Supplementary Information for: Secondary phenotype analysis in ascertained family designs: Application to the Leiden Longevity Study

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In this supplementary materials, we are presenting all the simulations results obtained to compare our retrospective likelihood approach to the naive mixed model approach in Section A. In section B are presented the association results between 41 selected SNPs and triglyceride levels obtained on the Leiden Longevity Study.

Contents

A Simulation results

A.1 Description of the simulation study

A simulation study has been set up to evaluate the performance of our proposed method in various settings for the correlation between the two outcomes, the disease prevalence, the strength of the association between the SNP and the primary phenotype and strength of the ascertainment mechanism. In addition, we contrasted our method with the naive approach which is typically followed in practice, namely analysis of the secondary trait without correcting for the sampling mechanism. In particular in this case we applied the standard linear mixed effects model for the secondary phenotype and explicitly modelled the familial relationship. The two methods have been compared in terms of Root Mean Square Error (RMSE), and 95% coverage probabilities. We simulate multiple cases family data and secondary phenotypes for sibships using the mixed-effects logistic regression:

$$\begin{aligned} Y_i^* &= \alpha_0 + \alpha_1 G_i + \sigma_{G_Y} b_i^Y + \sigma_u u_i + \epsilon_{Yi} \\ X_i &= \beta_0 + \beta_1 G_i + \sigma_{G_X} b_i^X + \sigma_u u_i + \sigma_\epsilon \epsilon_{Xi} \end{aligned} \tag{1}$$

With respect to the familial relationships we have simulated families with only siblings such that our simulation resembles more the LLS design. For the prevalence of the primary phenotype we considered two cases: the disease prevalence equals 1% which corresponds to $\alpha_0 \approx -2.32$ and 5% which corresponds to $\alpha_0 \approx -1.64$. The SNP effect on the primary phenotype measured by α_1, was taken equal to 0.1, 0.5 or 1. In addition the remaining variance parameters have been chosen such that they correspond to 50% heritability, i.e. ,σ_{G_X}=2, $\sigma_{G_Y} = \sqrt{3}$, $\sigma_u = \sqrt{2}$ and $\sigma_\epsilon = \sqrt{2}$. which corresponds to 0.78 correlation between the primary and the secondary phenotypes. Finally for the secondary phenotype we choose as fixed effects values :$\beta_0 = 3.5$ and $\beta_1 = 0.2$ or 2. Finally for each of the 4 scenarios (rare or low disease and low and higher SNP effect on the primary phenotype) we considered 4 ascertainment mechanisms, i.e. we assumed that families have been sampled provided that at least 1 or 2 out of the 5 members are affected. For each dataset 400 families were simulated.

A.2 Simulation study results for a SNP as genetic marker

A.2.1 Empirical bias and efficiency of association parameters for the secondary phenotype under genetic association with the primary phenotype.

Table S1: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a rare disease (prevalence around 1%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.612(1.444)(1.533)(0.859)	5.192(0.178)(1.702)(0.000)
β_1	0.200	0.211(0.204)(0.212)(0.936)	0.195(0.215)(218)(0.934)
σ_{GX}	2.000	1.841(0.479)(0.795)(0.622)	0.913(0.583)(1.131)(0.128)
σ_ϵ	1.414	1.552(0.145)(0.271)(0.662)	2.471(0.049)(1.063)(0.000)
α_0	-2.326	-2.279(1.650)(2.112)(0.773)	-
α_1	0.100	0.087(0.176)(0.196)(0.906)	-
σ_{GY}	1.732	1.317(0.677)(0.965)(0.844)	-
σ_u	1.414	0.995(0.687)(0.778)(0.730)	-

Table S2: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a rare disease (prevalence around 1%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.678(1.140)(1.172)(0.932)	5.099(0.185)(1.610)(0.000)
β_1	0.200	0.191(0.212)(0.228)(0.906)	0.108(0.198)(0.234)(0.893)
σ_{GX}	2.000	1.899(0.561)(0.693)(0.728)	0.974(0.458)(1.068)(0.106)
σ_ϵ	1.414	1.529(0.123)(0.231)(0.707)	2.444(0.051)(1.038)(0.000)
α_0	-2.326	-1.989(1.449)(1.814)(0.793)	-
α_1	0.500	0.416(0.173)(0.262)(0.905)	-
σ_{GY}	1.732	1.461(0.673)(1.044)(0.876)	-
σ_u	1.414	1.072(0.671)(0.730)(0.813)	-

Table S3: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a rare disease (prevalence around 1%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.439(1.190)(1.216)(0.912)	4.361(0.194)(0.883)(0.002)
β_1	0.200	0.200(0.209)(0.213)(0.955)	0.192(0.214)(0.216)(0.950)
σ_{GX}	2.000	1.983(0.257)(0.278)(0.679)	1.363(0.195)(0.683)(0.054)
σ_ϵ	1.414	1.552(0.119)(0.234)(0.644)	2.356(0.058)(0.951)(0.000)
α_0	-2.326	-2.470(1.951)(2.169)(0.902)	-
α_1	0.100	0.074(0.174)(0.191)(0.932)	-
σ_{GY}	1.732	1.553(0.574)(0.973)(0.765)	-
σ_u	1.414	0.973(0.677)(0.745)(0.862)	-

