



## Short Communication

# A performance evaluation study: Variant annotation tools - the enigma of clinical next generation sequencing (NGS) based genetic testing

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## ABSTRACT

Dramatically expanding our ability for clinical genetic testing for inherited conditions and complex diseases such as cancer, next generation sequencing (NGS) technologies are allowing for rapid interrogation of thousands of genes and identification of millions of variants. Variant annotation, the process of assigning functional information to DNA variants based on the standardized Human Genome Variation Society (HGVS) nomenclature, is a fundamental challenge in the analysis of NGS data that has led to the development of many bioinformatic algorithms. In this study, we evaluated the performance of 3 variant annotation tools: Alamut® Batch, Ensembl Variant Effect Predictor (VEP), and ANNOVAR, benchmarked by a manually curated ground-truth set of 298 variants from the medical exome database at the Molecular Diagnostics Laboratory at Lurie Children's Hospital. Of the 3 tools, VEP produces the most accurate variant annotations (HGVS nomenclature for 297 of the 298 variants) due to usage of updated gene transcript versions within the algorithm. Alamut® Batch called 296 of the 298 variants correctly; strikingly, ANNOVAR exhibited the greatest number of discrepancies (20 of the 298 variants, 93.3% concordance with ground-truth set). Adoption of validated methods of variant annotation is critical in post-analytical phases of clinical testing.

## Introduction

Genetic testing has witnessed a transformative revolution in the last decade with the introduction of next generation sequencing (NGS) technologies.<sup>1–3</sup> With its unprecedented cost-effective scalability, continuously improving efficiencies, and diagnostic yield, NGS has not only allowed for an exponential increase in the elucidation of genetic causes for both rare Mendelian and complex heterogenous disorders, but it is also proving to be an essential tool for identifying therapeutic targets in neoplasms, and screening for prenatal aneuploidy and pediatric onset disorders.<sup>4–7</sup> The rapid proliferation of diagnostic tests, from small hotspot mutation and large panels to whole exome and whole genome platforms, has allowed for rapid interrogation of tens of thousands of genes and identification of millions of variants, many of which we have never seen before.<sup>8–10</sup>

While whole genome sequencing results in the detection of about 4 million variants per individual, exome sequencing that covers only the 1% protein-coding portion of the genome, detects about 20 000 variants.<sup>11,12</sup> Though most of these variants likely only contribute to human population diversities since a single exome harbor only about 100–200 potential disease-causing changes, identification of the 1 or 2 disease-causing variants among these many alterations is a classic needle in the haystack

problem that ends being a meticulous and arduous process as part of the NGS bioinformatics pipeline.<sup>13</sup> Thus, translating sequencing information into clinical practice in this era of genomic testing is limited by the accurate annotation and interpretation of the variants rather than detection of variants alone. This discovery of novel genetic variants at this extraordinary pace has also reiterated the need of a standardized approach for describing, documenting, and communicating genetic variants, first recognized by the scientific community in the pre-NGS era,<sup>14,15</sup> and has resulted in the widespread adoption of the Human Genome Variation Society (HGVS) nomenclature system by research and clinical genetic testing laboratories.<sup>16–19</sup>

Variant annotation is the process of describing the nature and the effect of the genomic aberrations produced by a variant that involves adding auxiliary metadata to quality filtered raw putative variant calls.<sup>20</sup> The most basic annotations will classify variants based on their relationship to genes, their transcripts, and other key features such as exons, introns, and splice sites, in addition to frequently providing information regarding known allele frequencies, predicted deleterious effects, and involvement in known human diseases and phenotypes.<sup>21</sup>

Variant annotation is dependent wholly on the gene models within which they reside, and has seen a prolific growth in both capability and scale in the past decades, evolving into its own research field.<sup>21,22</sup> Conforming variant

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coordinates from the transcript to the genome, and vice versa, is a complicated process that is dependent on the genomic and transcript sequence accessions and versions, and the alignment tools used, consequently resulting in significantly different locations of the variants.<sup>23,24</sup>

Both theoretical and empirical based variant annotation approaches have been used. A series of empirically based tools including the open source SnpEff,<sup>25</sup> Variant Effect Predictor (VEP, Ensembl),<sup>26</sup> VarReporter,<sup>27</sup> ANNOVAR,<sup>28</sup> the commercial Alamut Batch,<sup>29</sup> and tools developed by individual laboratories such as Invitae,<sup>30</sup> for outputting HGVS syntax from next generation sequencing data have gained prominence over the years. A significant proportion of these tools enable variant annotation based on Ensembl transcripts while parallelly leveraging the rich annotations in dbSNP,<sup>31</sup> a database of curated annotations and functional effect predictions for all potential non-synonymous and splice site SNVs in the human reference genome.

