Improving the Diagnostic Yield in Patients Suspected for Primary Immunodeficiency

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Introduction

Finding the genetic diagnosis for patients with suspicion of immunodeficiency is becoming increasingly important in the management of primary immunodeficiency (PID) and estimating the risk for family members[1]. Here we report our experiences with nearly 2200 patients suspected with PIDs. Our data is based on simultaneous sequence and copy number variant (CNV) analysis combined with customized analysis for difficult to sequence regions in specific genes such as *NCF1*.

Methods

A retrospective review of 2162 cases sent for targeted panel testing related to PID at Blueprint Genetics was performed. Patients were tested with Blueprint Genetics next generation sequencing (NGS) related to immunology. A majority of the analyses (2095/2162, 97%) were performed combining both sequencing analysis and deletion/ duplication analysis utilizing NGS data (called as PLUS analyses). We have previously shown that our deletion/duplication analysis can identify both small deletions and duplications (1–5 exons) as well as larger CNVs. Specimens were sequenced with an Illumina NovaSeq 6000 platform.

Results

Diagnostic Yield

Diagnostic yield including all immunology related panels was 14.4% (311/2162). The highest diagnostic yield 19.9% (83/417) was observed in children from ages 0 to 5 years. The diagnostic yield was highly variable between the different panels.

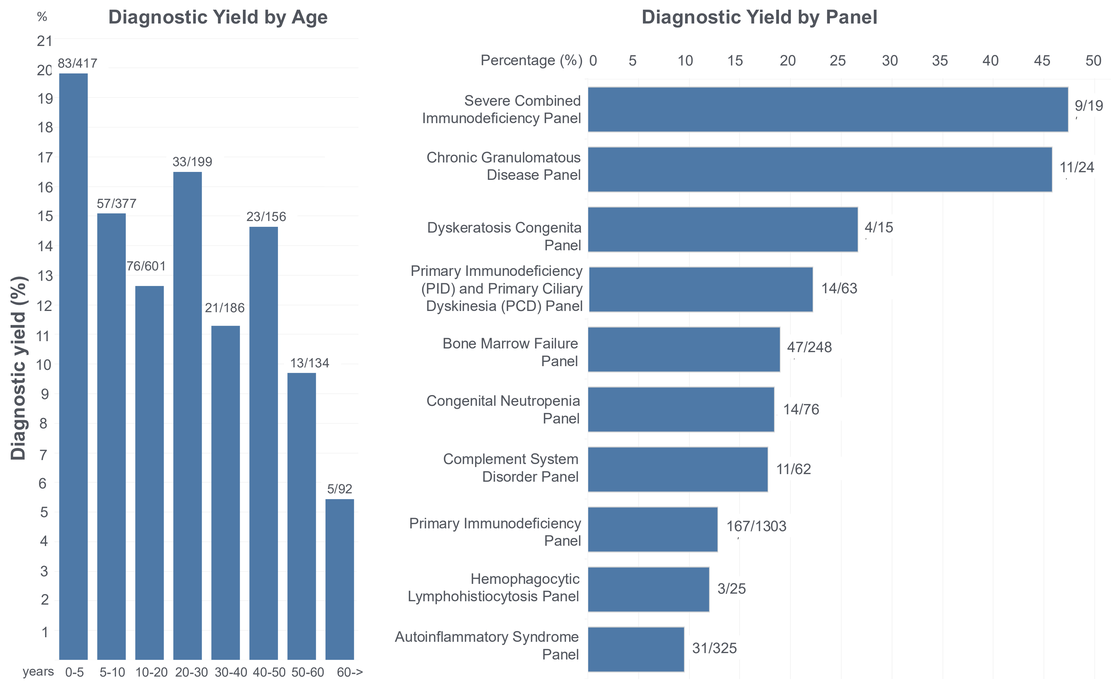

Fig. 1. Diagnostic yield in different age groups (A) and among different panels (B).

Diagnostic Sequence and CNV Findings

Out of the 270 reported pathogenic (P) and likely pathogenic (LP) diagnostic sequence variants, 217 (80%) were unique. VUS1 variants (VUS favoring likely pathogenic) were excluded from this data. Altogether 30 CNVs were reported for 29 patients.