Table S4: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a rare disease (prevalence around 1%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.377(0.940)(0.927)(0.949)	4.293(0.199)(0.816)(0.008)
β_1	0.200	0.195(0.218)(0.218)(0.943)	0.155(0.202)(0.218)(0.920)
σ_{GX}	2.000	1.995(0.233)(0.233)(0.704)	1.409(0.188)(0.641)(0.092)
σ_ϵ	1.414	1.544(0.115)(0.213)(0.674)	2.329(0.059)(0.924)(0.000)
α_0	-2.326	-2.342(1.530)(1.536)(0.961)	-
α_1	0.500	0.412(0.156)(0.283)(0.877)	-
σ_{GY}	1.732	1.578(0.556)(0.983)(0.787)	-
σ_u	1.414	1.027(0.597)(0.698)(0.888)	-

Table S5: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a common disease (prevalence around 5%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.397(1.463)(1.530)(0.901)	4.835(0.180)(1.356)(0.000)
β_1	0.200	0.202(0.204)(0.205)(0.939)	0.188(0.203)(0.207)(0.940)
σ_{GX}	2.000	1.919(0.467)(0.653)(0.650)	1.053(0.362)(0.990)(0.060)
σ_ϵ	1.414	1.543(0.131)(0.238)(0.699)	2.404(0.052)(0.998)(0.000)
α_0	-1.644	-1.755(1.801)(2.091)(0.874)	-
α_1	0.100	0.075(0.169)(0.222)(0.902)	-
σ_{GY}	1.732	1.470(0.669)(1.035)(0.837)	-
σ_u	1.414	0.991(0.730)(0.765)(0.798)	-

Table S6: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a common disease (prevalence around 5%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.393(1.179)(1.156)(0.952)	4.753(0.187)(0.1.266)(0.000)
β_1	0.200	0.197(0.212)(0.215)(0.946)	0.103(0.201)(0.221)(0.930)
σ_{GX}	2.000	2.003(0.327)(0.513)(0.700)	1.131(0.299)(0.912)(0.032)
σ_ϵ	1.414	1.523(0.114)(0.193)(0.702)	2.376(0.053)(0.970)(0.000)
α_0	-1.644	-1.623(1.432)(1.663)(0.952)	-
α_1	0.500	0.409(0.170)(0.276)(0.926)	-
σ_{GY}	1.732	1.530(0.570)(0.967)(0.864)	-
σ_u	1.414	1.033(0.725)(0.763)(0.878)	-

Table S7: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a common disease (prevalence around 5%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.285(1.203)(1.288)(0.904)	4.124(0.198)(0.653)(0.110)
β_1	0.200	0.203(0.209)(0.209)(0.946)	0.191(0.205)(0.217)(0.940)
σ_{GX}	2.000	1.999(0.237)(0.479)(0.660)	1.471(0.172)(0.586)(0.134)
σ_ϵ	1.414	1.559(0.116)(0.221)(0.672)	2.291(0.061)(0.887)(0.000)
α_0	-1.644	-1.991(1.870)(2.450)(0.920)	-
α_1	0.100	0.085(0.173)(0.221)(0.916)	-
σ_{GY}	1.732	1.623(0.573)(1.033)(0.742)	-
σ_u	1.414	0.960(0.738)(0.761)(0.894)	-

Table S8: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a common disease (prevalence around 5%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.156(0.939)(0.973)(0.946)	4.066(0.201)(0.599)(0.200)
β_1	0.200	0.194(0.222)(0.226)(0.944)	0.158(0.204)(0.234)(0.920)
σ_{GX}	2.000	2.035(0.245)(0.254)(0.712)	1.520(0.163)(0.540)(0.176)
σ_ϵ	1.414	1.543(0.124)(0.219)(0.654)	2.263(0.063)(0.861)(0.000)
α_0	-1.644	-1.982(1.518)(1.612)(0.960)	-
α_1	0.500	0.430(0.304)(0.574)(0.898)	-
σ_{GY}	1.732	1.680(0.574)(1.090)(0.772)	-
σ_u	1.414	0.986(0.666)(0.718)(0.894)	-

Table S9: Estimates with standard deviations and RMSE of the heritability of the secondary phenotype are given for a common disease (prevalence $\approx$ 5%), for the four ascertainment mechanisms and two values of α_1.

Ascertainment	α_1	Retrospective likelihood	Naive method
1.At least 2 cases			
	0.10	0.49(0.08)(0.21)	0.17(0.08)(0.34)
	0.50	0.50(0.08)(0.18)	0.19(0.08)(0.32)
2.At least 1 case			
	0.10	0.50(0.08)(0.17)	0.29(0.08)(0.22)
	0.50	0.52(0.08)(0.16)	0.31(0.08)(0.20)

Table S10: Estimates with standard deviations and RMSE of the heritability of the secondary phenotype are given for a rare disease (prevalence $\approx 1\%$), for the four ascertainment mechanisms and two values of α_1.

