CD3D and PRKCQ work together to discriminate between B-cell and T-cell acute lymphoblastic leukemia

Dongli Ma[1,2,*], Shan Zhong[1], Xiaorong Liu[1,2], Huirong Mai[1], Guoqin Mai[5], Cheng Xu[6], Fengfeng Zhou[3,4,*].

1. Shenzhen Children's Hospital, Shenzhen, Guangdong, 518038, China.

2. Shenzhen Children's Hospital, Shenzhen Engineering Laboratory for High-throughput Gene Sequencing of Pathogens, Shenzhen, Guangdong, 518038, China.

3. College of Computer Science and Technology, Jilin University, Changchun, Jilin 130012, China.

4. Key Laboratory of Symbolic Computation and Knowledge Engineering of Ministry of Education, Jilin University, Changchun, Jilin 130012, China.

5. Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518055, China.

6. College of Software, Jilin University, Changchun, Jilin 130012, China.

* Corresponding author: Fengfeng Zhou, e-mail: FengfengZhou@gmail.com, or ffzhou@jlu.edu.cn. Website: http://www.healthinformaticslab.org/ffzhou/. The mailing address is 2699 Qianjin Road, Jilin University, Changchun, Jilin 130012, China. Phone: +86-431-85166024, Fax: +86-431-85166024.

* Correspondence may also be addressed to Dongli Ma, Email: madl1234@126.com.

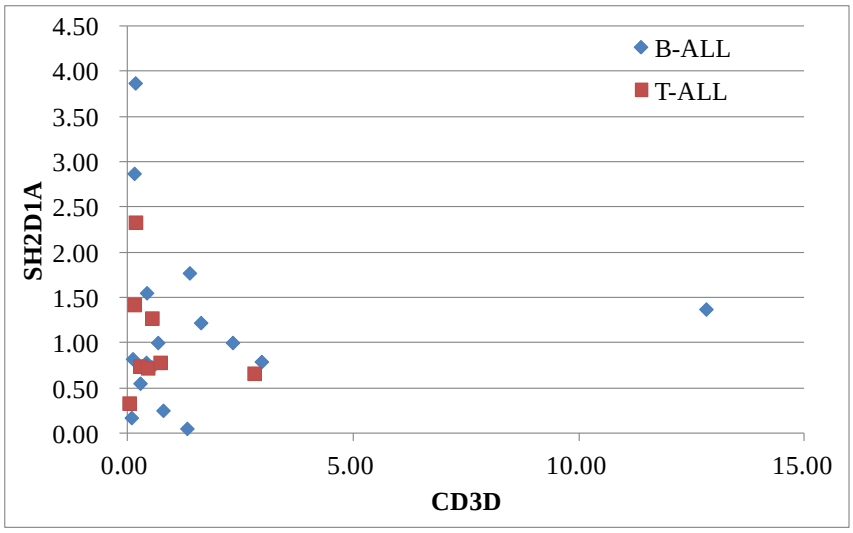
Supplementary Table S1

Primer sequences of the candidate biomarkers. The suffix FP, RP and probe refer to the forward primer, reverse primer and probe of each candidate biomarker/probeset in the next figure.

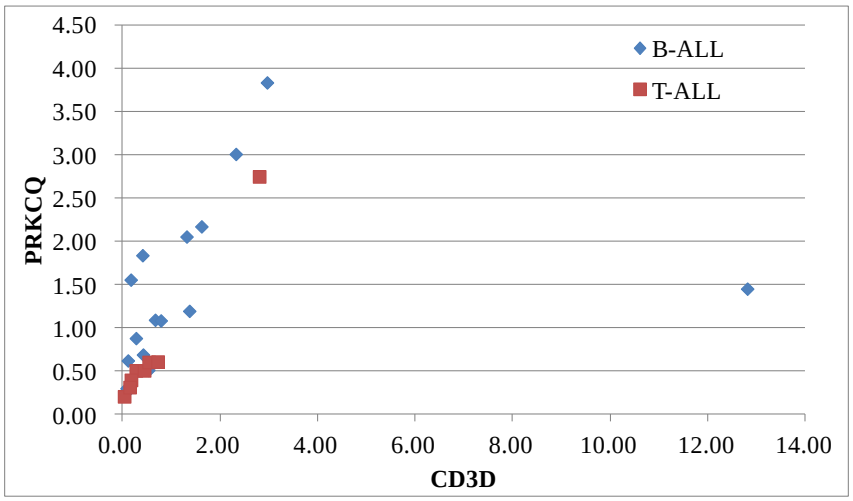
Type	Name	Primer (5' to 3')
Primer	38319_at_ CD3D_FP	GCAAGTGAGCCCCTTCAAGATAC
Primer	38319_at_ CD3D_RP	CTTGTAATGTCTGAGAGCAGTGTTC
Probe	38319_at_ CD3D_probe	AGAGGAACTTGAGGACAGAGTGTTTGTGAAT
Primer	38949_at_PRKCQ_FP	GGACTCAAGTGTGATGCATGTGG
Primer	38949_at_PRKCQ_RP	TAAGCAGCGAGCCTGTTGAGTG
Probe	38949_at_PRKCQ_probe	CATGAATGTGCATCATAGATGCCAGACAA
Primer	38147_at_SH2D1A_FP	CCTATGTGTGCTGTATCACGGTT
Primer	38147_at_SH2D1A_RP	GGAAATATCTTTTATGTACCCCAGG
Probe	38147_at_SH2D1A_probe	TTATACATACCGAGTGTCCCAGACAGAAACA
Primer	GAPDH_FP	ACGGATTTGGTCGTATTGGGC
Primer	GAPDH_RP	TTGACGGTGCCATGGAATTTG
Probe	GAPDH_probe	CCTGGTCACCAGGGCTGCTTTTAA

