

INFLUENCE OF CONTROL SELECTION IN GENOME-WIDE ASSOCIATION STUDY: THE EXAMPLE OF DIABETES IN THE FRAMINGHAM HEART STUDY.

Genetic Analysis Workshop 2008

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OBJECTIVES

Evaluate the influence of nested case-control design on results of genetic association studies.

Design 1: Selection by incidence density sampling

➤ Sample controls from the set of all participants at risk for disease at the time the case became affected.

- No prevalent cases
- Later cases can serve as controls for earlier cases

Design 2: Selection based on affection status at end of study follow up

- Prevalent & incident cases
- No one ever affected by end of study can serve as control (“case exclusion”)

FHS COHORT

- A community-based, multigenerational, longitudinal study of cardiovascular disease and its risk factors, including diabetes.
- Began in 1948 to investigate the causes of heart disease.
- 6752 subjects = 765 pedigrees with 2 to 301 genotyped subjects:
 - 134 pedigrees with 2
 - 123 with 3
 - 98 with 4
 - 85 with 5
 - 177 with 6 to 10
 - 72 with 11 to 15
 - 30 with 16 to 20 and
 - 46 with more than 20

EXAMPLE

- **T2D (Type 2 Diabetes)** is metabolic disorder primarily characterized by insulin resistance, relative insulin deficiency, and hyperglycemia.

WHO definition:

- Fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl)
or
- OGGT +2hrs plasma glucose ≥ 11.1 mmol/l (200 mg/dl)

In FHS:

- Original Cohort, casual blood sugar > 200 mg/dl or treatment for diabetes.
- Offspring and Gen3 Cohorts, fasting blood sugar > 125 mg/dl or on treatment.

18 SNPS IN ANALYSIS

➤ Analyzed SNP variants convincingly associated with T2D in recent studies

SNPs	Genes/Regions	OR	LowerCL	UpperCL	P-value
rs4607103	<i>ADAMTS9</i>	1.09	1.06	1.12	1.20E-08
rs4747969	<i>CDC123</i>	1.11	1.07	1.14	1.20E-10
rs10946398	<i>CDKAL1</i>	1.14	1.11	1.17	2.00E-18
rs7754840	<i>CDKAL1</i>	1.12	1.08	1.16	4.10E-11
rs10811661	<i>CDKN2A/2B</i>	1.20	1.14	1.25	2.00E-15
rs564398	<i>CDKN2A/2B</i>	1.12	1.07	1.17	1.20E-07
rs8050136	<i>FTO</i>	1.17	1.12	1.22	1.00E-12
rs9939609	<i>FTO</i>	1.11	1.02	1.20	1.43E-02
rs5015480	<i>HHEX</i>	1.15	1.10	1.19	7.00E-17
rs4402960	<i>IGF2BP2</i>	1.14	1.11	1.18	9.00E-16
rs864745	<i>JAZF1</i>	1.10	1.07	1.13	5.00E-14
rs5215	<i>KCNJ11</i>	1.14	1.10	1.19	5.00E-11
rs1801282	<i>PPARG</i>	1.14	1.08	1.20	2.0E-06
rs10885409	<i>TCF7L2</i>				1.20E-05
rs7901695	<i>TCF7L2</i>	1.37	1.31	1.43	1.00E-48
rs13431070	<i>THADA</i>	1.15	1.10	1.20	1.10E-09
rs7961581	<i>TSPAN8/LGR5</i>	1.09	1.06	1.12	1.10E-09
rs4689394	<i>WFS1</i>	1.11	1.08	1.16	1.00E-07

