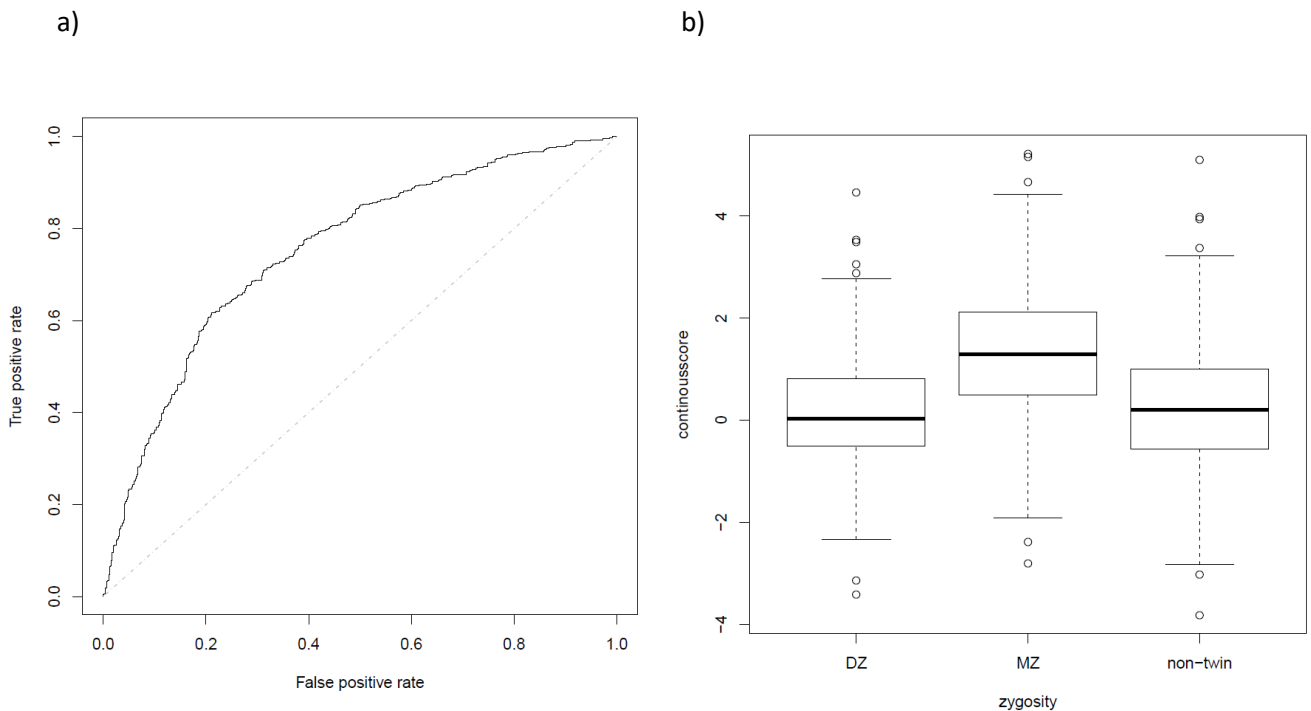


Examining the vanishing twin hypothesis of neural tube defects: Application of an epigenetic predictor for monozygotic twinning

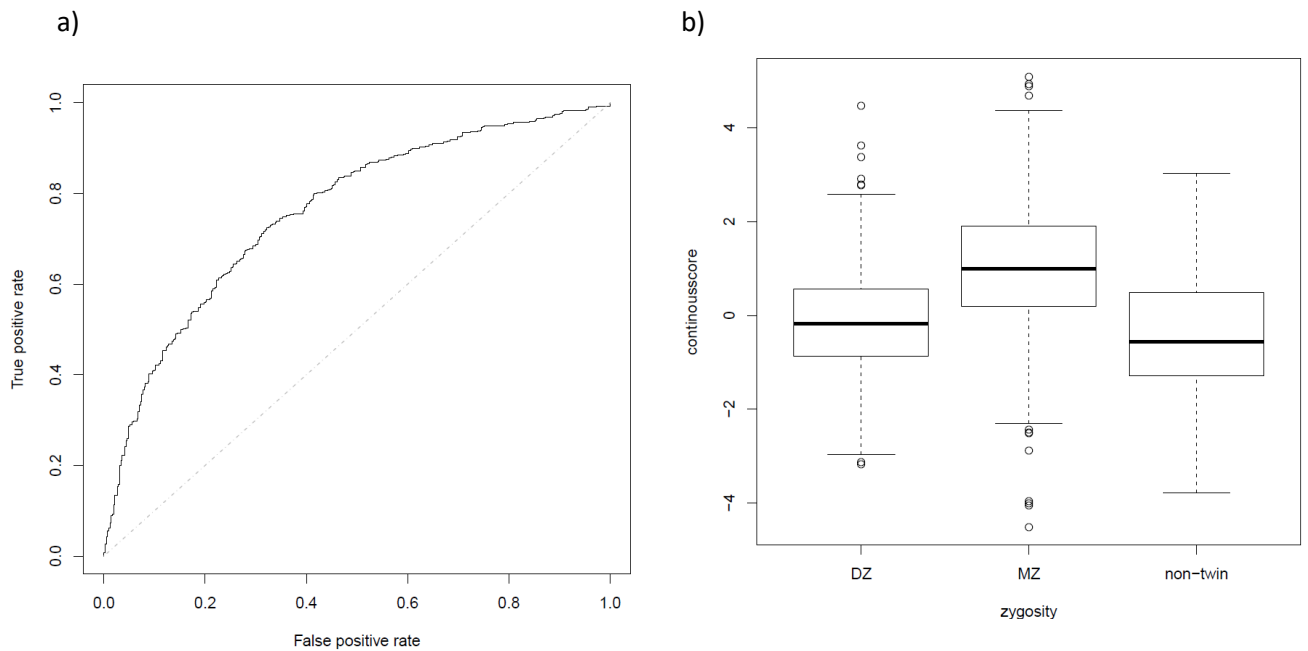
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Supplementary Figure S1 Performance of Predictor A in test data from the Netherlands Twin Register



