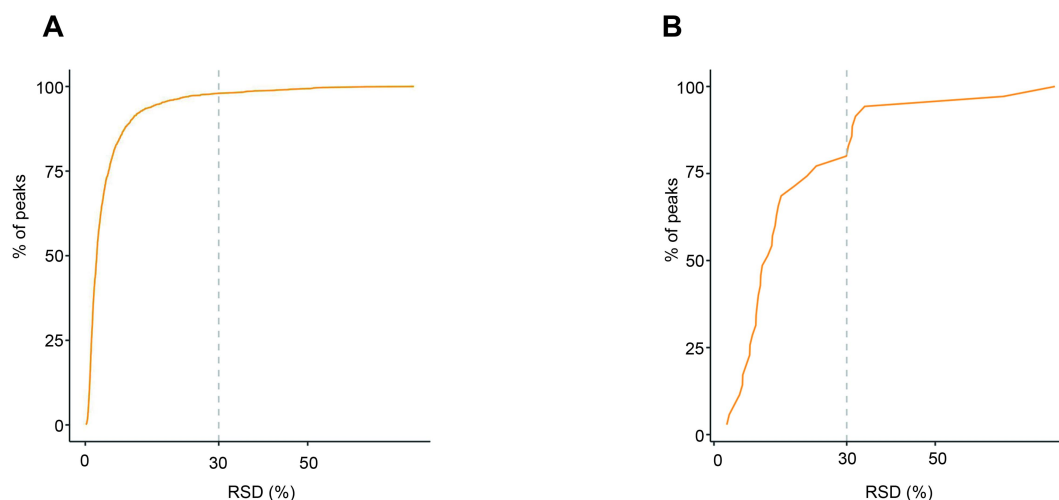


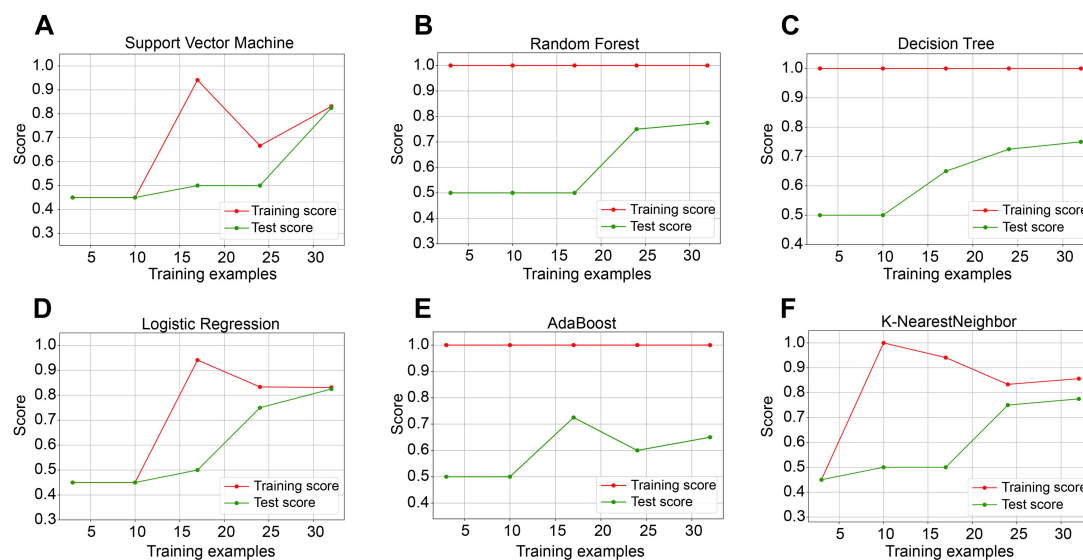
Title: Combining lipidomics and machine learning to identify lipid biomarkers for nonsyndromic cleft lip with palate

Supplemental Figure 1. QC RSD distribution of quality control samples in the lipidomic data.