18 SNPS IN ANALYSIS (2)

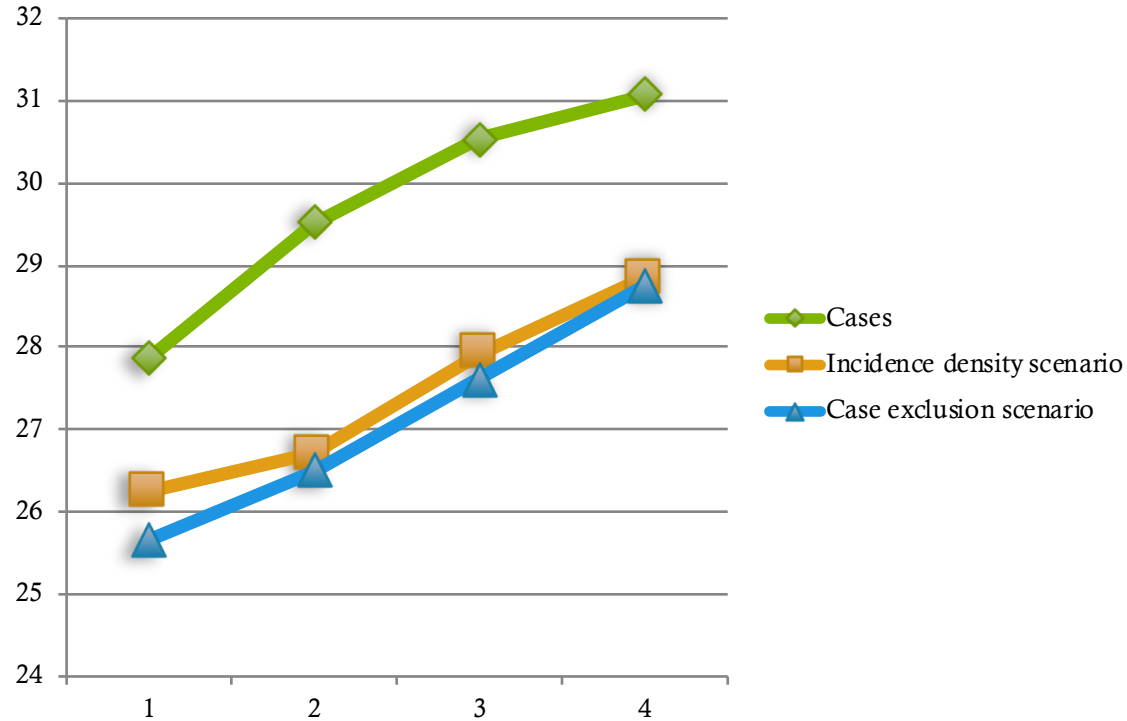
➤ For SNPs not available in our FHS data, we have used SNPs with a $r^2 > 0.8$ instead of them :

- *HHEX*; rs5015480 ($r^2=1$ with rs1111875)
- *TCF7L2*; rs7901695 ($r^2=0.8$ with rs7903146)
- *KCNJ11*; rs5215 ($r^2=0.89$ with rs5219)
- *CDC123*; rs4747969 ($r^2=0.83$ with rs12779790)
- *WFS1*; rs4689394 ($r^2=1$ with rs10010131)
- *THADA*; rs13431070 ($r^2=1$ with rs7578597)

