# Letter to the editor

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## The study of transcriptome sequencing in childhood immune thrombocytopenia

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To the editor,

Immune thrombocytopenia (ITP) is one of the most common bleeding disorders of childhood [1]. The pathogenesis of childhood ITP has been proven to be complex, and there is no genome-wide expression study through RNA sequencing (RNA-seq) to characterize the whole transcriptome change of childhood ITP. Thus, we performed RNA-seq of bone marrow samples in two childhood ITP patients (Table 1) and two normal children to identify differentially expressed genes (DEGs) and key biological processes.

We identified 1008 DEGs with at least two-fold change between ITP and control group (false discovery rate $< 0.001$), including 540 upregulated genes and 468 downregulated genes (Fig. 1a). These DEGs were involved in GO functions associated with immune, platelet and hematopoietic development, such as platelet aggregation (Fig. 1b). The dysregulation of immune system is observed in ITP patients. Through performing gene set enrichment analysis for these DEGs, we found that immune-related genes were almost down-expressed in ITP patients, consisting with immune dysregulation in ITP in previous studies [2]. In addition, we also found that the majority of platelet-related genes also showed up-expression, which may indicate dysfunction of platelet in the pathogenesis of ITP patients. The antiplatelet antibodies do not only influence the number of platelet but also affect platelet reactivity by modulating agonist stimulation and platelet secretory granule release. This observation may partially explain thrombotic events in ITP patients, perhaps due to procoagulant microparticles released by activated platelets [3]. *ACTN1* gene was one of the platelet-related genes which had three-fold change up-expression in ITP patients. ACTN1 variants have been reported to be linked with heritable thrombocytopenia [4]. However, this gene has not been studied in childhood ITP. Its high expression may have a possible role in low platelet count of ITP, further demonstrating the role of dysfunction of platelet in the pathogenesis of ITP.

The involvement of T cells in the pathogenesis of ITP has been known for many years. In our study, we found that most genes were significantly enriched with regulation of Tregs. Previous studies reported that decreased number of Tregs might be one of the mechanisms that cause immune regulation dysfunction in idiopathic ITP patients [5]. Our analysis further proved the role of Tregs in the pathogenesis of childhood ITP. Moreover, the majority of enriched genes were upregulated in ITP patients. These upregulated genes may influence the number and function of Tregs. natural killer (NK) cells play an important physiological role in controlling immune responses and regulate T-cell-mediated and B-cell-mediated adaptive immunity at multiple levels. Although studies that examined NK cells in ITP are few, decreased number of NK cells in pediatric ITP have been reported [6]. Our study further demonstrate that the NK-cell-mediated cytotoxicity is related with the pathogenesis of pediatric ITP patients. In addition, most genes enriched in the function of NK-cell-mediated cytotoxicity were up-expressed in ITP patients (Fig. 1c). Among these genes, *RAC2* gene showed 3.97-fold change up-expression. This gene participates in the NK-cell-mediated anti-cryptococcal killing [7]. The up-expression of *RAC2* gene may play a critical role in the abnormal function of T cell and NK cell in ITP patients.

Cytokine abnormalities have been reported to be associated with ITP. IFN-γ, TNF-α, IL-4, IL-6 and IL-10 were elevated significantly in ITP patients [8]. In our study, we also found that the DEGs were enriched in functions related with cytokines, such as chemokine-mediated signaling pathway. We found several known cytokines were differentially expressed, including FPR1 and TNFSF13, both of them showed up-expression. Compared with previous studies, our results discovered a new dysregulated cytokine profile in childhood ITP patients. Furthermore, we found that the whole transcriptome was positively enriched with genes involved in megakaryocyte development and platelet production. This is in accord with the theory that megakaryocyte s are targeted by autoantibodies and T cells, which leads to impaired megakaryocyte maturation and platelet production [9]. In our study, the enrichment genes including 13 member genes of