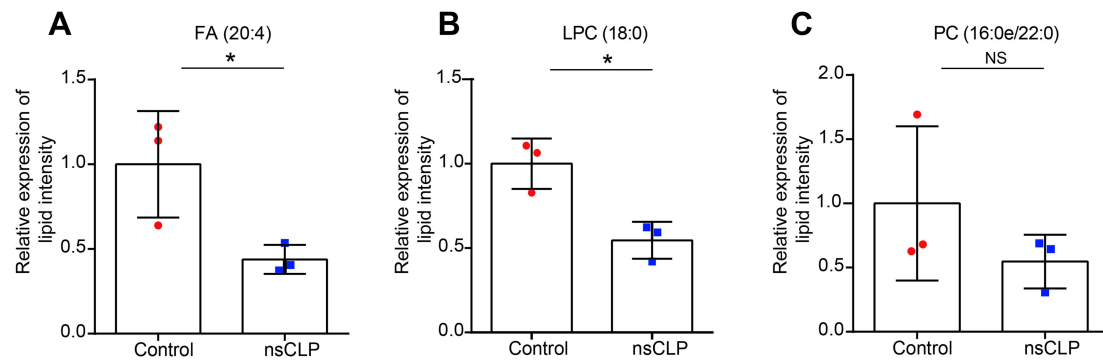
(A) QC RSD distribution of quality control samples in the untargeted lipidomics. **(B)** QC RSD distribution of quality control samples in the targeted lipidomics.

Supplemental Figure 2. Training curve analysis of the six different classification models, including SVM, RF, DT, LR, ADA and KNN.



(A) Learning curve analysis of the SVM classifier. **(B)** Learning curve analysis of the RF classifier. **(C)** Learning curve analysis of the DT classifier. **(D)** Learning curve analysis of the LR classifier. **(E)** Learning curve analysis of the ADA classifier. **(F)** Learning curve analysis of the KNN classifier.

Supplemental Figure 3. Relative expression of the three lipid features in the additional validation phase.



(A) Relative expression of the FA(20:4) in nsCLP compared with control group (data are presented as mean $\pm$ SD, by unpaired t-test; $n=3$, each group). **(B)** Relative expression of the LPC(18:0) in nsCLP compared with control group (data are presented as mean $\pm$ SD, by unpaired t-test; $n=3$, each group). **(C)** Relative expression of the PC(16:0e/22:0) in nsCLP compared with control group (data are presented as mean $\pm$ SD, by unpaired t-test; $n=3$, each group). * $P < 0.05$; NS, $P \geq 0.05$.

Supplemental Table 1. Fifteen pathways enriched by 35 dysregulated lipid features.

Pathway name	Pathway lipids	Converted lipids (number)	Converted lipids (percentage)	Converted lipids (list)	P-value	Benjamini correction	Bonferroni correction
Glycerophospholipid metabolism	26	7	58.33333333	C04438, C05973, C04230, C00157, C04233, C00350, C03819	1.87862E-07	2.81793E-06	2.81793E-06
Ferroptosis	11	3	25	C21480, C21481, C21484	0.001298164	0.00973623	0.01947246
Choline metabolism in cancer	5	2	16.66666667	C04230, C00157	0.004465587	0.022327933	0.0669838
Retrograde endocannabinoid signaling	8	2	16.66666667	C00157, C00350	0.012039813	0.045149299	0.180597194
Pathogenic Escherichia coli infection	1	1	8.333333333	C00350	0.02247191	0.06741573	0.337078652
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	3	1	8.333333333	C00350	0.066033129	0.123812117	0.990496933
Autophagy - other	3	1	8.333333333	C00350	0.066033129	0.123812117	0.990496933
Kaposi's sarcoma-associated herpesvirus infection	3	1	8.333333333	C00350	0.066033129	0.123812117	0.990496933
Autophagy - animal	4	1	8.333333333	C00350	0.087139725	0.130709587	1
Necroptosis	4	1	8.333333333	C00550	0.087139725	0.130709587	1
Sphingolipid signaling pathway	9	1	8.333333333	C00550	0.186265297	0.253998133	1
Sphingolipid metabolism	21	1	8.333333333	C00550	0.385271614	0.472457694	1
alpha-Linolenic acid metabolism	23	1	8.333333333	C00157	0.4137219	0.472457694	1
Linoleic acid metabolism	25	1	8.333333333	C00157	0.440960514	0.472457694	1
Arachidonic acid metabolism	75	1	8.333333333	C00157	0.840651573	0.840651573	1

Supplemental Table 2. Ten pathways enriched by 16 dysregulated lipid features.

