Yield of Clinically Relevant Candidates in Family Genomes in the UK 100,000 Genomes Project Using the Fabric Genomics Platform

Melanie Babcock[1], Charlene Son Rigby[1], Marco Falcioni[1], Andrew Guo[1], Josh Grigonis[1], Kyle Hart[1], Mark Yandell[2], and Martin G. Reese[1]. 1. mbabcock@fabricgenomics.com, Fabric Genomics™ Inc., Oakland, CA; 2. Department of Human Genetics, University of Utah, Salt Lake City, UT

ABSTRACT

The 100,000 Genomes Project, spearheaded by Genomics England (GeL), is a United Kingdom National Health Service sponsored study aimed at identifying disease-causing genetic variants in patients and families with rare genetic diseases and cancer using a whole genome sequencing (WGS) approach. For this study, clinical history was used to recruit patients into specific disease categories, each of which were associated with gene panels curated in the GeL PanelApp tool.

Fabric Genomics™, a clinical interpretation partner for the 100,000 Genomes Project, has analyzed over 1973 clinical cases using Opal™ Clinical, powered by Fabric Enterprise, a comprehensive platform for NGS data analysis and clinical reporting. The variant filtering and prioritization protocols utilized for case analysis include GeL's variant tiering methodology, ClinVar, and Fabric Genomics' proprietary variant and gene ranking algorithms VAAST (Variant Annotation, Analysis and Selection Tool) and Phevor (Phenotype Driven Variant Ontological Re-ranking Tool). We report results showing that by applying VAAST and Phevor we increase the clinical candidate yield compared to using the GeL tiering system alone. We identified candidate causal genes/variants in 49.8% of the cases. In 23.0% of these cases (9.4% overall) candidates were only obtained by using the VAAST/Phevor top 20 ranked genes/variants.

In a subset of 1191 cases, we reviewed the effects of providing parental genomes in the analysis and return rate of results. Interestingly, there was only small difference in clinical candidate yield between solo cases and true trios (proband and unaffected parents), but by further investigating how the causal candidates were determined, it showed an increase of candidates that were identified exclusively from VAAST/Phevor top 20 ranked genes in true trios compared to solos.

Interpretation Methodology

We have implemented a whole genome interpretation methodology within Opal™ Clinical that is comprehensive and rapid.

The workflow developed for GeL includes consideration of the following variants:

- Variants scored under different inheritance modes in the top 20 genes ranked by VAAST and Phevor, two sequentially applied proprietary algorithms: VAAST integrates sequence conservation, genetic consequence, and allele frequency in a probabilistic framework to identify disease-causing alleles, Phevor then combines HPO-based patient phenotype descriptions with the VAAST results to re-rank the variants
- Variants in phenotype matched panels curated in the GeL PanelApp tool and categorized using GeL's tiering methodology (https://bioinfo.extge.co.uk/crowdsourcing/PanelApp/): known pathogenic, protein truncating, and de novo protein alternating variants (Tier 1), and other protein altering variants (Tier 2)
- Variants with at least one ClinVar Pathogenic or Likely Pathogenic submission

The interpretation methodology concludes with an expert review by Fabric Genomics' Clinical Services team. Each case was interpreted by two primary reviewers. Identified candidates were scored using the ACMG variant interpretation guidelines.

The candidate genes/variants are further evaluated by the NHS Genomic Medicine Centre laboratories and these labs will determine which of those candidates are reported back to the patients.