COFOUNDING FACTORS

➤ BMI: Body Mass Index

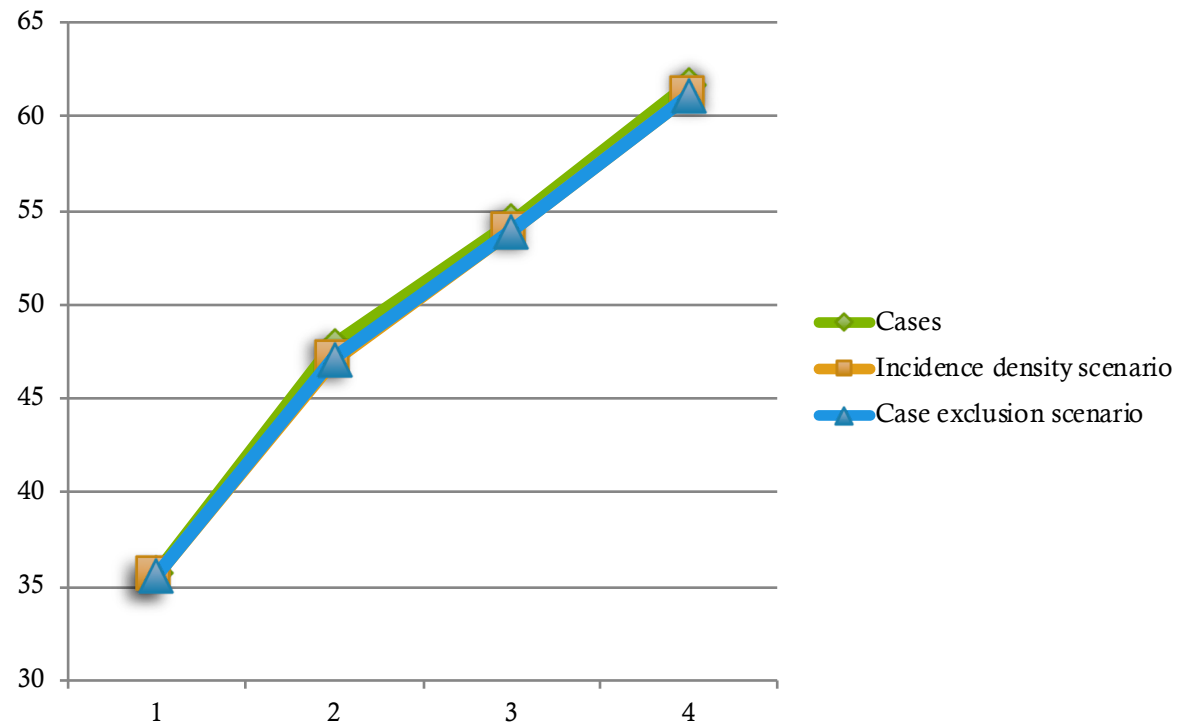
We have matched cases and controls on BMI at enrollment, i.e. BMI at the first visit.



COFOUNDING FACTORS (2)

➤ Age

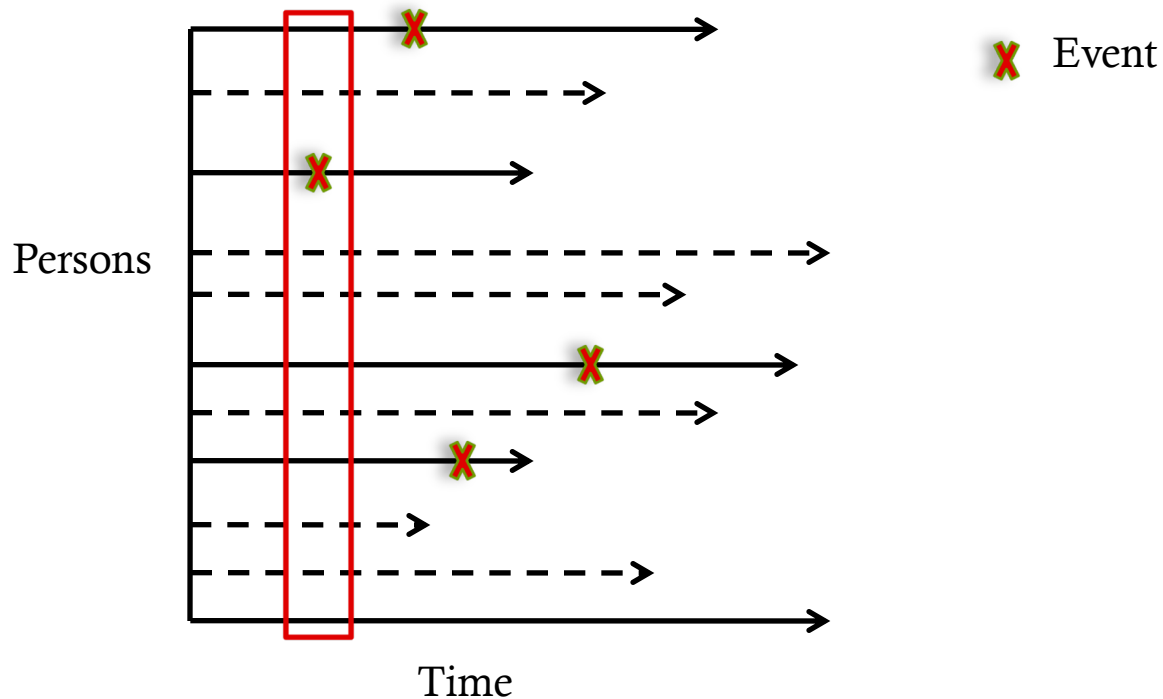
In our analysis, we have matched cases and controls on age at enrollment, i.e. age at the first visit.