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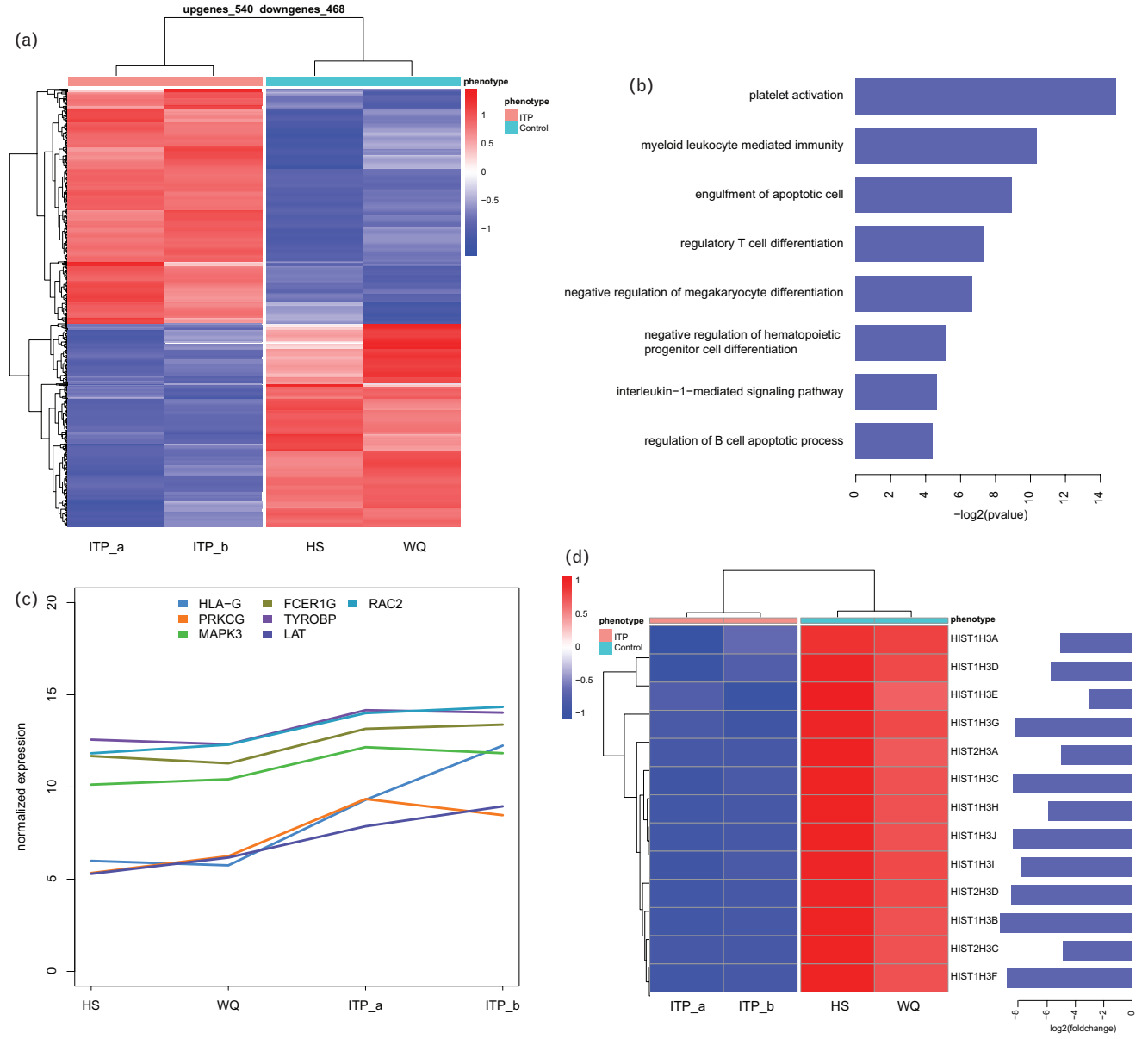
**Table 1 The clinical material of two childhood immune thrombocytopenia patients**

| Clinical material | Patient a | Patient b |
|---|---|---|
| Sex | Female | Female |
| Age (years) | 2 | 9 |
| Platelet ($\times 10^9$/l) | 38 | 69 |
| Clinical manifestation | Petechiae, purpura | Petechiae |
| Response to glucocorticoids | Sensitive | Sensitive |
| Follow-up time (months) | 58 | 77 |
| Whether stop medication | No | Yes |

histone cluster were all down-expressed. Aberrant histone methylation has been elucidated in the patients with ITP [10]. Thus, we speculate that the down-expression of histone genes may alter the histone state, which may finally induce the decrease of platelet production (Fig. 1d). Detecting new inducible costimulatory signal transduction pathway may provide a new theoretical basis for studying the pathogenesis and treatment of ITP. In our study, we identified several new pathways in

**Fig. 1**




(a) The heatmap of expression level of 1008 differentially expressed genes. (b) The significantly enriched functions of differentially expressed genes. Differentially expressed genes were involved in GO functions associated with immune, platelet and hematopoietic development. (c) The enriched genes of natural killer cell-mediated cytotoxicity. (d) 13 member genes of histone cluster, significantly enriched in megakaryocyte development and platelet production, were all down-expressed.

ITP, such as Fc gamma R-mediated phagocytosis, oxidative phosphorylation and Notch signaling pathway. Some genes involved in these pathways showed abnormal expression. For example, genes involved in the pathway of Fc-gamma R-mediated phagocytosis and Notch signaling pathway were both upregulation in ITP patients.

In conclusion, our results shed some light on the whole transcriptome change of childhood ITP patients and elucidated the genes and pathways consistently aberrant in ITP. These abnormal expressed genes, cytokines and pathways may play important roles in the pathogenesis of childhood ITP patients, and may serve as potential targets of diagnosis or treatment.

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### Conflicts of interest

There are no conflicts of interest.

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