GeL workflow for receiving interpretation requests, processing samples and returning clinical reports to Genomic Medicine Centres

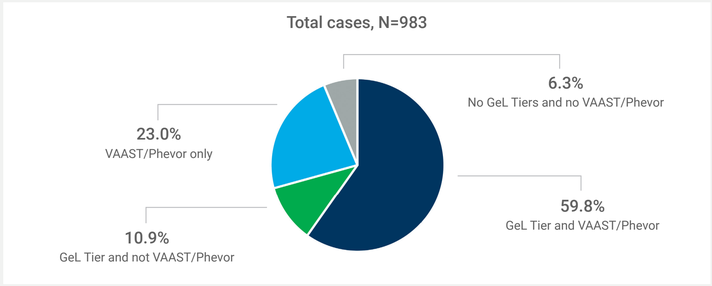
Fabric Genomics' Gene Ranking Algorithms: VAAST[1] and Phevor[2]

VAAST is an algorithm that was developed in collaboration with the University of Utah. Using VAAST for gene prioritization speeds diagnosis and improves diagnostic yield by providing a ranking of genes based on their likelihood to cause disease. Every variant is assessed for comparative functional impact on the protein product, conservation of the position across species, and the allele frequency.

Phevor re-ranks genes that have already been prioritized by VAAST by using the Human Phenotype Ontology (HPO) terms provided for the proband. Phevor starts by mapping phenotype terms to the Human Phenotype Ontology, Gene Ontology and other ontologies, then uses a unique network propagation approach to identify additional gene candidates. This process creates a ranked list of genes ordered by the specific phenotype provided. Phevor then combines this prioritized list of genes with the VAAST analysis to produce a combined ranking of candidate genes based on deleteriousness and the specific phenotype or phenotypes in question. This re-ranking allows rapid discovery of clinically relevant variants in genes related to the proband's phenotype, including genes not directly annotated to that phenotype.

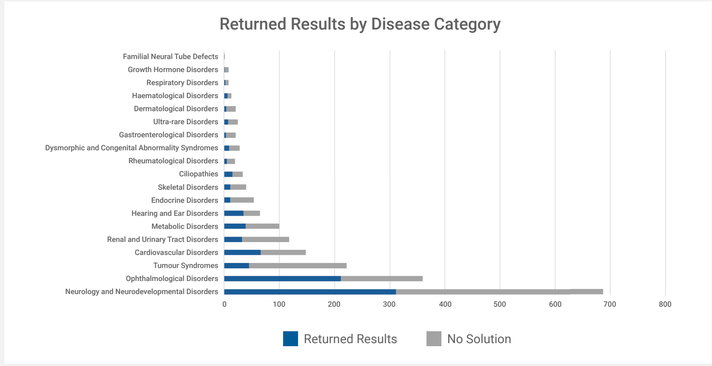
Return of Results in 49.8% of 1973 Cases

Of the 1973 cases, we returned 49.8% with causal candidate genes/variants. Of cases with causal candidates, 59.8% had at least one tiered and one VAAST/Phevor Top 20, and 23.0% were identified exclusively from VAAST/Phevor Top 20 (9.4% improvement).



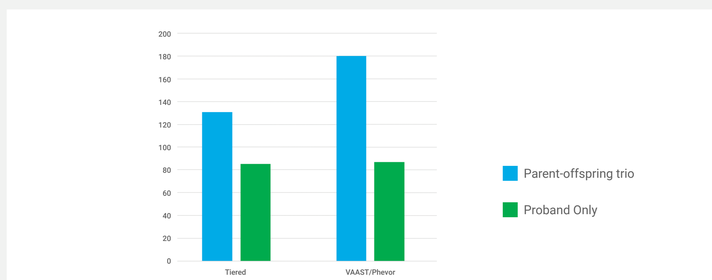
Return of Results by Recruited Disease Category

Recruited Disease Categories are defined by the recruiting Genomic Medicine Centres (GMC). The rate of return of results was highest for Cardiovascular Disorders, Opthalmological Disorders, and Hearing and Ear Disorders.



Return of Results Based on Family Structure

Of a subset of cases, 1191, we returned 38.6% with causal candidate genes/variants in proband only cases and 37.1% in parent-offspring trio cases (proband and 2 unaffected parents). When these results are broken down to the types of candidates, there is an increase of causal candidates that were identified exclusively from VAAST/Phevor Top 20 in true trio cases versus proband only.



