

Untargeted metabolic profiling in dried blood spots identifies disease fingerprint for pyruvate kinase deficiency

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Received: July 16, 2020.

Accepted: August 28, 2020.

Pre-published: September 10, 2020.

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Supplemental information

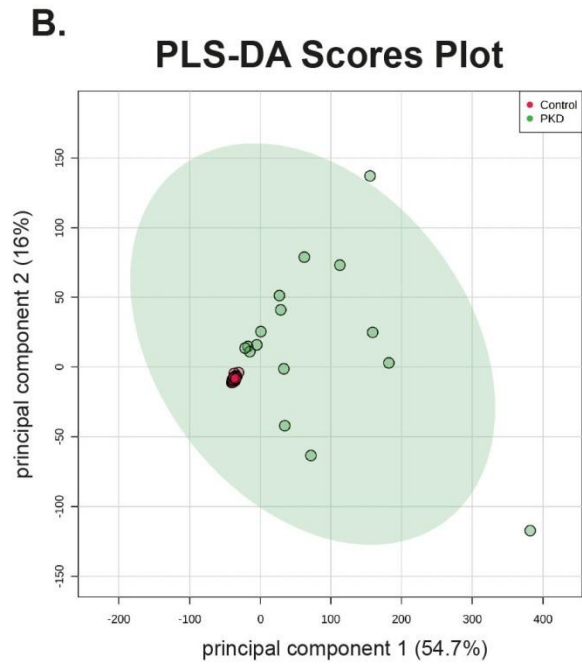
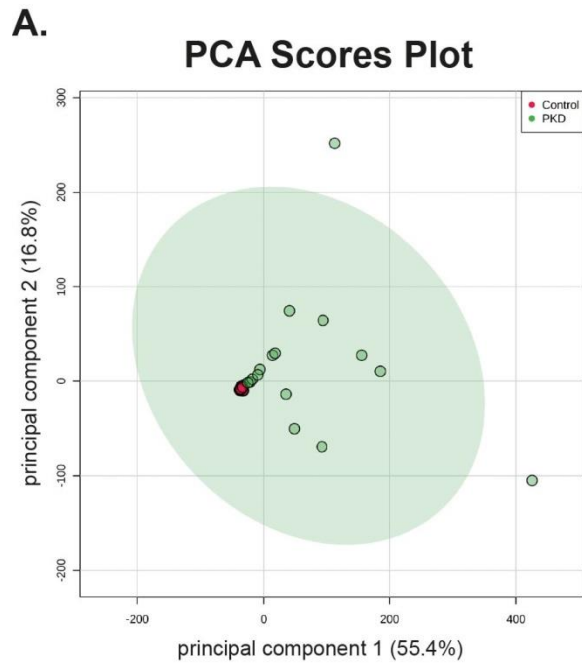
Supplementary Table 1. Clinical characteristics of additional PKD patients (test-cohort)

Age (yrs)	Gender	Hb (mmol/l)	RBC ($\times 10^{12}/L$)	Retics ($\times 10^9/L$)	WBC ($\times 10^9/L$)	Plts ($\times 10^9/L$)	Treatment	Allele 1	Allele 2
43	female	5.5	2.20	1045	11.6	714	splenectomy; no current treatment	c.401T>A; p.(Val134Asp)	c.1529G>A; p.(Arg510Gln)
44	female	5.7	2.75	756	9.3	841	splenectomy; regular transfusions	c.283G>A; p.(Gly95Arg)	c.401T>A; p.(Val134Asp)
32	female	6.5	3.49	338	9	464	splenectomy; regular transfusions	c.283G>A; p.(Gly95Arg)	c.401T>A; p.(Val134Asp)
26	male	5.0	2.33	930	15.7	780	splenectomy; sporadic transfusion	c.721C>T; p.(Glu241*)	c.1529G>A; p.(Arg510Gln)
4	<i>female</i>	7.6	4.00	194	7.8	275	<i>no current treatment</i>	<i>c.1529G>A; p.(Arg510Gln)</i>	<i>c.1529G>A; p.(Arg510Gln)</i>
54	male	6.2	3.08	166	5.3	150	no current treatment	c.142_159del; p.(Thr48_Thr53 del)	c.376-2A>C; p.(?)