APPROACH

- **Incidence density sampling method** in Longitudinal study:
Compare 1 case to 10 non-cases at time of event

Principle:



APPROACH (2)

Create a risk set for each case = Identify all members of the cohort at risk for the event at the time the case is diagnosed. Potential confounders could be added to definition of risk set (rs) (we used age, BMI and sex).

shareid	pedno	fshare	mshare	sex	age1	bmi1	diabetes	censor	rs
8888	138	19410	19996	2	40	23.69	1	1	9
4926	1236			2	37	23.59	0	0	9
8211	1524			2	45	23.05	0	0	9
17326	1495	26922	26923	2	41	25.51	0	0	9
2974	1524	25547	5650	2	44	22.14	0	0	9
4240	563	5748	10244	2	41	24.24	0	0	9
2155	872			2	40	24.21	0	0	9
12202	78			2	42	22.13	1	0	9
11460	582	16382	4596	2	37	25.97	0	0	9
12704	1524	26920	26921	2	43	25.7	1	0	9
22544	901	16918	4167	2	37	24.51	0	0	9

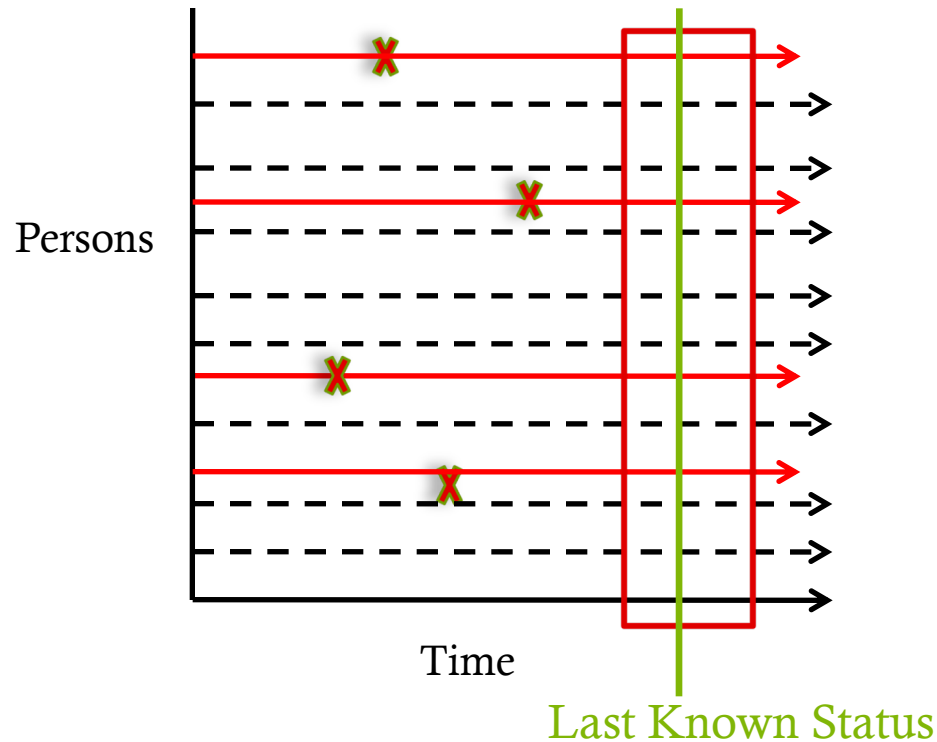
Criteria: Age \pm 5years; BMI \pm 2kg/m²

Cleaning data: 1976 individuals in our global sample, after removing duplicate = 719 individuals

APPROACH (3)

Case exclusion sampling method in Longitudinal study:
Compare 1 case to 10 non-cases at time of event

Principle:



APPROACH (4)

Select matched controls for each case = Identify all members of the controls cohort matched with the case included in the cases cohort. Potential confounders could be added. (we used age, BMI and sex)

shareid	pedno	fshare	mshare	age1	bmi1	diabetes	cancel	rs	
6598	74	20068	5747	36	23.56	1	1	1	3
16212	1528	26982	26983	35	23.03	0	0	0	3
23305	1506			33	24.03	0	0	0	3
22915	1348			40	24.33	0	0	0	3
15557	744			37	24.2	0	0	0	3
23437	1227	22312	16068	31	24.8	0	0	0	3
10924	1537	24997	21985	34	23.69	0	0	0	3
4915	425	7709	15894	37	25.51	0	0	0	3
9609	527			35	25.82	0	0	0	3
23721	1444	5672	26299	40	23.24	0	0	0	3
9629	521	17639	4213	40	24.27	0	0	0	3

RESULTS: INCIDENCE DENSITY

➤ All SNPs are in HWE for the 10 generated samples

SNPs	Sample 1			Sample 2			Sample 3			Sample 4			Sample 5			Sample 6			Sample 7			Sample 8			Sample 9			Sample 10		
	OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI	
rs13431070	0.732	0.461	1.161	0.494	0.312	0.784	0.701	0.450	1.092	0.626	0.392	1.002	0.647	0.419	1.000	0.708	0.449	1.116	0.541	0.341	0.857	0.610	0.388	0.960	0.575	0.361	0.918	0.654	0.419	1.019
rs1801282	0.839	0.569	1.235	0.889	0.616	1.283	1.107	0.753	1.627	0.899	0.619	1.307	0.912	0.628	1.324	1.024	0.708	1.481	0.877	0.601	1.281	0.876	0.601	1.276	0.994	0.681	1.453	1.097	0.746	1.613
rs4402960	1.055	0.819	1.359	0.975	0.761	1.250	1.042	0.812	1.337	1.002	0.781	1.287	1.151	0.896	1.477	0.959	0.748	1.229	1.171	0.914	1.502	1.161	0.905	1.489	1.150	0.898	1.472	1.114	0.870	1.427
rs4607103	0.929	0.699	1.235	0.850	0.644	1.122	0.896	0.675	1.191	0.949	0.718	1.255	0.950	0.716	1.260	0.976	0.734	1.299	1.057	0.798	1.401	1.040	0.781	1.385	1.066	0.802	1.417	0.987	0.744	1.308
rs468939	0.985	0.770	1.259	0.934	0.729	1.197	1.023	0.800	1.307	1.008	0.792	1.284	1.012	0.790	1.296	0.955	0.749	1.218	0.930	0.726	1.191	0.932	0.731	1.187	0.977	0.768	1.244	0.938	0.731	1.204
rs7754840	1.272	0.985	1.643	1.269	0.981	1.641	1.210	0.935	1.566	1.114	0.864	1.437	1.268	0.990	1.625	1.195	0.930	1.537	1.258	0.973	1.626	1.421	1.099	1.839	1.327	1.024	1.718	1.340	1.035	1.736
rs10946398	1.283	0.993	1.658	1.280	0.990	1.656	1.221	0.943	1.580	1.124	0.871	1.450	1.279	0.998	1.640	1.206	0.937	1.551	1.270	0.982	1.642	1.434	1.108	1.856	1.339	1.033	1.735	1.353	1.044	1.753