Example Cases Where Causal Candidates Were Only Found Using VAAST/Phevor

Main Recruited Disease Category	Condition	HPO/Phenotype	Panel Applied	Gene	Tier Status	VAAST Rank	Phevor Rank	ACMG Class	Family Style
Ophthalmological disorders	Noonan or Leopard	Retinal coloboma, visual impairment, retinal dystrophy, microphthalmia	Ocular coloboma	PTPN11	Not in the panel applied (present in another panel)	4	2	Pathogenic	True trio
Neurology and Neurodevelopmental Disorders	Intellectual developmental disorder with dysmorphic facies and ptosis	Global Developmental Delay, obesity, unilateral ptosis, short palpebral fissure, polyhydramnios	Intellectual Disability; RASopathies; Significant early-onset obesity +/- other endocrine features and short stature	BRPF1	Not in the panel applied (present in another panel)	2	3	Pathogenic	True trio
Ultra-rare disorders	Acampomelic campomelic dysplasia	Cleft palate, micrognathia, abnormality of the nervous system, small for gestational age, polyhydramnios, laryngomalacia, bilateral talipes equinovarus, gastroesophageal reflux, scoliosis, tracheomalacia, bronchomalacia, kyphosis, abnormality of the musculature, congenital septal defect, fixed elbow flexion, primum atrial septal defect, moderate intrauterine growth retardation, abnormality of limb bone	Intellectual Disability; Familial non syndromic congenital heart disease	SOX9	Not in the panel applied (present in another panel)	3	1	Pathogenic	True trio
Neurology and Neurodevelopmental Disorders	Mental retardation and microcephaly with pontine and cerebellar hypoplasia	Microcephaly, abnormality of the eye, autistic behavior, delayed speech and language development, intellectual disability, generalized hypotonia, small for gestational age, delayed gross motor development, proportionate short stature, unilateral strabismus	Intellectual Disability, Mitochondrial disorders, Undiagnosed metabolic disorders	CASK	No tier status	1	1	Pathogenic	True trio

CONCLUSIONS

Fabric Genomics' platform has provided GeL with potential causative candidates in 49.8% of cases. In the 1973 cases we present here, GeL's tiering system achieves a results return rate of 28.8%. By using our VAAST / Phevor algorithms we were able to increase the yield of candidate genes/variants by 23.0%, thus highlighting the complementary utility of the VAAST and Phevor algorithms and GeL's tier filtering methodology. There was a noticeable increased yield of causal candidates in true trios compared to solos that were identified only by VAAST/Phevor top 20. This further supports using our algorithms to help identify clinically relevant candidates for whole genome/exome analysis.

Fabric Genomics™ supports labs to maximize their diagnostic yield. The VAAST and Phevor ranking algorithms accelerate identification of disease-causing candidates. Fabric Genomics' clinical solutions are optimized to provide efficiency for variant scientist's time: the cases reported here each took less than three hours; however there are cases that took significantly less time. This time included both identification of candidate variants, and scoring using the ACMG 2015 Variant Interpretation Guidelines.

This makes Fabric Enterprise ideal for hard to solve rare genetic disease cases, and for large scale country projects.

1. Using VAAST to identify an X-linked disorder resulting in lethality in male infants due to N-terminal acetyltransferase deficiency. Rope et al. Am J Hum Genet. 2011 Jul 15;89(1):28-43.

2. Phevor combines multiple biomedical ontologies for accurate identification of disease-causing alleles in single individuals and small nuclear families. Singleton et al. Am J Hum Genet. 2014 Apr 3;94(4):599-610

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Integration of PHEVOR algorithm module to aid candidate gene identification in a clinical WES analysis pipeline: Prospective review across 74 consecutive cases

Wenjie Chen, PhD; Merry Ferre, MS, CGC; Merica Gellerman, MS, CGC; Ekaterina Bogdanova, PhD; Sabrina A. Gardner, PhD; Wei S. Kelly, PhD; Ildiko Thibodeau, PhD; Katelyn S. Weymouth, PhD; Ian King, PhD; Alecia Willis, PhD, FACMG; Narasimhan Nagan, PhD, FACMG
Integrated Genetics, Laboratory Corporation of America® Holdings, Westborough, MA and Raleigh, NC