Alamut® Batch (Sophia Genetics, formerly Interactive Biosoftware), a licensed gene annotation software, is widely used in clinical laboratories, including Lurie Children's Molecular Diagnostics Lab, for supporting variant annotation and classification using HGVS standards. However, the efficiency of performing variant annotation by Alamut® Batch is limited by its licensing structure, and thus prompted our evaluation of other potential, well supported open-source replacement tools like VEP. In this study, we compare the concordance of variant nomenclature generated by the open source ANNOVAR and Variant Effect Predictor (VEP), and the commercial Alamut® Batch.

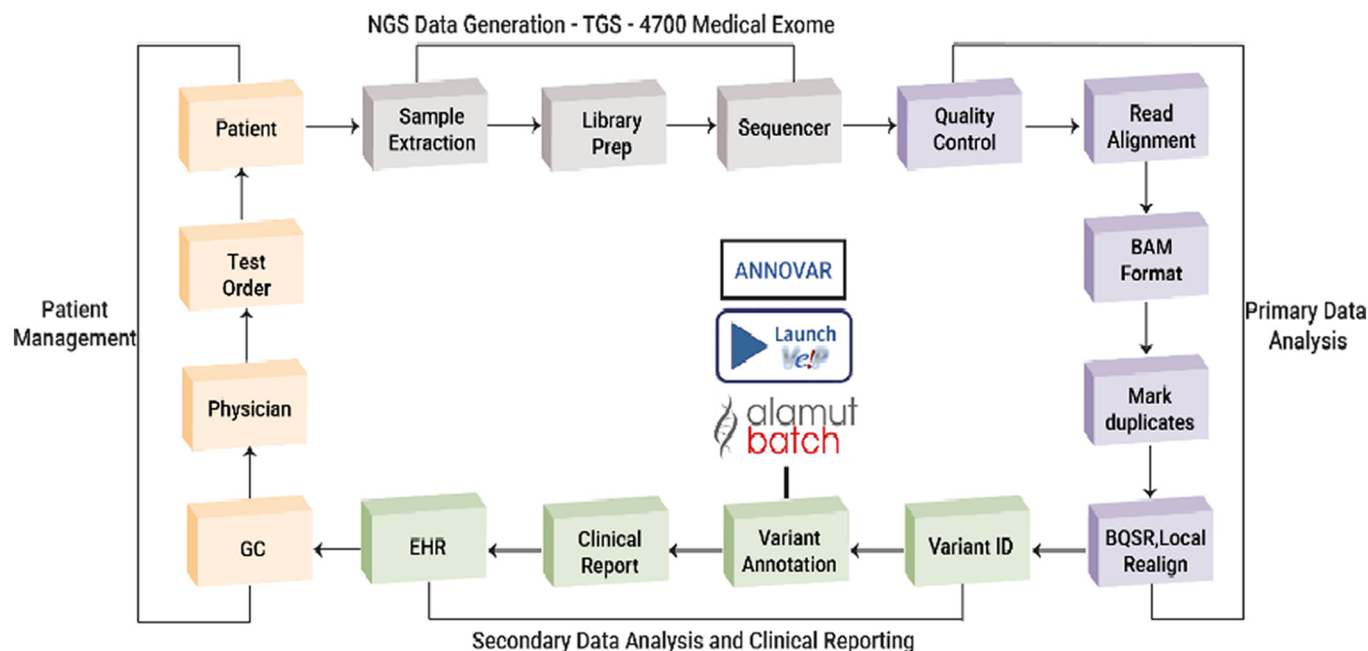
## Materials and methods

A test set of 298 intronic and exonic variants across 191 genes, previously classified and reviewed in clinical reports by the Molecular Diagnostics Laboratory at Lurie Children's Hospital was curated and used for this study. These variants were generated by targeted gene panel sequencing using the ~4700 gene medical exome panel on 105 patients [Fig. 1]. Briefly, 150 bp paired end reads generated using the Illumina NextSeq 550 at an average depth of 250 per sample were mapped to the human

genome GRCh38/hg38 using Burrows Wheeler aligner (BWA) v0.7.12, sequence alignment and map (SAM) files converted to binary alignment and map (BAM) format and sorted using SAMtools v1.9. Local realignment around the known indels was performed by GATK v4.1.4.1 on the sorted BAM files and Picard-tools v2.18.27 was used to remove Polymerase chain Reaction (PCR) duplicates. Finally, base quality score recalibration was performed using GATK again to generate the VCF (Variant Call File), a de facto standard used in reporting genetic variants.

