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Letter to the Editor

Rethinking newborn screening for severe combined immunodeficiency: Lessons from an international partnership for patients with primary immunodeficiencies in Pakistan

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To the Editor,

Early diagnosis of primary immunodeficiencies (PIDs) reduces infectious complications, guides therapeutic decision-making, and minimizes iatrogenic risks [1]. T cell receptor excision circle (TREC) analysis is the most widely utilized screening test for severe combined immunodeficiencies characterized by T cell lymphopenia [1]. An abnormal newborn TREC screen prompts assessment of T cell numbers and function, followed by genetic sequencing in patients with impaired function [1]. This tiered approach is cost-efficient for populations with low pre-test probabilities of PID and access to multi-disciplinary laboratory expertise in immunophenotyping, assays of immune function, and genetic sequencing. However, many countries with the highest prevalence of PIDs lack these diagnostic technologies. The majority of known PIDs are autosomal recessive, monogenic disorders, and are most prevalent in areas of Northern Africa, the Middle East, and southeast Asia, all of which have a high frequency of consanguineous unions and founder mutations [2]. Compared to countries with resources for implementing widespread newborn TREC screening or next-generation DNA sequencing (NGS), resource-limited regions have distinctly different health care delivery models and challenges in the diagnosis of PIDs [2].

We tested the feasibility of an international partnership for providing NGS to patients with PIDs in Pakistan, which has the highest infant mortality rate in the world [3]. The mortality rate of neonates before 28 days of life is 1 in 22.7 live births, while the mortality rate of children from one to five years of life is 1 in 9.5 live births [3]. Over 60% of unions are consanguineous [4]. Pakistan does not have government-sponsored initiatives for PID patients, such as registries or newborn screening programs. NGS for PIDs is available at one center in Pakistan for approximately $1500 USD per patient. Hematopoietic stem cell transplantation (HSCT) has been available in Pakistan since 1995 [5]. Transplantation is limited to family donors since there is no national bone marrow donor registry [5]. Over 350 transplants were performed from 1999 to 2009 in Pakistan, during which mortality was 10–20%, compared to global mortality rates of 10–50% [5]. Supportive therapies for PIDs, including intravenous immunoglobulin replacement and antibiotics, are also available in Pakistan.

Clinical histories and immunophenotyping on 10 patients were performed by the Armed Forces Institute of Pathology, Rawalpindi, the largest independent laboratory serving a catchment area of over 50 military hospitals. Targeted NGS for a panel of 264 genes associated with PIDs was performed at Boston Children's Hospital at a cost of $250 USD per patient, funded by a philanthropic grant. Recurrent infections occurred in 80% of patients, respiratory infections occurred in 60% of patients, failure to thrive in 40% of patients (Table 1). Nearly all patients (90%) were from consanguineous unions and 40% had a family member die due to symptoms shared by the proband.

The average delay from symptom onset until local immunologic testing was 16 months. A genetic cause of PID was identified after targeted NGS in nine patients (90%), comprised of eight autosomal recessive and one X-linked PID. Combined immunodeficiencies were the most common type of PID (80%). All of these patients had T cell lymphopenia severe enough to be identified by TREC analysis, if it had been performed, and would thus have prompted functional and genetic testing. This supports the utility of using NGS as the initial diagnostic test. Live vaccines are contraindicated in patients with combined immunodeficiency; however, seven (70%) patients in our cohort with a combined immunodeficiency received live vaccines as part of the national immunization program prior to diagnosis. Eight patients (80%), nearly all of whom had a combined immunodeficiency, died by the conclusion of this 16-month study, reflecting the known urgency of early diagnosis for patients with severe PIDs [1].

Studies have noted the widening global disparities in genomics expertise and technologies [6]. Early partnership models allocated the work of sample collection to collaborators in resource limited areas, while investigative studies were performed in centers with scientific expertise [6]. More recently, training programs have been developed to

Abbreviations: PID, primary immunodeficiency disease; NGS, next generation sequencing; TREC, T cell receptor excision circle; HSCT, hematopoietic stem cell transplantation

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Table 1
Clinical and laboratory features of patient cohort.