Integrated GENETICS
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I. Abstract

Whole exome sequencing (WES) has been increasingly used to identify disease-causing alleles in novel and well-established genes to facilitate the clinical diagnosis of rare diseases. Tens of thousands of variants can be identified in any patient sample using WES; only one or a few of which are expected to be causative. A candidate gene prioritization strategy that ranks the underlying variants and the genes they affect, with those most likely related to the patient phenotype remains the corner stone for an unequivocal WES diagnosis. Omicia Opal Clinical™, a recently developed application, uses VAAST and PHEVOR algorithms to analyze WES data and prioritize variants and genes involved in disease. In this study, we report our experience with Omicia Opal Clinical™ to identify disease-causing variants across a set of 74 consecutive clinical WES cases. We identified at least one variant in an associated/novel possibly associated gene or a candidate gene in 43 out of the first 74 (58%) clinical WES cases analyzed. An additional set of 10 positive cases were obtained from our validation cohort for a total of 53 cases to assess the performance of the VAAST and PHEVOR algorithms. These 53 cases comprised a total of sixty-four identified genes spanning *de novo* (n=12), autosomal recessive (n=24), autosomal dominant (n=20), autosomal unknown (n=3), and X-linked (n=5) modes of inheritance. Ninety-eight percent (63/64) of the reported genes harboring the disease-associated variants were ranked by the VAAST-PHEVOR analysis. In the absence of HPO terms corresponding to the patient's phenotype, the percentage of genes that ranked within the top 20 was 66%. After incorporating HPO terms corresponding to the patient's phenotype in the VAAST-PHEVOR analysis, this figure increased to 83%. Furthermore, restricting the review to the Phenotype/Gene Association score in PHEVOR analysis improved the percentage of genes ranking within the top 20 to 88%, which is comparable to the ranking result obtained by the VarElect (free trial version, LifeMap Sciences) (89%) across the set of 53 identical cases. The impact on candidate gene ranking by PHEVOR analysis was more significant for singleton/duo cases than in trio cases. As a limitation, the VAAST-PHEVOR analysis does not rank genes with a missing 2nd variant in cases with possible recessive inheritance, requiring alternative approaches to rank these genes. In conclusion, the VAAST-PHEVOR analysis is an efficient adjunct for identification of disease-causing genes and variants in clinical whole exome sequencing analyses

II. Methods

Clinical Samples

Seventy-four clinical cases and ten validation cases were included in this study.

Whole Exome Sequencing and Sanger Confirmation

Genomic DNA is isolated from the provided specimens. The DNA samples are fragmented through sonication and exonic regions are enriched using Agilent SureSelect XT with custom content.
The enriched targeted DNA is sequenced on an Illumina HiSeq 2500 system. Sequence data is mapped and aligned to Human Genome Build GRCh37/hg19 using CLC Bio software. Variants included in the report are confirmed through targeted Sanger sequence analysis. This test was developed and its performance characteristics determined by LabCorp.

Data Interpretation and Reporting

Candidate genes/variants were selected based on variant type, inheritance modeling, presence in the literature, frequency in exome aggregation consortium (ExAC) population database, and phenotype correlation. Reported candidate genes were categorized as: disease genes related to phenotype (category 1), disease genes possibly related to phenotype (category 2), findings in strong candidate genes (category 3). VAAST and Phevor analysis were performed using Omicia Opal Clinical™. VarElect analysis was performed using the free trial version (http://varelect.genecards.org/). To compare the candidate gene ranking either PHEVOR or VarElect were applied to the output of VAAST analysis derived from Omicia Opal Clinical™ using identical HPO terms.