The 298 variants represented the following 191 genes: ACTA2, ADAM17, ADAMTS10, ADAMTS17, ADAMTSL4, ADSL, AICDA, AK2, ALDH5A1, ALDH7A1, ANKRD26, AP3D1, ASNS, ATM, ATRX, BGN, BLOC1S6, BTK, C1QC, C1S, C2, C3, C5, C6, C9, CACNA2D2, CARMIL2, CCDC151, CD247, CD46, CD79A, CDAN1, CFB, CFHR4, CHD7, CIITA, CLN6, CLPB, CNTN2, CNTNAP2, COL12A1, COL1A2, COL3A1, COL5A1, COL5A2, COLEC11, CR2, CSF2RB, CSF3R, CTC1, CTSC, CXCR4, DEPDC5, DGKE, DNAH11, DNAH5, DOCK8, DRC1, DYNC1H1, ELANE, ENG, EPG5, EPHB4, ERCC6L2, FANCA, FANCB, FANCD2, FANCL, FANCL, FASN, FAT4, FBN1, FBN2, FCGR2A, FOXP3, GABBR2, GABRD, GAMT, HRAS, HYOU1, ICAM1, IFIH1, IFNGR2, IKBKG, IL10RA, IL12RB1, IL17RA, IL17RC, IL7R, JAK3, JMJD1C, KCND2, KCNT1, KMT2D, KRIT1, LCK, LIG1, LPIN2, LRBA, LTBP2, LYST, MASP1, MASP2, MECP2, MEFV, MICU1, MKL1, MLH1, MSH2, MSH6, MTHFD1, MVK, MYH11, MYLK, NALCN, NF1, NFKBIA, NLR4, NLRP1, NOD2, NOTCH1, NRXN1, OFD1, PARN, PIGQ, PLCB1, PLCG2, PLOD1, PNKD, POLE2, POLG, PRICKLE1, PSTPIP1, PTPN11, PTPRC, RAF1, RBCK1, RECQL4, RFXANK, RFXAP, RMRP, RNASEH2B, RNF213, RNF31, RTEL1, RUNX1, RYR3, SBDS, SCN3A, SCN8A, SERPINA1, SERPING1, SETD2, SLC13A5, SLC25A22, SLC29A3, SLC2A10, SMARCAL1, SOS1, SOS2, SPINK5, ST3GAL3, STAT3, STAT5B, STRADA, SZT2, TBK1, TGFBR1, TICA1, TINF2, TIRAP, TMC6, TMC8, TNFRSF13B, TNFRSF1A, TNXB, TPP1, TPP2, TRAF3IP2, TSC1, TSC2, TTC37, TTC7A, TYK2, UNC13D, VPS13B, VPS45, WRAP53, ZBTB24, ZCCHC8, and ZNF341.

The 298 variants were manually reviewed using Alamut® Visual (v2.15, Sophia Genetics, formerly Interactive Biosoftware) and Integrative