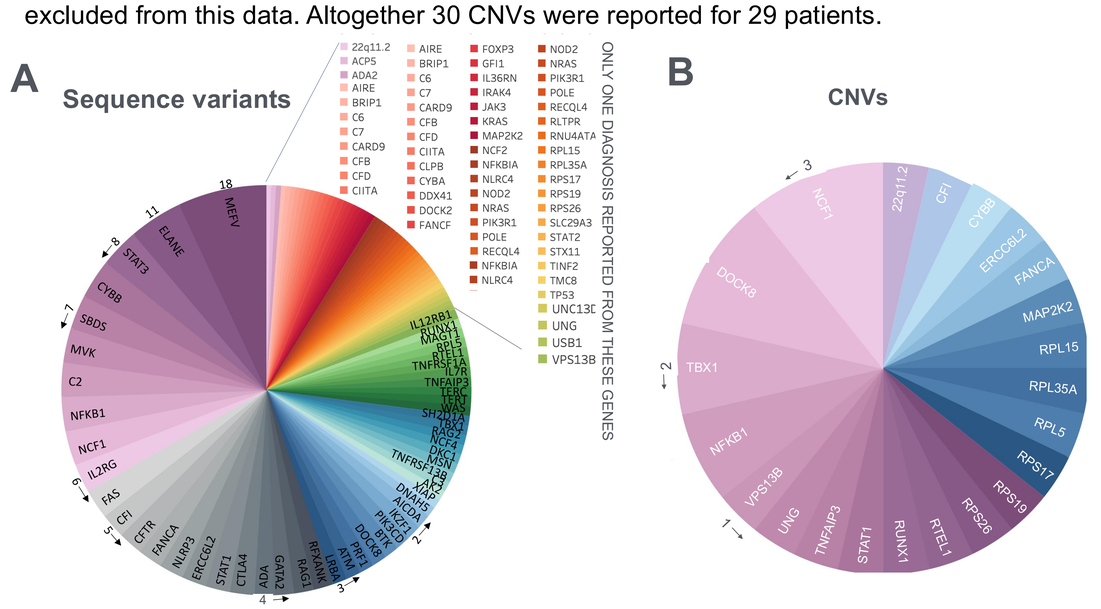

Fig. 2. Number of diagnostic findings (LP/P) in different genes; sequence variants (A) and CNVs (B).

NCF1 Custom Analysis

Homozygous deletion of *NCF1* was confirmed in three chronic granulomatous disease patients by custom analysis. *NCF1* gene is complicated by two highly homologous pseudogenes *NCF1B* and *NCF1C*. Homozygous deletions were detected by our CNV detection algorithm and confirmed by Sanger sequencing with primers that specifically bind to either *NCF1* or the pseudogenes. Additional bioinformatic analysis targeting two specific coding positions in exons 4 and 7 that differ between the *NCF1* gene and the two pseudogenes showed that all reads in those positions originated from the pseudogenes, and thus, supported the original finding.

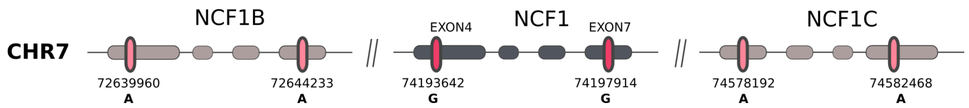

Figure 3. *NCF1* and pseudogenes *NCF1B* and *NCF1C*. Differing positions in *NCF1B*, *NCF1C* and *NCF1* exons 4 and 7 used for bioinformatic analysis are highlighted.

Conclusions

- **Diagnostic yield in PID patients was 14.4%, and highest in children from ages 0 to 5 years 19.9%.**
- **Diagnostic yield varied greatly between different panels related to PID.**
- **Genetic diagnoses in PID patients are highly heterogeneous.**
- **Our customized analysis identified 3 patients with homozygous deletion in *NCF1*.**

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References:
1. Bousfiha A, Jeddane L et al. (2020) Human Inborn Errors of Immunity: 2019 Update of the IUIS Phenotypical Classification. *J Clin Immunol.*

Conflict of interest statement: All authors are employed by Blueprint Genetics.