rs864745	0.848	0.671	1.071	0.782	0.615	0.994	0.840	0.665	1.060	0.821	0.648	1.039	0.850	0.673	1.074	0.843	0.663	1.072	0.851	0.672	1.078	0.908	0.720	1.145	0.902	0.714	1.140	0.843	0.667	1.066
rs10811661	0.812	0.584	1.129	0.927	0.670	1.282	0.775	0.556	1.079	0.779	0.558	1.087	0.829	0.592	1.162	0.907	0.654	1.258	0.712	0.512	0.989	0.764	0.551	1.060	0.854	0.614	1.189	0.813	0.579	1.141
rs564398	1.126	0.873	1.451	1.062	0.825	1.367	1.055	0.824	1.351	1.035	0.804	1.332	1.030	0.800	1.328	1.058	0.820	1.365	1.137	0.887	1.459	0.998	0.775	1.287	0.999	0.783	1.273	1.110	0.861	1.432
rs5015480	0.897	0.705	1.141	0.849	0.664	1.085	0.808	0.633	1.031	0.895	0.699	1.146	0.843	0.663	1.073	0.775	0.604	0.993	0.909	0.713	1.158	0.852	0.668	1.086	0.895	0.704	1.138	0.811	0.634	1.036
rs10885409	0.733	0.574	0.936	0.746	0.589	0.944	0.789	0.618	1.008	0.740	0.584	0.939	0.682	0.538	0.866	0.761	0.595	0.973	0.785	0.618	0.998	0.747	0.584	0.955	0.750	0.589	0.955	0.711	0.556	0.910
rs7901695	1.455	1.128	1.875	1.453	1.130	1.869	1.323	1.031	1.700	1.399	1.085	1.803	1.529	1.194	1.957	1.509	1.170	1.946	1.360	1.058	1.747	1.474	1.148	1.891	1.439	1.120	1.849	1.505	1.164	1.946
rs4747969	0.763	0.544	1.071	0.756	0.538	1.062	0.841	0.605	1.168	0.708	0.508	0.988	0.871	0.624	1.214	0.866	0.621	1.209	0.775	0.557	1.080	0.836	0.602	1.161	0.816	0.587	1.135	0.840	0.599	1.178
rs5215	0.935	0.730	1.199	1.009	0.786	1.296	0.962	0.746	1.241	0.998	0.776	1.282	1.023	0.796	1.313	0.983	0.766	1.260	0.923	0.719	1.186	0.928	0.722	1.194	0.918	0.710	1.186	0.918	0.709	1.190
rs7961581	1.013	0.783	1.311	0.990	0.761	1.289	1.048	0.803	1.367	1.078	0.833	1.395	0.951	0.736	1.230	1.086	0.834	1.415	0.987	0.760	1.283	1.008	0.781	1.301	1.130	0.873	1.463	1.081	0.831	1.407
rs9939609	1.061	0.826	1.363	1.051	0.819	1.347	1.065	0.827	1.372	1.109	0.864	1.422	1.092	0.852	1.400	1.064	0.832	1.360	1.092	0.852	1.399	1.061	0.827	1.361	1.156	0.901	1.483	1.200	0.934	1.543
rs8050136	1.065	0.829	1.367	1.051	0.819	1.347	1.062	0.824	1.368	1.095	0.854	1.405	1.084	0.845	1.390	1.059	0.829	1.354	1.096	0.856	1.404	1.058	0.824	1.357	1.148	0.895	1.474	1.197	0.931	1.538
Cases (n)	144			145			136			147			142			143			139			139			137			135		
Controls (n)	572			580			573			574			571			568			583			582			581			587		