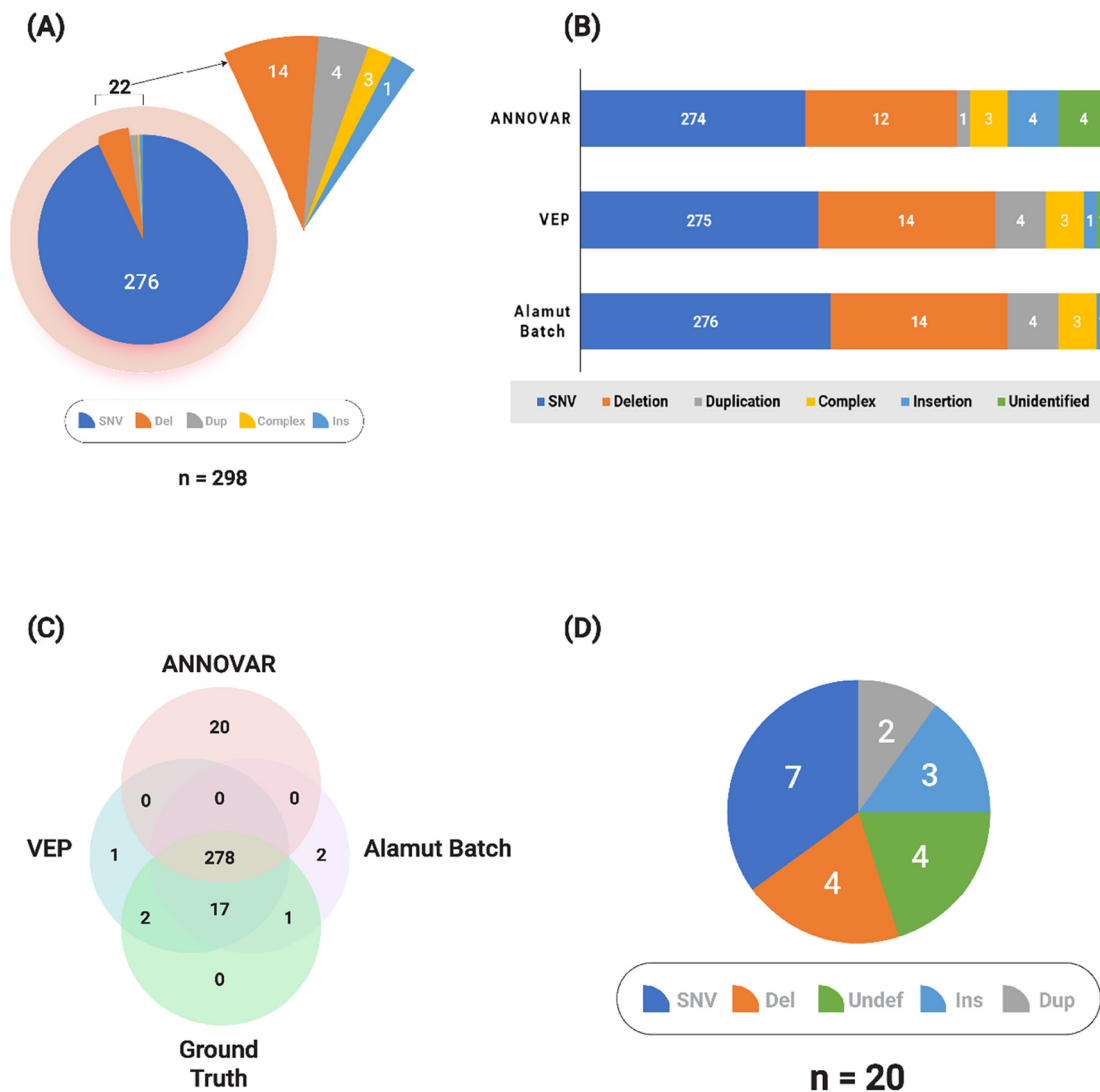
**Fig. 1.** Overview of the targeted gene panel sequencing pipeline using the ~4700 gene medical exome panel on 105 patients used to generate the test set of 298 intronic and exonic variants across 191 genes evaluated in this study. Briefly, 150 bp paired end reads generated using the Illumina NextSeq 550 at an average depth of 250 per sample were mapped to the human genome GRCh38/hg38 using BWA v0.7.12 and GATK v4.1.4.1 used to generate the variant call file (VCF). The 298 variants were manually reviewed using Alamut® Visual (v2.15, Sophia Genetics) and Integrative Genomics Viewer (IGV v2.10.2),<sup>[10]</sup> and orthogonally confirmed with Sanger sequencing when necessary. After the validation process, the variants were distributed with respect to their classification (Single nucleotide variants (SNVs), Deletions, Duplications, Insertions, and Complex variants), thereby constituting the ground-truth set, and subsequently independently annotated using Alamut® Batch (v1.4.2, July 2015, Sophia Genetics), Ensembl Variant Effect Predictor (VEP v105.0, 2017) and ANNOVAR (v. October 24, 2019). Custom functions were written in python to compare the results of the 3 software packages.

Genomics Viewer,<sup>32</sup> (IGV v2.10.2), and orthogonally confirmed with Sanger sequencing when necessary. As a HGVS nomenclature tool, Mutalyzer,<sup>33</sup> (v2.0.35), was used to verify the outputted data with respect to the standard HGVS nomenclature guidelines. After this validation process, the variants were distributed with respect to their classification (Single nucleotide variants (SNVs), Deletions, Duplications, Insertions, and Complex variants), thereby constituting the ground-truth set.