Ascertainment	α_1	Retrospective likelihood	Naive method
1.At least 2 cases			
	0.10	0.48(0.07)(0.22)	0.13(0.07)(0.37)
	0.50	0.48(0.07)(0.22)	0.14(0.07)(0.36)
2.At least 1 case			
	0.10	0.50(0.08)(0.17)	0.25(0.08)(0.25)
	0.50	0.50(0.08)(0.17)	0.27(0.08)(0.24)

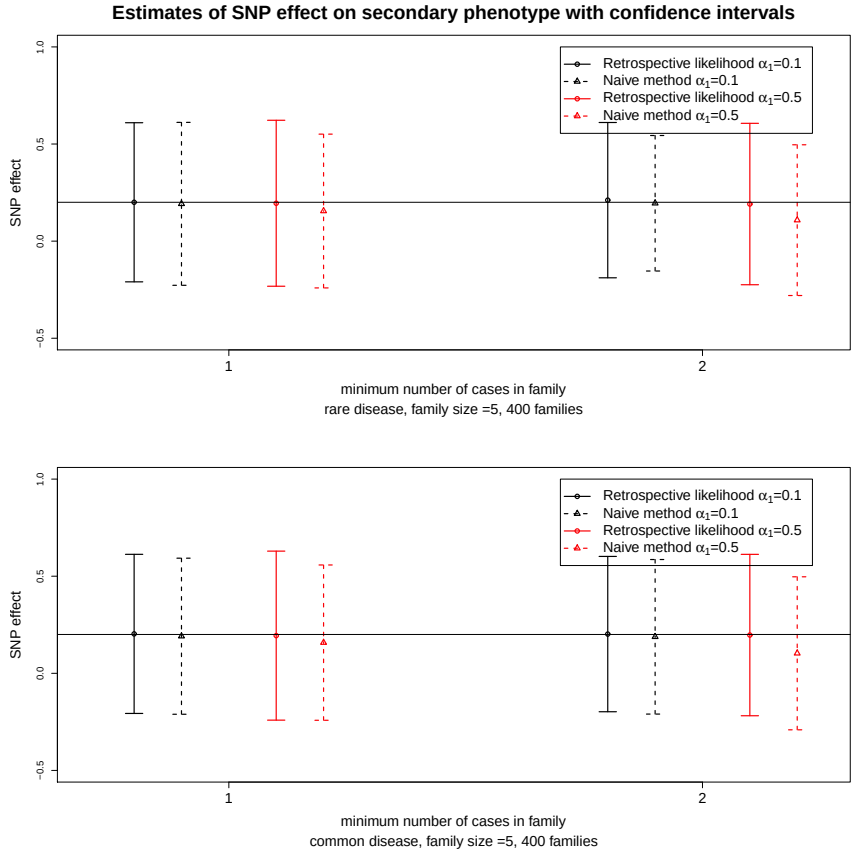

Figure S1: Estimates and 95% confidence intervals for the SNP effect on the secondary phenotype for the retrospective likelihood approach and the naive method. Results are obtained from 500 simulated datasets of 100 families for 2 ascertainment schedules. The top and bottom panel correspond to a rare or common primary phenotype with a prevalence around 1% and 5% respectively. In black and red are represented results for small (α_1=0.5) and large (α_1=1) effect sizes of the SNP on the primary phenotype respectively. The horizontal line corresponds to the true SNP effect on the secondary phenotype.

A.2.2 Robustness of the retrospective likelihood methods to violation of the probit model assumption for the primary phenotype

Table S11: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a rare disease (prevalence around 1%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood
		Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.586(0.897)(0.910)(0.905)
β_1	0.200	0.199(0.103)(0.104)(0.948)
σ_{GX}	2.000	1.887(0.155)(0.296)(0.623)
σ_ϵ	1.414	1.571(0.050)(0.178)(0.257)
α_0	-2.326	-1.366(0.838)(1.342)(0.659)
α_1	0.100	0.053(0.094)(0.250)(0.901)
σ_{GY}	1.732	1.019(0.250)(0.764)(0.129)
σ_u	1.414	0.988(0.644)(0.526)(0.948)

Table S12: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a rare disease (prevalence around 1%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood
		Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.720(0.583)(0.625)(0.905)
β_1	0.200	0.197(0.106)(0.110)(0.945)
σ_{GX}	2.000	1.909(0.139)(0.271)(0.625)
σ_ϵ	1.414	1.560(0.047)(0.162)(0.245)
α_0	-2.326	-1.161(0.528)(1.245)(0.311)
α_1	0.500	0.287(0.221)(0.232)(0.913)
σ_{GY}	1.732	1.052(0.221)(0.715)(0.094
σ_u	1.414	1.009(0.501)(0.601)(0.975)

Table S13: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a rare disease (prevalence around 1%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood
		Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.630(0.726)(0.730)(0.909)
β_1	0.200	0.200(0.104)(0.107)(0.961)
σ_{GX}	2.000	1.887(0.133)(0.266)(0.623)
σ_ϵ	1.414	1.572(0.050)(0.176)(0.248)
α_0	-2.326	-1.377(0.784)(1.339)(0.657)
α_1	0.100	0.056(0.090)(0.231)(0.900)
σ_{GY}	1.732	1.010(0.231)(0.771)(0.116)
σ_u	1.414	0.998(0.495)(0.514)(0.761)

