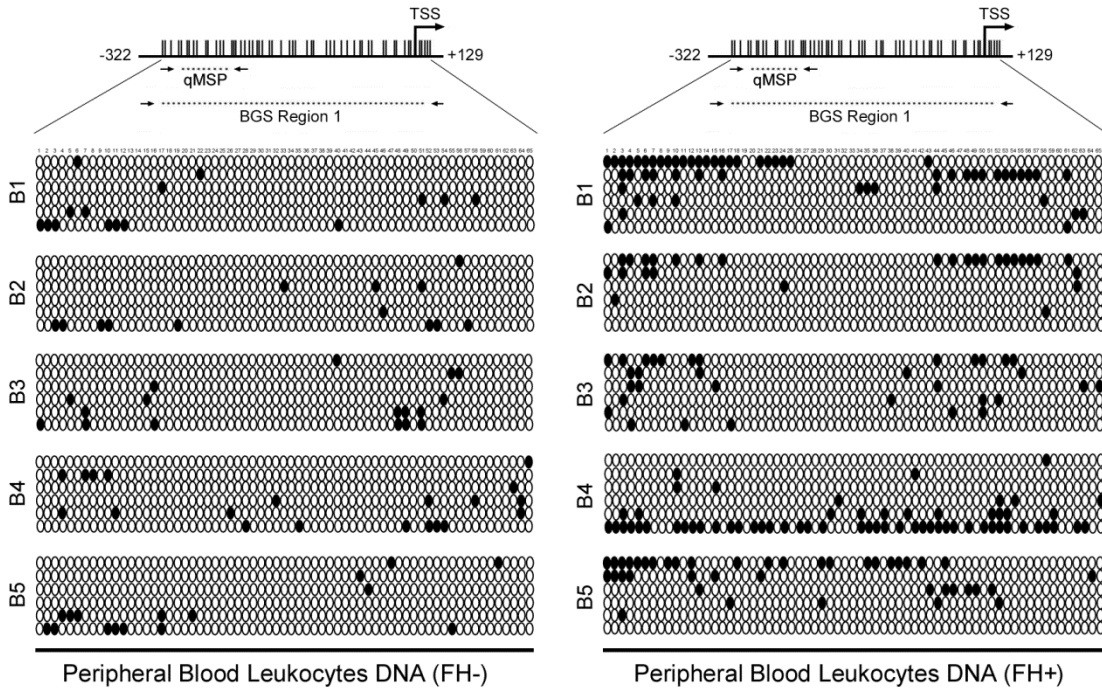
<sup>a</sup>Minor allele; <sup>b</sup>Minor allele frequency; <sup>c</sup>Frequency of minor allele in cases; <sup>d</sup>Frequency of minor allele in controls; <sup>e</sup>6q SNP significantly associated with ESCC risk in FH+ cases near the gene *SLC22A3* is in bold.

## Supplementary Figures



Xiong et al. Suppl. Figure 1

**Figure S1. Bisulfite sequencing of the *SLC22A3* proximal promoter in nontumorous esophageal epithelia from FH- and FH+ ESCCs.** Detailed methylation profiles of the *SLC22A3* BGS region 1 (-322 to +129), comprised of 65 CpG sites, in nontumorous esophageal epithelia from FH- ESCCs (n = 4, left) and FH+ ESCCs (n = 4, right). 8-10 colonies were sequenced for each DNA sample. Each allele is represented by a horizontal row, within which each CpG is represented by a circle. Methylated CpGs are shown in black and unmethylated CpGs in white.



Xiong et al. Suppl. Figure 2

**Figure S2. Bisulfite sequencing of the *SLC22A3* proximal promoter in peripheral blood leukocytes (PBLs) from FH- and FH+ ESCCs.** Detailed methylation profiles of the *SLC22A3* BGS region 1 (-322 to +129), comprised of 65 CpG sites, in PBLs from FH- ESCCs (n = 5, left) and FH+ ESCCs (n = 5, right). 6-8 colonies were sequenced for each DNA sample. Each allele is represented by a horizontal row, within which each CpG is represented by a circle. Methylated CpGs are shown in black and unmethylated CpGs in white.