

Experience of germline genetic testing for inborn errors of immunity: using multigene panel testing compared to exome sequencing at a diagnostic laboratory

Daniel E. Pineda-Alvarez, Trevor J. Williams, Yi-Lee Ting
Labcorp (formerly Invitae Corporation), San Francisco, CA

Despite the growing number of genes associated with IEIs, the increase in molecular diagnosis rate from ES cannot be exclusively attributed to novel IEI-related genes



Background

- Next-generation sequencing (NGS) has proven a valuable tool to diagnose inborn errors of immunity (IEI) because it can interrogate many genes concurrently and has enabled a quick expansion of IEI-related genes.
- Currently, both multigene panel testing (MGPT) and exome sequencing (ES) are available. While ES can analyze novel and established IEI genes, fixed MGPTs are still broadly used.
- The aim of this study was to examine the molecular diagnosis (MoDx) rate from both MGPT and ES, and the phenotypic pattern of patients referred for ES.

Methods

- Patients were referred for MGPT or ES between March 2017-May 2024 at a diagnostic laboratory.
- MGPT contained up to 574 genes and were curated based on the International Union of Immunological Societies (IUIS) phenotypic classification list of genes related to IEIs¹ and expert opinion.
- Patients in the ES cohort were selected based on clinician-provided ICD-10 and Human Phenotype Ontology (HPO) terms, grouped under their top-level HPO terms. We required patients in the ES cohort to have at least one HPO term under "Abnormality of the immune system" to be included.
- Variants were classified using Sherloc², a validated variant classification framework based on the ACMG/AMP variant classification guidelines³.
- MoDx was defined by one pathogenic/likely pathogenic (P/LP) variant in a gene with an autosomal dominant, X-linked dominant or X-linked recessive (male only) inheritance pattern or two or more P/LP variants in trans in a gene with an autosomal recessive inheritance pattern.
- Odds ratios (OR) and p-values were calculated using G-tests; p-values < 0.05 were considered statistically significant.

Results

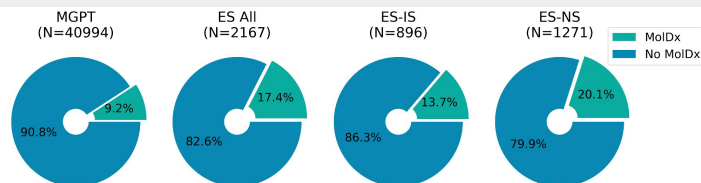


Figure 1: Comparison of MoDx. The overall MoDx was higher in the ES cohort (378/2,167; 17.4%) versus MGPT (3,754/40,994; 9.2%) (OR 2.1, $p < 2.2 \times 10^{-16}$). Patients in the ES-IS cohort had a lower MoDx rate (123/896; 13.7%) than the ES-NS cohort (255/1,271; 20.1%; OR 1.58, $p = 0.0001$) but a higher MoDx rate than the MGPT cohort (OR 1.58, $p = 1.1 \times 10^{-5}$).

- The most frequent top-level HPO term was "Abnormal nervous system" so we further stratified the ES cohort into individuals with higher proportions of terms under abnormality of the immune system (ES-IS cohort) versus those with a higher proportion of terms under abnormal nervous system (ES-NS cohort).
- A subset of 121 patients had both MGPT and ES (Figure 2):
 - 110/121 had a concordant diagnostic result e.g. Positive MoDx on MGPT and ES is diagnostic. Carrier in MGPT and Negative on ES were considered concordant since those are non-diagnostic results
 - 8/121 had discordant diagnostic results with a positive result on ES, typically in a gene not related to the patient's clinical indication (Not IEI-related finding in ES)
 - 1/121 had a discordant diagnostic result with a positive result on MGPT due to phenotypic overlap (Not IEI-related finding in MGPT)
 - 1/121 had a discordant diagnostic result due to technical differences in calling variant types between MGPT and ES
 - 1/121 had a discordant diagnostic result with a positive result on ES due to a gene not on panel

Conclusion

- The MoDx in patients with IEI tested using ES is higher compared to those tested via MGPT, as expected.
- This difference may be explained by the indication for testing, which suggests patients who present with an IEI phenotype and involvement with another organ system may benefit from ES.
- Granular characterization of the phenotypic spectrum of patients who receive a MoDx from ES is warranted.

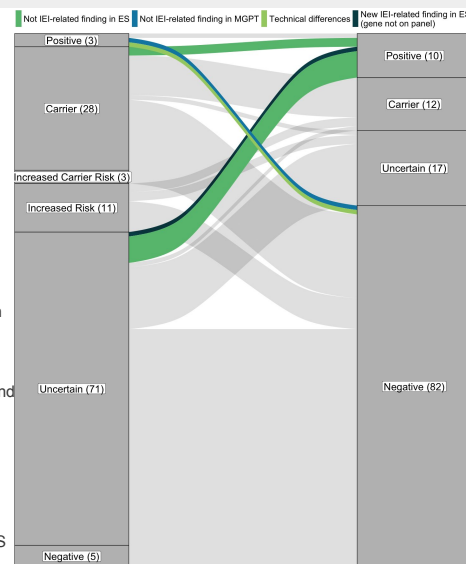


Figure 2: Concordance of diagnostic results from patients who had both MGPT and ES. Discordant diagnostic results are shown in the legend.