Title: Multiple Findings in Diagnostic Exome Sequencing: Separate or Intertwined Diagnoses?

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Diagnostic Exome Sequencing (DES) allows simultaneous interrogation of all protein-coding genes and therefore can be especially useful for diagnosing patients with complex clinical presentations, and is uniquely effective in the identification of multiple diagnoses in a single patient. Among 6061 patients who underwent DES at Ambry Genetics, 1406 patients (23%) received at least one genetic diagnosis, *i.e.* at least one variant that was either likely pathogenic (VLP) or pathogenic (P) in a gene with significant clinical overlap with characterized disease. Within the cohort of patients who received a genetic diagnosis, 122 patients (8.7%) received multiple gene findings (additional findings could be variants of unknown significance or have uncertain clinical relevance). These patients with multiple results make up 2.0% of the total cohort of patients tested. Of patients with multiple results, 108 patients had potentially relevant findings in 2 genes, 13 patients had relevant findings in 3 genes, and 1 had relevant findings in 4 genes. We reviewed these cases to clarify the types of cases that receive multiple diagnoses.

Of all diagnosed patients, non-trios were more likely to receive multiple diagnoses than parent-proband trios: 13% (40 patients) of all non-trios received multiple diagnoses, while 8% (82 patients) of all trios received multiple diagnoses (p < 0.05). In cases who received a single diagnosis, more than half (53%) of the variants identified were de novo. In cases who received multiple diagnoses, however, de novo variants were in the minority (43%), despite each patient having at least twice as many diagnostic variants as cases with single diagnoses. Of all the genetic diseases identified in the patients with multiple potential relevant gene findings, 56.9% were autosomal dominant, 27.8% were autosomal recessive, and 14.9% were X-linked, and 0.4% were mitochondrial. These rates are similar to the overall diagnostic rates of the whole cohort.

Most patients who received multiple diagnoses had complex clinical presentations, involving an average of 4.1 organ systems per patient, but this was not significantly different from the average of 3.7 organ systems per patient involved in the whole cohort tested. The most commonly involved systems were neurologic, musculoskeletal, craniofacial, gastrointestinal, and opthalmologic. Given the diversity of organ systems involved per patient on average, it can be difficult to select a disease-specific gene panel enabling the identification of the correct single or multiple diagnoses, so DES is a good strategy.

Twenty patients received two definitive molecular diagnoses with exclusively VLP or P variants in a gene with significant clinical overlap with characterized disease. For 20% (4 patients), the diagnoses explained completely separate symptoms, suggesting the presence of two different Mendelian diseases in the same patient (for instance, *ADNP*-Helsmoortel-van der Aa syndrome and *PKD1*-Polycystic kidney disease). In 80% (16 patients), each diagnosis could be responsible for some unique symptoms, but the two diagnoses had sufficient symptom overlap as to appear intertwined (eg, *SATB2*-SATB2-associated-syndrome and *CAMTA1*- Cerebellar ataxia, nonprogressive, with intellectual disability). In patients with completely overlapping, or "competing" diagnoses, additional clinical evaluation of the proband or discovery of the new pathognomonic features of each disease might allow a more precise diagnosis for

patients with completely overlapping, or "competing" diagnoses. In many cases with dual definitive findings, however, it is likely that both genetic diseases are contributing to the overall phenotype, and oligogenic effects may affect the clinical presentation Our results also suggest that the number of genetic diseases to be discovered in a patient does not correlate with the number of organ systems involved, and that for many patients, a single diagnosis may not be the whole story.

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