a) AUC curve in test data (whole blood) from the Netherlands Twin Register for a classifier based on predictor A, which was trained on whole blood DNA methylation data to distinguish MZ twins from DZ twins, and is based on 213 methylation sites. The AUC was 0.75. b) Boxplots of continuous scores for predictor A in test data (whole blood) from the Netherlands Twin Register in twins and family members. Zygosity and familial relationships were confirmed by DNA. MZ=Monozygotic twins, DZ=Dizygotic twins, non-twin=Parents and siblings of twins. The y-axis shows the continuous scores in each group based on the 213-methylation site predictor, illustrating a higher average score in MZ twins compared to DZ twins and non-twins.

Supplementary Figure S2 Performance of Predictor B in test data from the Netherlands Twin Register



a) AUC curve in test data (whole blood) from the Netherlands Twin Register for a classifier based on predictor B, which was trained on whole blood DNA methylation data to distinguish MZ twins from all others (DZ twins + non-twins), and is based on 242 methylation sites. The AUC was 0.76. b) Boxplots of continuous scores for predictor B in test data (whole blood) from the Netherlands Twin Register in twins and family members. Zygosity and familial relationships were confirmed by DNA. MZ=Monozygotic twins, DZ=Dizygotic twins, non-twin=Parents and siblings of twins. The y-axis shows the continuous scores in each group based on the 242-methylation site predictor, illustrating a higher average score in MZ twins compared to DZ twins and non-twins.

Supplementary Table S1: Results from gee model with main and interaction effects of diagnosis and tissue for Predictor A (trained to distinguish MZ from DZ twins)

Spina Bifida versus controls					Anencephaly versus controls				
Parameter	Estimate	Robust S.E.	Robust z	p-value	Parameter	Estimate	Robust S.E.	Robust z	p-value
Intercept	0.705	0.275	2.561	0.010	Intercept	0.490	0.257	1.904	0.057
Diagnosis spina bifida	0.063	0.533	0.118	0.906	Diagnosis anencephaly	-0.043	0.383	-0.113	0.911
Tissue kidney	-0.215	0.377	-0.572	0.568	Tissue muscle	0.095	0.419	0.227	0.820
Tissue muscle	-0.120	0.431	-0.279	0.781	Tissue placental chorionic villi	-0.015	0.394	-0.037	0.971
Tissue placental chorionic villi	-0.230	0.406	-0.565	0.572	Tissue spinal cord	0.463	0.513	0.902	0.367
Tissue spinal cord	0.247	0.522	0.474	0.636	Tissue muscle X Diagnosis anencephaly	0.431	0.687	0.627	0.531
Tissue kidney X Diagnosis spina bifida	0.490	0.648	0.756	0.450	Tissue placental chorionic villi X Diagnosis anencephaly	0.163	0.536	0.304	0.762
Tissue muscle X Diagnosis spina bifida	0.087	0.747	0.116	0.908	Tissue spinal cord X Diagnosis anencephaly	-0.578	0.841	-0.688	0.492
Tissue placental chorionic villi X Diagnosis spina bifida	0.471	0.640	0.735	0.462					
Tissue spinal cord X Diagnosis spina bifida	-0.256	0.806	-0.318	0.751					

Supplementary Table S2: Results from gee model with main and interaction effects of diagnosis and tissue for Predictor B (trained to distinguish MZ twins from all others including parents/sibs)

Spina Bifida versus controls					Anencephaly versus controls				
Parameter	Estimate	Robust S.E.	Robust z	p-value	Parameter	Estimate	Robust S.E.	Robust z	p-value
Intercept	0.560	0.358	1.564	0.118	Intercept	0.557	0.356	1.564	0.118
Diagnosis spina bifida	-0.175	0.701	-0.249	0.804	Diagnosis anencephaly	-0.873	0.477	-1.831	0.067
Tissue kidney	-0.003	0.505	-0.006	0.995	Tissue muscle	-0.040	0.546	-0.072	0.942
Tissue muscle	-0.043	0.547	-0.078	0.938	Tissue placental chorionic villi	-0.204	0.490	-0.417	0.677
Tissue placental chorionic villi	-0.207	0.491	-0.422	0.673	Tissue spinal cord	0.518	0.593	0.873	0.383
Tissue spinal cord	0.515	0.594	0.866	0.387	Tissue muscle X Diagnosis anencephaly	0.982	0.884	1.110	0.267
Tissue kidney X Diagnosis spina bifida	0.358	0.852	0.420	0.674	Tissue placental chorionic villi X Diagnosis anencephaly	0.789	0.621	1.271	0.204
Tissue muscle X Diagnosis spina bifida	-0.023	0.901	-0.026	0.979	Tissue spinal cord : Diagnosis anencephaly	-0.279	0.840	-0.332	0.740
Tissue placental chorionic villi X Diagnosis spina bifida	0.533	0.804	0.663	0.508					
Tissue spinal cord X Diagnosis spina bifida	-0.536	0.974	-0.550	0.582					