Sex	Age at testing	Consanguinity	Family death due to recurrent infections	OPV/BCG vaccine	Clinical presentation	Cell counts	Referral delay[a]	Outcome	Inheritance	Mutation	Category of disease
F	3 m	+	−	+	Recurrent infections, respiratory infections	$CD3^+$: 0 (2500–5500) $CD19^+$: 0 (300 − 200) $CD56^+$: 2040 (170–1100)	3 m	Deceased	AR	*DCLRE1C:* deletion of exons 12,13,14	Severe combined immunodeficiency
M	5 m	+	−	+	Recurrent infections, respiratory infections, fever, cough, dyspnea	$CD3^+$: 0 (1900–5900) $CD19^+$: 0 (430–3000) $CD56^+$: 151 (9170–830)	3 m	Deceased	AR	*DCLRE1C:* deletion of exons 1,2,3	Severe combined immunodeficiency
M	3 m	+	+	+	Recurrent infections, failure to thrive	$CD3^+$: 0 (2500–5500) $CD19^+$: 568 (300–200) $CD56^+$: 37 (170–1100)	3 m	Deceased	AR	*IL7R:* c. 83–1 G > T	Severe combined immunodeficiency
M	7 m	+	+	+	Respiratory infections, fever, cough, failure to thrive	$CD3^+$: 148 (1900–5900) $CD19^+$: 8 (610–2600) $CD56^+$: 538 (160–950)	3 m	Alive	AR	*RAG1:* p. R759C	Severe combined immunodeficiency
F	2 m	+	+	+	Oral thrush	$CD3^+$: 0 (2500–5500) $CD19^+$: 0 (300–200) $CD56^+$: 526 (170–1100)	1 m	Deceased	AR	*RAG1:* p. R737H	Severe combined immunodeficiency
M	3 m	+	−	+	Chronic diarrhea, failure to thrive, chronic cough	$CD3^+$: 0 (2500–5500) $CD19^+$: 0 (300–200) $CD56^+$: 22 (170–1100)	3 m	Deceased	AR	*ADA:* p. G239D	Severe combined immunodeficiency
M	10 m	+	+	+	Recurrent fever	$CD3^+$: 0 (1900–5900) $CD19^+$: 198 (610–2600) $CD56^+$: 0 (160–950)	9 m	Deceased	AR	*JAK3:* deletion of exon 10	Severe combined immunodeficiency
F	5y	−	−	−	Recurrent infections, skin lesions, respiratory infections, pancolitis	$CD3^+$: 1940 (2300–5400) $CD19^+$: 776 (390–1400) $CD56^+$: 60 (130–720)	5y	Loss to follow up	AR	*DOCK8:* p. L510Rfs	Combined immunodeficiency
M	7y	+	−	+	Recurrent infections, tuberculosis, failure to thrive	$CD3^+$: 2369 (1200–2600) $CD19^+$: 0 (270–860) $CD56^+$: 24 (100–480)	5y	Deceased	XL	*BTK:* c. 240 + 1G > T	Antibody deficiency

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Table 1 (*continued*)

Sex	Age at testing	Consanguinity	Family death due to recurrent infections	OPV/BCG vaccine	Clinical presentation	Cell counts	Referral delay[a]	Outcome	Inheritance	Mutation	Category of disease
M	2.25y	+	–	–	Recurrent skin infections, fever	$CD3^+$: 6271 (1400–3700) $CD19^+$: 4378 (390–1400) $CD56^+$: 828 (130–720)	1.75y	Deceased	N/A	N/A	Leukocyte adhesion deficiency

[a] The average delay from symptom onset until local immunologic testing.

disseminate genomics expertise and NGS technologies globally [6]. However, challenges encountered by trainees of these programs include the time needed for trainees to acquire the necessary expertise and the limited research infrastructure encountered by researchers returning to their home countries [6]. Our study provides a third model of international collaboration built on the strengths of each collaborating center. Given the high rate of childhood mortality in Pakistan, the clinical expertise of local physicians is essential for distinguishing patients with potential PIDs from those with other causes of childhood illness. The capacity for performing immunophenotyping locally is important for the interpretation of NGS data, since transit times and shipping impair the viability and function of immune cells [7]. International centers with a high volume of NGS can provide immunogenomics expertise and technology at lower cost. Due to the pace of change in the fields of both NGS and PIDs, it is less costly and more feasible to update a limited number of central sequencing hubs than to establish and maintain a large number of local sequencing facilities. Additionally, sequencing mutations in the Pakistani population in conjunction with development of a national registry can identify at-risk kindreds. In a study of extended family screening for hemoglobin disorders in Pakistan, 31% of relatives of index cases were carriers; 40% of married carriers were married to another carrier [8]. Quantifying the incidence of combined immunodeficiencies in Pakistan would also highlight the importance of delaying live vaccines in this population. Despite the burden of genetic diseases in Pakistan, genetic counseling is not readily available [4]. In a study surveying genetic expertise in Pakistan, only four clinical geneticists were identified for the country's population of over 180 million individuals; furthermore, 88% of the physicians surveyed self-identified their knowledge of genetics in medicine as “fair” or “poor.” [4] Increasing the local capacity for immunophenotyping and clinical genetics builds on the existing infrastructure to address the need for improved diagnostics for patients with PIDs in Pakistan.

Larger studies are needed to determine the applicability of our findings to the Pakistani population at large and to other genetic disorders. Our study suggests that resource-limited regions with a high prevalence of PIDs may require screening strategies that utilize immunophenotyping and NGS rather than TREC analysis, an endeavor that can be facilitated by global diagnostic partnerships.

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Jacqueline G. Wallace[a,1], Hamid Nawaz Tipu[b,1], Kelsey Stafstrom[a], Mohammed F. Alosaimi[a,c], Michel J. Massaad[a,d], Wayne Bainter[a], Raif S. Geha[a], Janet Chou[a,*]
[a] *Division of Immunology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA*
[b] *Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan*
[c] *Department of Pediatrics, King Saud University, Riyadh, Saudi Arabia*
[d] *Faculty of Medicine, American University of Beirut, Beirut, Lebanon*
E-mail address: janet.chou@childrens.harvard.edu (J. Chou).

* Corresponding author at: Division of Immunology, Boston Children's Hospital, Karp building 10th floor, 1 Blackfan Circle, Boston, MA 02115, USA.
[1] Equal contribution.