Table S14: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a rare disease (prevalence around 1%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood
		Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.440(0.366)(0.467)(0.996)
β_1	0.200	0.199(0.107)(0.111)(0.960)
σ_{GX}	2.000	1.892(0.108)(0.231)(0.670)
σ_ϵ	1.414	1.4(0.045)(0.171)(0.284)
α_0	-2.326	-1.453(0.472)(0.940)(0.446)
α_1	0.500	0.295(0.190)(0.222)(0.948)
σ_{GY}	1.732	1.049(0.190)(0.711)(0.074)
σ_u	1.414	1.000(0.572)(0.494)(0.680)

Table S15: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a common disease (prevalence around 5%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood
		Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.500(0.822)(0.871)(0.961)
β_1	0.200	0.193(0.105)(0.105)(0.951)
σ_{GX}	2.000	1.884(0.140)(0.278)(0.597)
σ_ϵ	1.414	1.572(0.047)(0.175)(0.236)
α_0	-1.644	-1.021(0.804)(1.050)(0.813)
α_1	0.100	0.051(0.098)(0.230)(1.000)
σ_{GY}	1.732	1.040(0.230)(0.736)(0.131)
σ_u	1.414	0.987(0.517)(0.602)(0.931)

Table S16: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a common disease (prevalence around 5%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood
		Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.501(0.589)(0.449)(0.996)
β_1	0.200	0.186(0.107)(0.108)(0.940)
σ_{GX}	2.000	1.886(0.125)(0.246)(0.606)
σ_ϵ	1.414	1.568(0.044)(0.168)(0.174)
α_0	-1.644	-0.995(0.508)(0.767)(0.650)
α_1	0.500	0.289(0.207)(0.232)(0.904)
σ_{GY}	1.732	1.054(0.207)(0.709)(0.088)
σ_u	1.414	1.015(0.480)(0.517)(0.910)

Table S17: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a common disease (prevalence around 5%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood
		Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.509(0.716)(0.720)(0.921)
β_1	0.200	0.197(0.106)(0.108)(0.949)
σ_{GX}	2.000	1.884(0.117)(0.254)(0.587)
σ_ϵ	1.414	1.576(0.046)(0.178)(0.297)
α_0	-1.644	-1.069(0.730)(1.078)(0.823)
α_1	0.100	0.055(0.094)(0.204)(0.905)
σ_{GY}	1.732	1.036(0.204)(0.741)(0.109)
σ_u	1.414	0.988(0.499)(0.512)(0.724)

Table S18: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a common disease (prevalence around 5%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood
		Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.254(0.421)(0.449)(0.982)
β_1	0.200	0.196(0.107)(0.108)(0.942)
σ_{GX}	2.000	1.868(0.099)(0.226)(0.652)
σ_ϵ	1.414	1.575(0.043)(0.175)(0.260)
α_0	-1.644	-1.249(0.440)(0.540)(0.936)
α_1	0.500	0.302(0.181)(0.217)(0.948)
σ_{GY}	1.732	1.044(0.181)(0.711)(0.046)
σ_u	1.414	1.022(0.383)(0.450)(0.770)

Table S19: Estimates with standard deviations and RMSE of the heritability of the secondary phenotype are given for a common disease (prevalence $\approx$ 5%), for the four ascertainment mechanisms and two values of α_1.

Ascertainment	α_1	Retrospective likelihood	Naive method
1.At least 2 cases			
	0.100	0.499(0.102)(0.102)	0.17(0.08)(0.34)
	0.500	0.498(0.089)(0.089)	0.19(0.08)(0.32)
2.At least 1 case			
	0.100	0.499(0.089)(0.089)	0.29(0.08)(0.22)
	0.500	0.492(0.076)(0.077)	0.31(0.08)(0.20)

Table S20: Estimates with standard deviations and RMSE of the heritability of the secondary phenotype are given for a rare disease (prevalence $\approx 1\%$), for the four ascertainment mechanisms and two values of α_1.

Ascertainment	α_1	Retrospective likelihood
1.At least 2 cases		
	0.100	0.499(0.107)(0.107))
	0.500	0.505(0.104)(0.104)
2.At least 1 case		
	0.100	0.499(0.092)(0.092))
	0.500	0.501(0.084)(0.084)

A.3 Simulation study for a polygenic score

Table S21: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a rare disease (prevalence around 1%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.483(0.899)(0.907)(0.899)	5.221(0.067)(1.723)(0.000)
β_1	0.200	0.193(0.065)(0.073)(0.932)	0.175(0.062)(0.070)(0.922)
σ_{GX}	2.000	2.013(0.190)(0.357)(0.657)	0.990(0.146)(1.018131)(0.000)
σ_ϵ	1.414	1.529(0.055)(0.140)(0.559)	2.467(0.025)(1.054)(0.000)
α_0	-2.326	-1.932(0.921)(1.013)(0.839)	-
α_1	0.100	0.076(0.054)(0.069)(0.814)	-
σ_{GY}	1.732	1.398(0.328)(0.548)(0.793)	-
σ_u	1.414	1.170(0.778)(0.869)(0.920)	-