III. Results

Figure 1: A. The set of 74 consecutive clinical WES cases includes 55 trios (74%), 8 duos (11%), 11 singletons (15%). **B.** At least one pathogenic/likely pathogenic variant was identified in 15 cases (20%). At least one VUS variant was identified in 28 cases (38%)

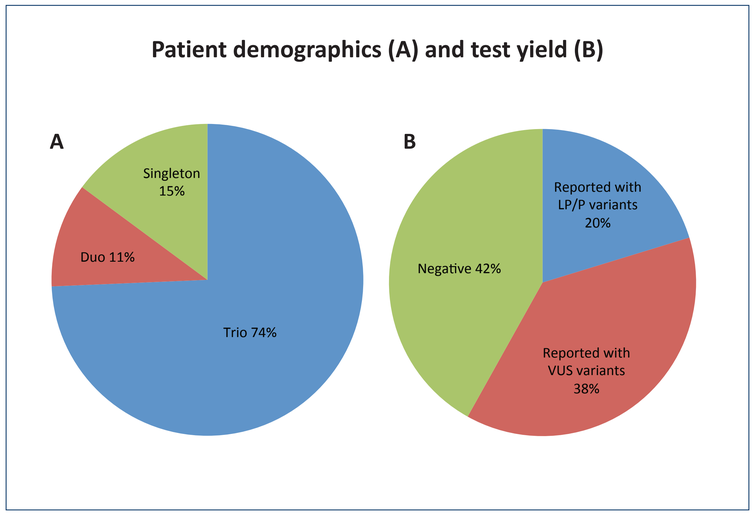

Table 1: Breakdown of test yield by number of reported genes per case

Total positive cases	43
Total reported genes across all positive cases	54
Cases with 1 reported gene	33
Cases with 2 reported genes	9
Cases with 3 reported genes	1

Figure 2: Ranking of 64 reported genes (43 clinical cases and 10 validation cases) by Opal VAAST, VAAST-Phevor, Phevor Phenotype/Gene Association (P/G), and VarElect.

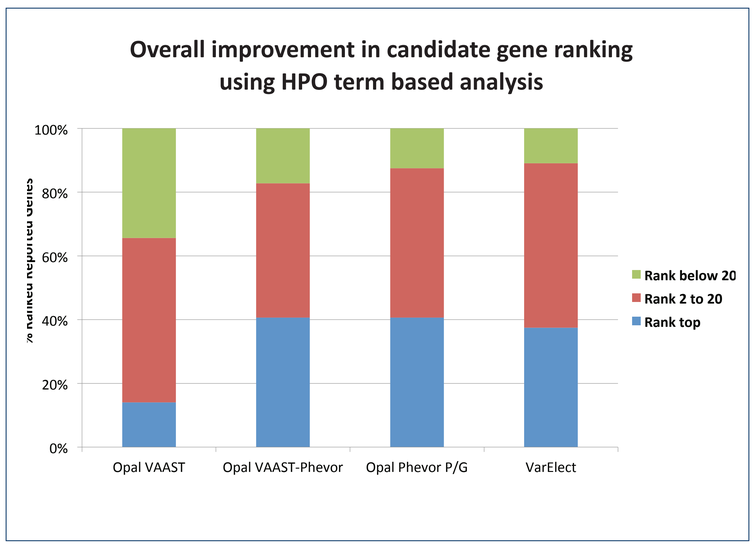

Figure 3: Ranking of 29 reported genes in trio cases and 35 reported genes in singleton/duo cases by Opal VAAST, VAAST-Phevor, Phevor Phenotype/Gene Association (P/G), and VarElect.
* p<0.05 when compared to candidate gene ranking using Opal VAAST.