RESULTS: CASE EXCLUSION

➤ All SNPs are in HWE for the 10 generated samples

SNPs	Sample 1			Sample 2			Sample 3			Sample 4			Sample 5			Sample 6			Sample 7			Sample 8			Sample 9			Sample 10					
	OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI		OR	CI				
rs13431070	0.626	0.407	0.963	0.641	0.420	0.976	0.633	0.412	0.971	0.635	0.410	0.985	0.697	0.450	1.080	0.648	0.415	1.010	0.738	0.474	1.150	0.637	0.414	0.980	0.639	0.413	0.990	0.681	0.442	1.051			
rs1801282	1.221	0.836	1.785	1.014	0.698	1.472	0.966	0.666	1.402	1.000	0.688	1.453	0.898	0.623	1.296	1.061	0.724	1.555	1.058	0.736	1.520	1.041	0.718	1.509	1.123	0.778	1.622	0.897	0.617	1.303			
rs4402960	1.144	0.893	1.467	0.959	0.749	1.228	1.015	0.792	1.300	1.030	0.804	1.320	1.042	0.813	1.334	0.979	0.764	1.254	1.129	0.880	1.447	1.012	0.789	1.299	1.042	0.811	1.340	1.039	0.808	1.337			
rs4607103	0.952	0.718	1.263	0.935	0.704	1.243	0.938	0.707	1.245	0.992	0.744	1.322	1.007	0.761	1.333	1.029	0.777	1.363	0.890	0.671	1.180	0.965	0.730	1.277	0.983	0.738	1.309	0.881	0.664	1.168			
rs468939	0.835	0.657	1.063	0.853	0.671	1.084	0.878	0.686	1.124	0.805	0.630	1.028	0.758	0.593	0.968	0.847	0.666	1.076	0.867	0.681	1.104	0.902	0.706	1.153	0.854	0.667	1.094	0.835	0.656	1.063			
rs7754840	1.193	0.934	1.523	1.228	0.956	1.579	1.163	0.910	1.487	1.247	0.969	1.605	1.140	0.891	1.459	1.204	0.933	1.553	1.109	0.861	1.429	1.236	0.960	1.591	1.054	0.822	1.353	1.204	0.938	1.546			
rs10946398	1.197	0.937	1.529	1.239	0.964	1.593	1.173	0.918	1.500	1.255	0.975	1.616	1.147	0.896	1.469	1.205	0.933	1.556	1.113	0.864	1.435	1.247	0.968	1.606	1.058	0.824	1.358	1.216	0.947	1.561			
rs864745	0.856	0.678	1.079	0.846	0.668	1.071	0.833	0.662	1.049	0.807	0.637	1.023	0.825	0.649	1.048	0.871	0.689	1.101	0.785	0.622	0.990	0.808	0.639	1.022	0.915	0.725	1.154	0.780	0.616	0.986			
rs10811661	0.806	0.582	1.117	0.840	0.601	1.173	0.792	0.568	1.105	0.813	0.584	1.134	0.847	0.608	1.180	0.842	0.604	1.173	0.805	0.578	1.122	0.784	0.563	1.093	0.854	0.613	1.191	0.837	0.598	1.172			
rs564398	0.946	0.739	1.211	0.937	0.733	1.198	1.019	0.793	1.308	1.122	0.872	1.442	1.086	0.843	1.400	0.988	0.768	1.271	0.965	0.753	1.237	1.019	0.794	1.307	1.011	0.794	1.289	1.085	0.844	1.394			
rs5015480	0.875	0.688	1.111	0.902	0.710	1.146	0.836	0.655	1.067	0.869	0.681	1.108	0.838	0.656	1.071	0.857	0.670	1.096	0.877	0.687	1.119	0.800	0.626	1.023	0.875	0.686	1.116	0.842	0.657	1.079			
rs10885409	0.770	0.607	0.976	0.797	0.629	1.008	0.779	0.612	0.992	0.804	0.633	1.021	0.787	0.621	0.998	0.790	0.621	1.005	0.796	0.630	1.006	0.815	0.640	1.038	0.846	0.667	1.072	0.801	0.631	1.018			
rs7901695	1.653	1.289	2.122	1.480	1.157	1.893	1.494	1.157	1.930	1.443	1.130	1.842	1.399	1.095	1.788	1.442	1.128	1.844	1.436	1.125	1.834	1.581	1.231	2.032	1.417	1.104	1.820	1.492	1.167	1.907			
rs4747969	0.710	0.508	0.992	0.703	0.507	0.975	0.667	0.477	0.933	0.784	0.558	1.101	0.721	0.514	1.010	0.699	0.501	0.976	0.675	0.482	0.945	0.702	0.502	0.982	0.695	0.499	0.968	0.705	0.504	0.987			
rs5215	0.916	0.716	1.173	0.963	0.756	1.227	0.975	0.766	1.241	0.957	0.745	1.228	0.917	0.717	1.173	0.933	0.730	1.192	0.948	0.745	1.206	0.913	0.712	1.171	0.873	0.687	1.109	0.898	0.703	1.149			
rs7961581	1.158	0.896	1.497	1.145	0.886	1.479	1.140	0.879	1.479	1.156	0.891	1.500	1.111	0.855	1.444	1.108	0.857	1.431	1.230	0.947	1.599	1.147	0.882	1.493	1.168	0.897	1.520	1.143	0.883	1.481			
rs9939609	1.016	0.796	1.297	1.038	0.814	1.322	1.110	0.878	1.405	0.999	0.783	1.276	1.050	0.822	1.341	1.214	0.943	1.562	1.025	0.804	1.307	1.024	0.807	1.301	1.111	0.866	1.424	1.033	0.805	1.326			
rs8050136	1.016	0.796	1.296	1.031	0.811	1.312	1.114	0.881	1.409	1.006	0.789	1.285	1.034	0.811	1.317	1.208	0.939	1.555	1.025	0.804	1.307	1.032	0.813	1.309	1.110	0.866	1.422	1.024	0.799	1.313			
Cases (n)	188			188			188			188			188			188			188			188			188			188			188		
Controls (n)	599			591			597			577			580			573			604			576			600			577					