Table S22: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a rare disease (prevalence around 1%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.962(0.541)(0.618)(0.834)	5.178(0.069)(1.679)(0.000)
β_1	0.200	0.193(0.074)(0.079)(0.940)	0.096(0.063)(0.122)(0.610)
σ_{GX}	2.000	2.084(0.165)(0.346)(0.664)	1.037(0.135)(0.972)(0.000)
σ_ϵ	1.414	1.518(0.053)(0.130)(0.592)	2.452(0.025)(1.039)(0.000)
α_0	-2.326	-1.871(0.522)(1.0314)(0.758)	-
α_1	0.500	0.391(0.074)(0.134)(0.772)	-
σ_{GY}	1.732	1.471(0.273)(0.475)(0.796)	-
σ_u	1.414	1.176(0.670)(0.744)(0.924)	-

Table S23: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a rare disease (prevalence around 1%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.338(0.573)(0.655)(0.862)	4.370(0.076)(0.850)(0.000)
β_1	0.200	0.190(0.067)(0.067)(0.954)	0.189(0.066)(0.066)(0.948)
σ_{GX}	2.000	1.925(0.163)(0.364)(0.645)	1.386(0.088)(0.626)(0.000)
σ_ϵ	1.414	1.460(0.057)(0.171)(0.676)	2.364(0.028)(0.952)(0.000)
α_0	-2.326	-2.219(0.774)(0.922)(0.889)	-
α_1	0.100	0.073(0.058)(0.071)(0.829)	-
σ_{GY}	1.732	1.359(0.307)(0.583)(0.611)	-
σ_u	1.414	1.186(0.623)(0.714)(0.926)	-

Table S24: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a rare disease (prevalence around 1%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.542(0.361)(0.411)(0.963)	4.346(0.076)(0.850)(0.000)
β_1	0.200	0.197(0.074)(0.078)(0.944)	0.157(0.066)(0.079)(0.904)
σ_{GX}	2.000	2.018(0.127)(0.278)(0.663)	1.412(0.186)(0.600)(0.000)
σ_ϵ	1.414	1.538(0.052)(0.147)(0.582)	2.352(0.029)(0.940)(0.000)
α_0	-2.326	-1.876(0.425)(631)(0.797)	-
α_1	0.500	0.401(0.074)(0.128)(0.838)	-
σ_{GY}	1.732	1.435(0.240)(0.473)(0.619)	-
σ_u	1.414	1.209(0.466)(0.574)(0.948)	-

Table S25: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a common disease (prevalence around 5%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.370(0.854)(0.854)(0.933)	4.854(0.069)(1.356)(0.000)
β_1	0.200	0.192(0.067)(0.067)(0.952)	0.177(0.063)(0.063)(0.948)
σ_{GX}	2.000	2.018(0.127)(0.278)(0.663)	1.113(0.118)(0.896)(0.000)
σ_ϵ	1.414	1.538(0.052)(0.147)(0.682)	2.405(0.026)(0.992)(0.000)
α_0	-1.644	-1.470(0.875)(0.897)(0.897)	-
α_1	0.100	0.073(0.057)(0.068)(0.879)	-
σ_{GY}	1.732	1.477(0.295)(0.500)(0.772)	-
σ_u	1.414	1.099(0.997)(1.139)(0.945)	-

Table S26: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 2 cases of a common disease (prevalence around 5%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.653(0.502)(0.593)(0.932)	4.823(0.070)(1.325)(0.000)
β_1	0.200	0.192(0.073)(0.075)(0.954)	0.103(0.064)(0.115)(0.656)
σ_{GX}	2.000	2.055(0.149)(0.309)(0.664)	1.148(0.113)(0.861)(0.000)
σ_ϵ	1.414	1.530(0.051)(0.140)(0.614)	2.395(0.026)(0.982)(0.000)
α_0	-1.644	-1.367(0.495)(0.599)(0.848)	-
α_1	0.500	0.394(0.072)(0.131)(0.868)	-
σ_{GY}	1.732	1.462(0.255)(0.456)(0.762)	-
σ_u	1.414	1.172(0.645)(0.733)(0.954)	-

Table S27: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a common disease (prevalence around 5%) and α_1=0.1. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.088(0.524)(0.701)(0.896)	4.134(0.077)(0.639)(0.000)
β_1	0.200	0.196(0.066)(0.0.066)(0.960)	0.193(0.066)(0.071)(0.962)
σ_{GX}	2.000	1.936(0.144)(0.364)(0.694)	1.482(0.080)(0.531)(0.000)
σ_ϵ	1.414	1.568(0.052)(0.218)(0.607)	2.296(0.030)(0.884)(0.000)
α_0	-1.644	-1.955(0.669)(0.941)(0.953)	-
α_1	0.100	0.077(0.056)(0.065)(0.847)	-
σ_{GY}	1.732	1.444(0.285)(0.410)(0.687)	-
σ_u	1.414	1.244(0.321)(0.415)(0.937)	-

Table S28: Simulations results obtained on 500 datasets with 400 families of size 5 with at least 1 case of a common disease (prevalence around 5%) and α_1=0.5. Into brackets are respectively standard deviations, root mean square errors and the coverage probabilities.