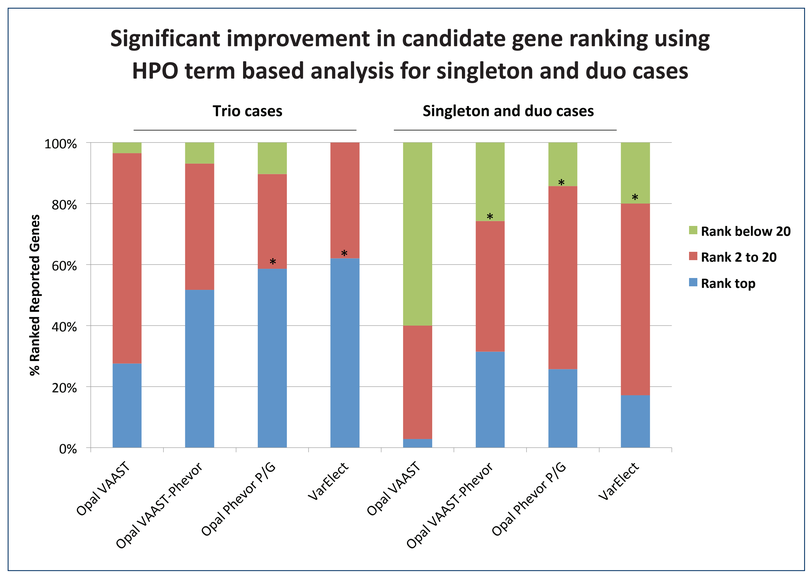

Figure 4: A. Category 1: disease genes related to phenotype; Category 2: disease genes possibly related to phenotype; Category 3: findings in strong candidate genes. **B.** P: pathogenic, LP: likely pathogenic, VUS: variant of unknown significance

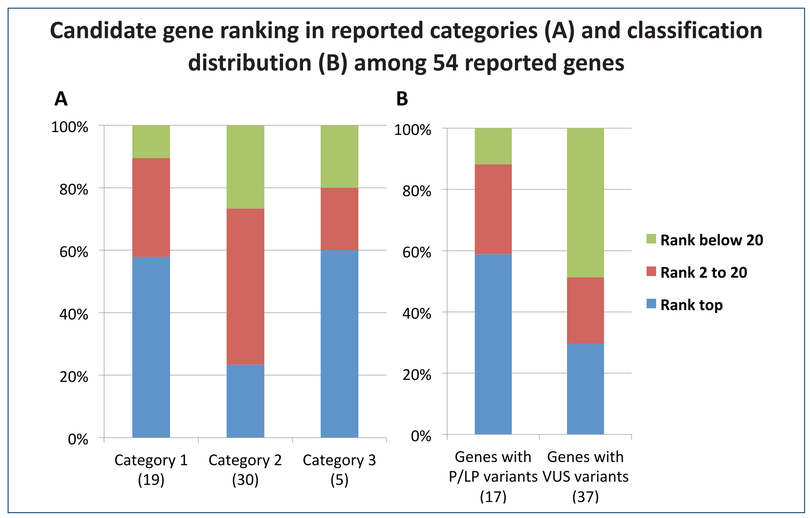

Table 2. Representative candidate genes

Gene	Total Genes in VAAST	Opal VAAST	Opal VAAST-Phevor	Opal P/G	VarElect
DYNC1H1	257	221	72	4	5
COL6A1	511	428	59	1	2
*DHCR7**	351	208	31	2	5
TTN	430	405	134	3	1
PTCH2	302	2	1	1	83
C5ORF42	33	5	31	31	1
C10ORF2	408	149	392	383	1
ADCY5	Not included in VAAST-Phevor due to low allele fraction (mosaicism)				

*The second allele is missing in DHCR7 gene. This gene was ranked in autosomal dominant mode in Opal VAAST and VAAST-Phevor.

IV. Conclusion

- HPO term based analysis significantly increases the percentage of top ranked candidate genes in all cases and the percentage of candidate genes ranked within the top 20 in singleton and duo cases.
- The VAAST-PHEVOR analysis does not rank candidate genes with a missing 2nd variant in cases with possible recessive inheritance. The alternative is to rank these genes in autosomal dominant mode.
- Overall, the VAAST-PHEVOR analysis is an efficient adjunct for identification of disease-causing genes and variants in clinical whole exome sequencing analyses.

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