Supplementary Figure S1

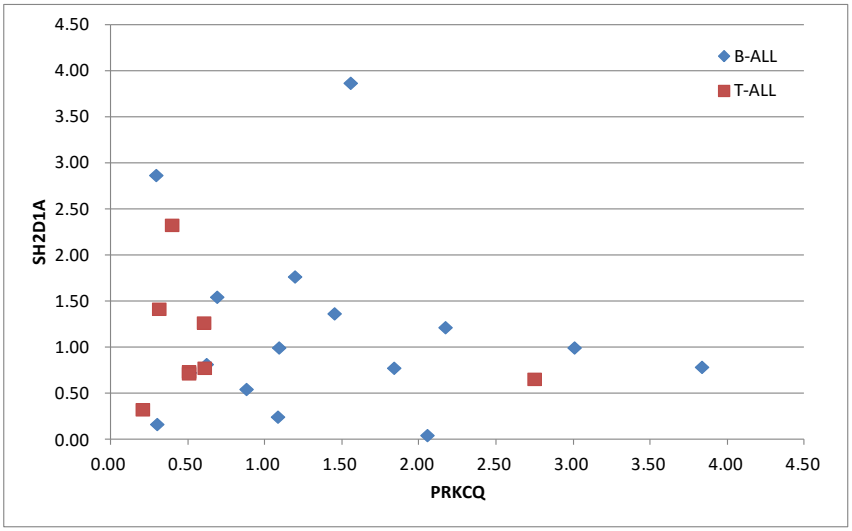
Dotplots of quantitative real-time PCR levels of the two biomarker pairs. The value of each gene is the final PCR based expression level. (a) SH2D1A versus CD3D, (b) PRKCQ versus CD3D, and (c) SH2D1A versus PRKCQ.



(a)



(b)


4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
SH2D1A
0.00
0.50
1.00
1.50
2.00
2.50
3.00
3.50
4.00
4.50
PRKCQ
B-ALL
T-ALL

(c)

Quantitative real-time PCR

Two ml blood sample was extracted from each participant, and put in a 15 ml centrifuge tube with 3 ml lymphocyte separation medium at 20 °C. The lymphocyte separation medium was provided by the Sangon Biological Engineering Co. Ltd. The tube was centrifuged for 20 minutes at 400g in 20 °C. The second layer of the solution was transferred to another 15 ml tube, added with 5 ml PBS buffer, and centrifuged for 10 minutes at 400g in 20 °C. Abandoned the supernatant and added 2 ml PBS buffer to the suspended cells, before being centrifuged for 10 minutes at 400xg and 20 °C. 0.5 ml PBS was added to the suspended cells, after abandoning the supernatant. The cell solution was transferred to a 1.5 ml centrifuge tube, and centrifuged for 5 minutes at 400g in 20 °C. After abandoning the supernatant, the rest of the solution was kept for further processing.

Total RNA was purified from the lymphocyte solutions. The cell membrane was disrupted by 350 µl buffer RLT with beta-ME. The lysate was homogenized by pipetting the solution with 5 ml injector for 5 times, and transferred to a 2 ml collection tube. One volume of 70% ethanol was added to the homogenized lysate, and the solution was mixed well by pipetting without centrifuging. Transferred up to 700 µl of the sample, including any precipitate if being formed during the process, to an RNeasy MinEluteR spin column (Micro) in a 2 ml collection tube. Closed the lid gently and centrifuge for 15 seconds at 9000g. The flow-through may be discarded. 700 µl buffer RW1 was added to the solution, and centrifuge for 15 seconds at 9000g. Discarded the flow-through. Two more rounds of centrifuge operations were conducted, with 500 µl buffers added but for 15 seconds and 2 minutes, respectively. Both centrifuge operations use 9000g. After centrifuging, the flow-through was discarded. The RNeasy spin column was placed in a new 2 ml collection tube, and centrifuge for 1 minute at 15,000g. Centrifuged the solution for 1 minute at 9000g after 30 µl RNase-free water was added. Repeat this centrifuge operation using the elute. The elute was kept for further analysis.

Mixed 7 μl of the above elute and 1 μl oligo-primer at 65 °C, and incubated for 5 minutes. Then put the mixture for ice bath for 2 minutes. Mixed 10 μl Ts-UniReaction Mix, 1 μl TransScript-Uni RT/RI Enzyme Mix, and 1 μl gDNA remover. The solution was blended and centrifuged instantaneously. Let the reverse transcription reaction run at 50 °C for 15 minutes and at 85 °C for 15 seconds. Stored the solution at -20 °C for further analysis.

The quantitative real-time PCR reaction was conducted on the Roche Lightcycle 96 system with the TaqMan Master Mix (Thermo Fisher Scientific Inc.). Put the TaqMan Master Mix 12.5 μl, Forward primer 0.75 μl, Reverse primer 0.75 μl, TaqMan fluorescence probe 0.5 μl, cDNA 1 μl and DI water 9.5 μl in the tube. The Real-time PCR conditions were as follows: 1 cycle of 50℃ for 2 min, 95 °C for 10 min; 40 cycles of 95 °C for 20 s, 59 °C for 20 s, 72 °C for 30 s. Data acquisition was performed during the annealing/extension step at 72 °C. The PCR reactions were performed for 3 times.