	Real Value	Retrospective likelihood	Naive method
		Est (SD) (RMSE) (Cov Pr)	Est (SD) (RMSE) (Cov Pr)
β_0	3.500	3.278(0.341)(0.408)(0.958)	4.118(0.077)(0.623)(0.000)
β_1	0.200	0.187(0.072)(0.078)(0.953)	0.159(0.067)(0.074)(0.928)
σ_{GX}	2.000	1.952(0.118)(0.285)(0.610)	1.508(0.078)(0.506)(0.004)
σ_ϵ	1.414	1.552(0.049)(0.161)(0.607)	2.286(0.030)(0.874)(0.000)
α_0	-1.644	-1.648(0.395)(0.567)(0.988)	-
α_1	0.500	0.392(0.071)(0.140)(0.798)	-
σ_{GY}	1.732	1.373(0.230)(0.504)(0.660)	-
σ_u	1.414	1.223(0.328)(0.337)(0.943)	-

Table S29: Estimates with standard deviations and RMSE of the heritability of the secondary phenotype are given for a rare disease (prevalence $\approx$ 1%), for the four ascertainment mechanisms and two values of α_1.

Ascertainment	α_1	Retrospective likelihood	Naive method
1.At least 2 cases			
	0.10	0.50(0.03)(0.13)	0.14(0.03)(0.36)
	0.50	0.52(0.03)0.12)	0.15(0.03)(0.34)
2.At least 1 case			
	0.10	0.48(0.04)(0.12)	0.25(0.03)(0.24)
	0.50	0.50(0.04)(0.10)	0.26(0.04)(0.23)

B Results analysis Leiden Longevity Study on triglyceride levels

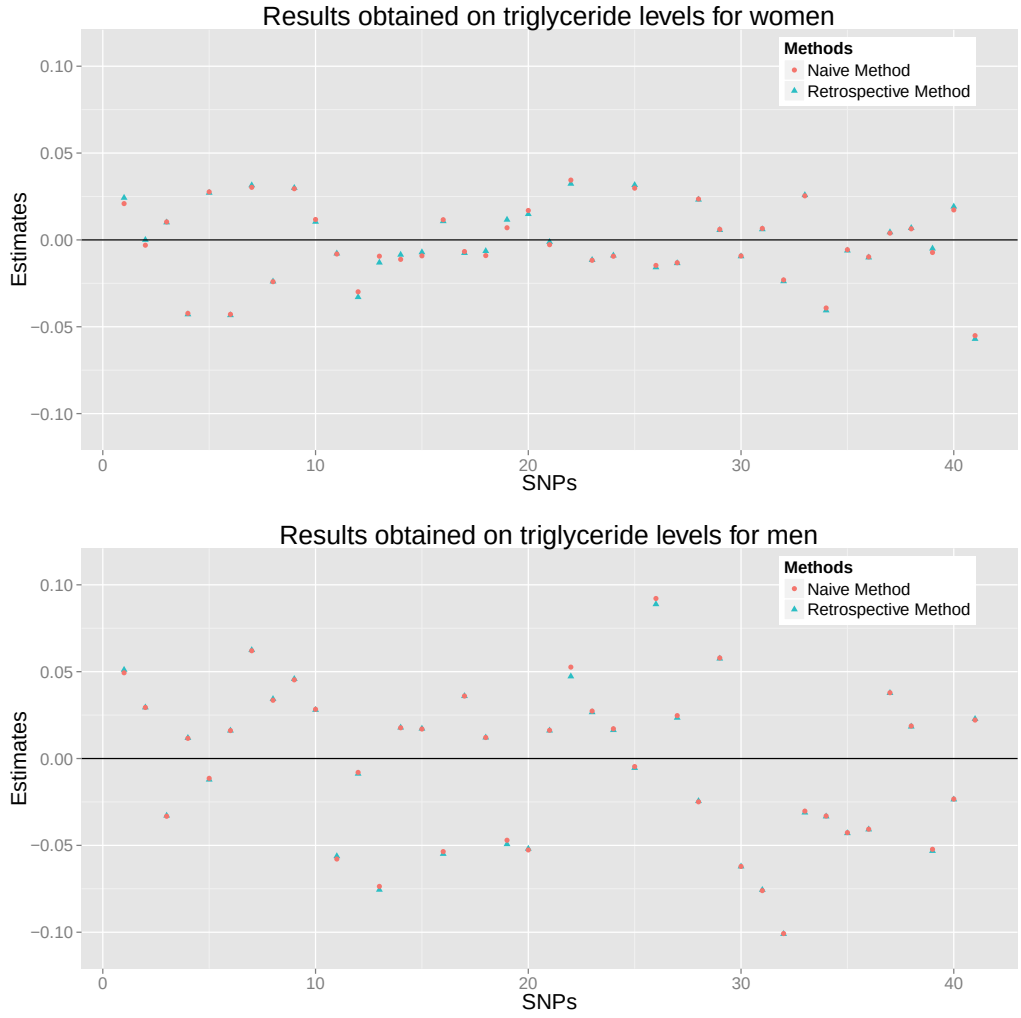

Figure S2: Estimates for the association of 41 SNPs with triglyceride in the LLS. In the top and bottom are the estimates of the 41 SNPs for women and for men respectively. The black line represents no SNP effect on triglyceride.