Pathway name	Pathway lipids	Converted lipids (number)	Converted lipids (percentage)	Converted lipids (list)	<i>P</i> -value	Benjamini correction	Bonferroni correction
Glycerophospholipid metabolism	26	6	66.66666667	C04230, C03819, C00157, C05973, C04233, C04438	5.41132E-07	5.41132E-06	5.41132E-06
Choline metabolism in cancer	5	2	22.22222222	C04230, C00157	0.002436551	0.012182753	0.024365507
Necroptosis	4	1	11.11111111	C00550	0.065551286	0.218504285	0.655512856
Retrograde endocannabinoid signaling	8	1	11.11111111	C00157	0.127254856	0.284206144	1
Sphingolipid signaling pathway	9	1	11.11111111	C00550	0.142103072	0.284206144	1
Ferroptosis	11	1	11.11111111	C21484	0.171127574	0.285212623	1
Sphingolipid metabolism	21	1	11.11111111	C00550	0.303564456	0.390034524	1
alpha-Linolenic acid metabolism	23	1	11.11111111	C00157	0.327670026	0.390034524	1
Linoleic acid metabolism	25	1	11.11111111	C00157	0.351031071	0.390034524	1
Arachidonic acid metabolism	75	1	11.11111111	C00157	0.74459572	0.74459572	1

Supplemental Table 3. Sex features of foetuses included in lipidomic analysis.

Foetuses sex	Discovery cohort			Validation cohort			Additional validation cohort		
	Control (n = 30)	nsCLP (n = 30)	<i>P</i>	Control (n = 20)	nsCLP (n = 20)	<i>P</i>	Control (n = 3)	nsCLP (n = 3)	<i>P</i>
male	13	16	0.44	11	8	0.34	1	2	1.00
female	17	14		9	12		2	1	

Supplemental Data. Details of lipid features at every step of the machine learning approach.

untargeted lipidomics (n=1302)	FC ≥ 1.2 or $\leq$ 0.83, $P < 0.05$ (n=181)	15 lipids were excluded for could not be validated in the targeted lipidomics (n=166)	feature selection (n=148)	44 lipids were excluded for isomers (n=104)	feature selection (n=103)	targeted lipidomics (n=35)	FC ≥ 1.2 or $\leq$ 0.83, $P < 0.05$ (n=16)	panel of lipid biomarkers (n=3)
M524T237_POS	M520T154_POS	M520T154_POS	M943T993_NEG	M943T993_NEG	M524T237_POS	M524T237_POS	M802T858_POS	M303T298_NEG (FA[20:4])
M546T256_POS	M450T167_POS	M704T329_POS	M480T204_POS	M480T204_POS	M546T256_POS	M546T256_POS	M303T298_NEG	M524T237_POS (LPC[18:0])
M524T256_POS	M704T329_POS	M661T226_POS	M482T213_POS	M524T237_POS	M538T308_POS	M538T308_POS	M327T272_NEG	M849T948_NEG (PC[16:0e/22:0])
M496T169_POS	M424T153_POS	M606T230_POS	M524T237_POS	M580T219_POS	M480T204_POS	M480T204_POS	M524T237_POS	
M496T182_POS	M661T226_POS	M772T519_POS	M508T291_POS	M965T1019_NEG	M510T214_POS	M510T214_POS	M548T205_POS	
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M667T1327_POS	M380T250_POS	M496T182_POS	M869T846_POS	M773T765_POS	M606T474_POS	M606T474_POS	M815T710_NEG	
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					M801T572_1_PO
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M767T580_POS	M705T710_POS	M805T566_POS	M793T678_POS
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