Normal range* 7.4-10.7 3.6-5.5 25-120 4.0-13.5 150-450

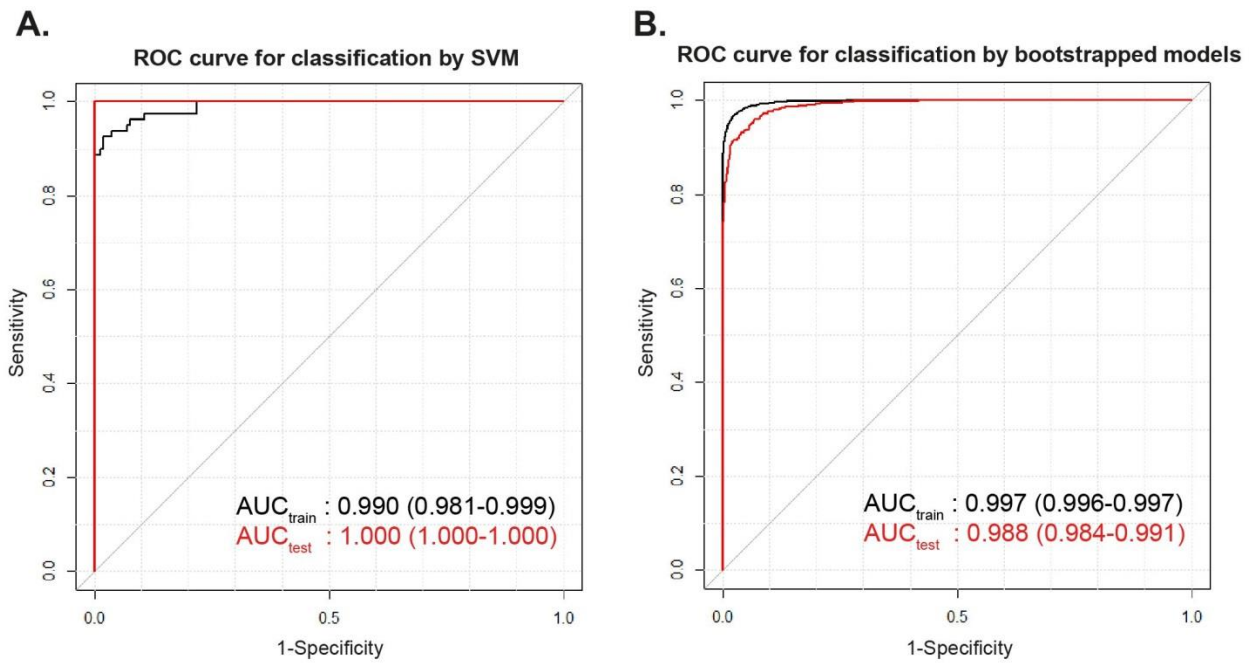
Abbreviations: Hb = hemoglobin, Retics = reticulocytes, Plts = platelets. Regular transfusions defined as ≥ 6 per 12 months.

Italic: patient who was predicted as control in predictive algorithm



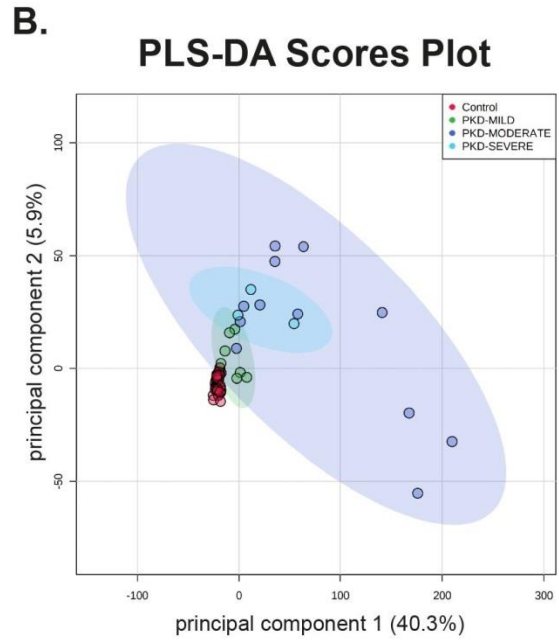
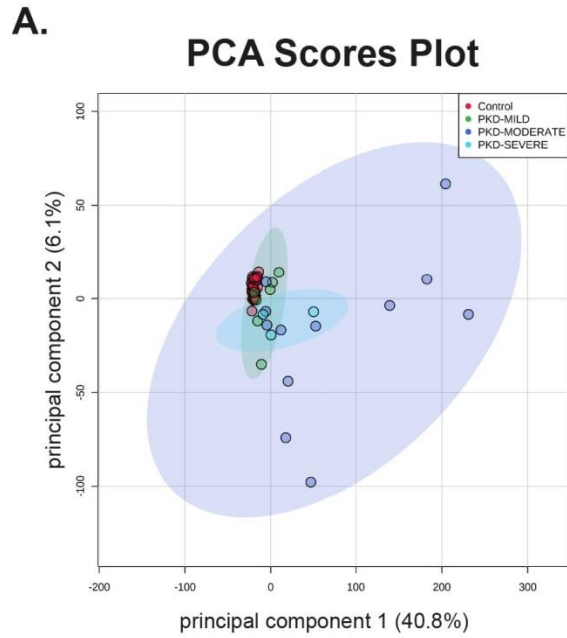
Supplementary Figure 1. Scores plots for PCA and PLS-DA

A) Principal component analysis (PCA) of PKD and control groups. **B)** Partial least square discriminant analysis (PLS-DA) of PKD and control groups.



Supplementary Figure 2. Receiver Operator Characteristic curves (ROC) for classification of training and test sets by SVM models.

A. Classification performance of samples in training and test set according to AUC. Note that AUC is a measure of the ability to rank samples according to the probability of class membership, meaning that even falsely classified samples can have a higher rank towards the correct class compared to other samples. **B.** Classification performance of samples in bootstrap models (n=100) of the complete data set.



Supplementary Figure 3. Multivariate analysis with distinction of phenotype severity

A. PCA plot, and **B.** PLS-DA plot distinguishing between disease phenotypes based on transfusion dependence and splenectomy. Most resemblance in metabolic profile is clear for mild phenotypes, followed by severely affected PKD patients (possibly related to interference of frequent transfusions).