Table S30: Leiden Longevity Study: Estimates of the association between the 41 selected SNPS and triglyceride levels for womend and for three different approaches. The retrospective likelihood approach with same variance assumed for the shared random effect, with different variances, and the naive approach. Are also presented the absolute difference between the estimates of the two last approaches with the first one. Into brackets are the standard errors.

SNPs	Constrained approach	Not Constrained approach		Naive Likelihood	
	Estimates (SE)	Estimates (SE)	Difference	Estimates (SE)	Difference
rs3863318_A	.0242(.0205)	.0245(.0205)	.0003	.0209(.0205)	.0033
rs2512139_A	.0000(.0215)	.0000(.0345)	.0000	-.0031(.0214)	.0031
rs7103514_G	.0101(.0365)	.0101(.0275)	.0000	.0104(.0367)	.0003
rs2512158_A	-.0428(.0206)	-.0430(.0208)	.0002	-.0423(.0207)	.0005
rs11216648_G	.0271(.0206)	.0272(.0206)	.0001	.0276(.0207)	.0005
rs4936414_G	-.0433(.0212)	-.430 (.0212)	.0003	-.0429(.0213)	.0004
rs4252287_A	.0314(.0345)	.0313(.0385)	.0001	.0303(.0347)	.0011
rs947889_G	-.0239(.0202)	-.0238(.0199)	.0001	-.0241(.0203)	.0002
rs2512154_A	.0299(.0307)	.0298(.0215)	.0001	.0295(.0308)	.0004
rs4936417_G	.0104(.0223)	.0104(.0233)	.0000	.0118(.0223)	.0014
rs652107_G	-.0078(.0275)	-.0075(.206)	.0003	-.0081(.0276)	.0003
rs12576767_A	-.0329(.0432)	-.320(.0367)	.0009	-.0299(.0433)	.0030
rs1786186_A	-.0131(.0287)	-.0132(.0216)	.0001	-.0094(.0287)	.0037
rs3825050_A	-.0085(.0219)	-.0083(.0205)	.0002	-.0112(.0219)	.0027
rs10160375_G	-.0071(.0214)	-.0072(.0203)	.0001	-.0092(.0215)	.0021
rs689264_A	.0108(.0368)	.0107(.0431)	.0001	.0116(.0370)	.0008
rs948461_A	-.0075(.0216)	-.0072(.205)	.0003	-.0067(.0217)	0008
rs2276123_A	-.0064(.0218)	.0070(.0243)	.0006	-.0090(.0218)	.0026
rs948466_A	.0116(.0385)	.0069(.0509)	.0047	.0070(.0387)	.0046
rs881122_A	.0149(.0243)	.0149(.0217)	.0000	.0169(.0244)	.0020
rs2276129_A	-.0012(.0217)	-.0008(.0217)	.0004	-.0028(.0218)	.0016
rs2155857_A	.0323(.0509)	.0320(.0200)	.0003	.0344(.0511)	.0021
rs1894177_A	-.0115(.0200)	-.0130(.0456)	.0015	-.0118(.0201)	.0003
rs596134_G	-.0090(.0202)	-.0094(.205)	.0004	-.0094(.0203)	.0004
rs3741311_G	.0316(.0215)	.0311(.0364)	.0005	.0298(.0216)	.0018
rs1941637_A	-.0158(.054)	-.0159(.0214)	.0001	-.0147(.0542)	.0011
rs658624_G	-.0134(.0205)	-.136(.0203)	.0002	-.0131(.0206)	.0003
rs625464_A	.0232(.0200)	.0234(.286)	.0002	.0236(.0200)	.0004
rs11216788_A	.0057(.0204)	.0057(.219)	.0000	.0061(.0204)	.0004
rs679327_G	-.0094(.0217)	-.0092(.251)	.0002	-.0092(.0218)	.0002
rs678957_A	.0061(.0457)	.0053(.0209)	.0008	.0066(.0459)	.0005
rs7944321_A	-.0238(.0252)	-.0238(.0327)	.0000	-.0230(.0253)	.0008
rs7949751_G	.0258(.0233)	.0257(.0267)	.0001	.0254(.0234)	.0004
rs11216816_A	-.0406(.0268)	-.0399(.0201)	.0007	-.0392(.0268)	.0014
rs1805_A	-.0061(.0200)	-.0060(.0201)	.0001	-.0056(.0201)	.0005
rs3759001_A	-.0101(.0205)	-.103(.214)	.0002	-.0098(.0206)	.0003
rs4938493_C	.0044(.0203)	.0044(.217)	.0000	.0039(.0204)	.0005
rs2853009_A	.0068(.0201)	.0068(.0222)	.0000	.0064(.0202)	.0004
rs12282721_A	-.0050(.0327)	-.0052(.0199)	.0002	-.0072(.0328)	.0022
rs676134_A	.0192(.0210)	.0185(.0200)	.0007	.0173(.0210)	.0019
rs7083_A	-.0570(.0205)	-.0572(.0205)	.0002	-.0551(.0206)	.0019

Table S31: Leiden Longevity Study: Estimates of the association between the 41 selected SNPS and triglyceride levels for men and for three different approaches. The retrospective likelihood approach with same variance assumed for the shared random effect, with different variances, and the naive approach. Are also presented the absolute difference between the estimates of the two last approaches with the first one. Into brackets are the standard errors.