CONCLUSION

SNPs	Genes/Regions	Previous studies				Incidence density sampling				Case exclusion sampling			
		OR	LowerCL	UpperCL	P-value	OR	LowerCL	UpperCL	P-value	OR	LowerCL	UpperCL	P-value
rs4607103	ADAMTS9	1.09	1.06	1.12	1.20E-08	0.97	0.7311	1.2873		0.9572	0.7214	1.2703	
rs4747969	CDK123	1.11	1.07	1.14	1.20E-10	0.8072	0.5785	1.1266		0.7061	0.5052	0.9869	3.98E-02
rs10946398	CDKAL1	1.14	1.11	1.17	2.00E-18	1.2789	0.9899	1.6521		1.185	0.9226	1.5223	
rs7754840	CDKAL1	1.12	1.08	1.16	4.10E-11	1.2674	0.9816	1.6368		1.1778	0.9174	1.5125	
rs10811661	CDKN2A/2B	1.20	1.14	1.25	2.00E-15	0.8172	0.587	1.1376		0.822	0.5899	1.146	
rs564398	CDKN2A/2B	1.12	1.07	1.17	1.20E-07	1.061	0.8252	1.3645		1.0178	0.7933	1.3057	
rs8050136	FTO	1.17	1.12	1.22	1.00E-12	1.0915	0.8506	1.4004		1.06	0.8309	1.3525	
rs9939609	FTO	1.11	1.02	1.20	1.43E-02	1.0951	0.8534	1.405		1.062	0.8318	1.3561	
rs5015480	HHEX	1.15	1.10	1.19	7.00E-17	0.8534	0.6687	1.0887		0.8571	0.6716	1.0936	
rs4402960	IGF2BP2	1.14	1.11	1.18	9.00E-16	1.078	0.8404	1.3829		1.0391	0.8103	1.3326	
rs864745	JAZF1	1.10	1.07	1.13	5.00E-14	0.8488	0.6708	1.0739		0.8326	0.6585	1.0523	
rs5215	KCNJ11	1.14	1.10	1.19	5.00E-11	0.9597	0.746	1.2347		0.9293	0.7277	1.1869	
rs1801282	PPARG	1.14	1.08	1.20	2.0E-06	0.9514	0.6522	1.388		1.0279	0.7084	1.4917	
rs10885409	TCF7L2				1.20E-05	0.7444	0.5845	0.9484	1.99E-02	0.7985	0.6291	1.0134	
rs7901695	TCF7L2	1.37	1.31	1.43	1.00E-48	1.4446	1.1228	1.8583	6.50E-03	1.4837	1.1583	1.9012	2.74E-03
rs13431070	THADA	1.15	1.10	1.20	1.10E-09	0.6288	0.3992	0.9909	5.3E-02	0.6575	0.4257	1.0156	
rs7961581	TSPAN8/LGR5	1.09	1.06	1.12	1.10E-09	1.0372	0.7995	1.3461		1.1506	0.8873	1.4923	
rs4689394	WFS1	1.11	1.08	1.16	1.00E-07	0.9694	0.7586	1.2387		0.8434	0.6613	1.0757	