SNPs	Constrained approach	Not Constrained approach		Naive Likelihood	
	Estimates (SE)	Estimates (SE)	Difference	Estimates (SE)	Difference
rs3863318_A	.0511(.0236)	.0501(.0236)	.0010	.0494(.0236)	.0017
rs2512139_A	.0294(.0249)	.0285(.0249)	.0009	.0294(.0249)	.0000
rs7103514_G	-.0329(.0426)	-.0324(.0427)	.0005	-.0332(.0426)	.0003
rs2512158_A	.0119(.0241)	.0118(.0241)	.0001	.0116(.0241)	.0003
rs11216648_G	-.0121(.0244)	-.0124(.0244)	.0003	-.0114(.0244)	.0007
rs4936414_G	.0161(.0244)	.0162(.0245)	.0001	.0161(.0244)	.0000
rs4252287_A	.0624(.0386)	.0631(.0386)	.0007	.0620(.0386)	.0004
rs947889_G	.0343(.0237)	.0335(.0238)	.0008	.0335(.0237)	.0008
rs2512154_A	.0458(.0351)	.0444(.0353)	.0014	.0453(.0352)	.0009
rs4936417_G	.0280(.0256)	.0280(.0256)	.0000	.0283(.0256)	.0003
rs652107_G	-.0562(.0310)	-.0568(.0311)	.0006	-.0579(.0310)	.0017
rs12576767_A	-.0087(.0468)	-.0086(.0472)	.0001	-.0080(.0469)	.0007
rs1786186_A	-.0755(.0356)	-.0748(.0357)	.0007	-.0736(.0357)	.0019
rs3825050_A	.0178(.0250)	.0182(.0252)	.0004	.0177(.0251)	.0001
rs10160375_G	.0172(.0248)	.0172(.0249)	.0000	.0170(.0248)	.0002
rs689264_A	-.0549(.0466)	-.0549(.0465)	.0000	-.0536(.0466)	.0013
rs948461_A	.0360(.0249)	.0352(.025)	.0008	.0360(.0249)	.0000
rs2276123_A	.0121(.0253)	.0121(.0253)	.0000	.0121(.0253)	.0000
rs948466_A	-.0493(.0498)	-.0491(.0499)	.0002	-.0470(.0499)	.0023
rs881122_A	-.0519(.0280)	-.0523(.028)	.0004	-.0526(.0280)	.0007
rs2276129_A	.0161(.0250)	.0161(.0251)	.0000	.0162(.0250)	.0001
rs2155857_A	.0473(.0633)	.0482(.0634)	.0009	.0526(.0633)	.0053
rs1894177_A	.0267(.0232)	.0263(.0233)	.0004	.0273(.0232)	.0006
rs596134_G	.0164(.0238)	.0160(.0238)	.0004	.0172(.0238)	.0008
rs3741311_G	-.0054(.0247)	-.0052(.0248)	.0002	-.0046(.0247)	.0008
rs1941637_A	.0888(.0667)	.0897(.0668)	.0011	.0921(.0667)	.0033
rs658624_G	.0235(.0239)	.0232(.0239)	.0003	.0247(.0239)	.0012
rs625464_A	-.0245(.0239)	-.0241(.024)	.0004	-.0249(.0240)	.0004
rs11216788_A	.0575(.0239)	.0572(.024)	.0003	.0579(.0240)	.0004
rs679327_G	-.0622(.0270)	-.0623(.0271)	.0001	-.0621(.0270)	.0001
rs678957_A	-.0756(.0583)	-.0768(.0585)	.0012	-.0761(.0583)	.0005
rs7944321_A	-.1010(.0315)	-.1012(.0315)	0002	-.1008(.0315)	.0002
rs7949751_G	-.0311(.0270)	-.0310(.027)	.0001	-.0303(.0270)	.0008
rs11216816_A	-.0334(.0330)	-.0333(.033)	.0001	-.0331(.0330)	.0003
rs1805_A	-.0430(.0243)	-.0428(.0243)	.0002	-.0427(.0243)	.0003
rs3759001_A	-.0408(.0241)	-.0406(.0242)	.0002	-.0407(.0241)	.0001
rs4938493_C	.0377(.0236)	.0377(.0237)	.0000	.0379(.0236)	.0002
rs2853009_A	.0184(.0234)	.0184(.0234)	.0000	.0187(.0234)	.0003
rs12282721_A	-.0532(.0379)	-.0534(.038)	.0002	-.0523(.0379)	.0009
rs676134_A	-.0236(.0243)	-.0234(.0244)	.0002	-.0233(.0243)	.0003
rs7083_A	.0228(.0244)	.0229(.0245)	.0001	.0221(.0244)	.0007