Subsequently, these 298 variants were independently annotated using Alamut® Batch (v1.4.2, July 2015, Sophia Genetics, formerly Interactive Biosoftware), Ensembl Variant Effect Predictor (VEP v105.0, 2017) and ANNOVAR (v. October 24, 2019). For single nucleotide variations (SNVs), the substitutions were further divided into missense variants (219 variants), which causes a substitution in the amino acid residues, and is a non-synonymous change; nonsense variants (19 variants), which prematurely end the protein with a stop codon, also a non-synonymous change; and silent variants (19 variants), which had no effect on the

amino acid, and is a synonymous change. The effect of 39 variants is not predicted to have an effect on the amino acid since those variants were in the intronic region. Genomic variants were recognized as duplications if the alternate nucleotides were a repeat of the sequence prior to the genomic location of the variant. Complex variants were identified when multiple base pairs were involved and can be described as having deleted nucleotide(s) as well as inserted nucleotide (s). Custom functions were written in Python to compare the results from the 3 software packages (<https://github.com/sachT19/Variant-Nomenclature-Comparator>).

Previous manual evaluation for each of the software was completed by Lurie's Bioinformatics team. Alamut Batch annotations were part of clinical testing at Lurie Children's, therefore a standardized operating procedure was followed by different bioinformaticians. VEP annotations were run by a bioinformatician and ANNOVAR was added as an additional tool subsequently and the annotation process performed by ST.



**Fig. 2.** Relative concordance of variant nomenclature generated by ANNOVAR, Variant Effect Predictor (VEP), and Alamut® Batch. A. Distribution of variant types in the ground-truth set. Variant types evaluated were: single nucleotide variants (SNVs), deletions (del), duplications (dup), insertions (Ins), and complex (complex) variants. The majority of the variants were classified as SNVs, followed by deletions, duplications, complex, and insertions. B. Variant distribution profile of the 3 annotation packages - Alamut® Batch, Variant Effect Predictor (VEP), and ANNOVAR. The relative annotation profiles are very similar between the 3 software, although VEP was unable to identify one of the variants, and ANNOVAR was unable to annotate 4 changes. C. Exact concordance of HGVS syntax at the coding level between the ground truth and the 3 variant annotation tools - Alamut® Batch, Variant Effect Predictor (VEP), and ANNOVAR. A total 278 of the 298 variants were found to be concordant between the 3 software packages. VEP and Alamut® Batch were 99% concordant, however, ANNOVAR produced 20 discrepancies. D. Distribution profile of the 20 discrepant variants called by ANNOVAR. Variant types evaluated were: single nucleotide variants (SNVs), deletions (del), duplications (dup), insertions (Ins), and undefined (UnDef). The majority of the discrepancies were classified as SNVs and ANNOVAR was unable to annotate four variants.

**Table 1**  
Detailed characterization of the functional annotation of the discrepancies between the 3 variant annotation packages - Alamut® Batch, Variant Effect Predictor (VEP), and ANNOVAR. A majority of the discrepancies were due to the various transcripts used by the 3 different software.

Chrom	Gene	Alamut Batch cNomen	Alamut Batch pNomen	VEP cNomen	VEP pNomen	ANNOVAR cNomen	ANNOVAR pNomen	Ground Truth cNomen	Ground Truth pNomen	Ground Truth Location	Pathogenicity
<i>Discordant variants between alamut batch and ground truth</i>											
1	CSF3R	NM_000760.3:c.2092C > T	p.Arg698Cys	NM_000760.4: c.2041-30C > T	.	NM_000760:exon17:c.2041-30C > T	.	NM_00760.4: c.2041-30C > T	p.?	Intronic	VUS
9	ERCC6L2	NM_020207.4:c.1408G > T	p.Val470Phe	NM_020207.7:c.1375G > T	NP_064592.3:p.Val459Phe	NM_020207:exon8:c.1375G > T	p.V459F	NM_020207.7:c.1375G > T	p.Val459Phe	Exonic	VUS
<i>Discordant variants between VEP and ground truth</i>											
X	BTK	NM_000061.2:c.-4456C > T	p.?	.	.	NM_000061.2: c.-4456C > T	.	NM_000061.2:c.-4456C > T	p.?	5'UTR	VUS
<i>Discordant variants between ANNOVAR and ground truth</i>											
2	FANCL	NM_018062.3:c.1096_1099dup	p.Thr367 Asnfs*13	NM_018062.4: c.1096_1099dup	NP_060532.2:p.Thr367 AsnfsTer13	NM_018062:exon14: c.1099_1100insATTA	p.T367fs	NM_018062.3: c.1096_1099dup	p.Thr367 Asnfs*13	exonic	Pathogenic
3	IL17RC	NM_153461.3:c.1674_1676del	p.Leu559del	NM_153461.4: c.1674_1676del	NP_703191.2:p.Leu559del	NM_153461:exon17: c.1672_1674del	p.558_558del	NM_153461.3: c.1674_1676del	p.Leu559del	exonic	VUS
7	COL1A2	NM_000089.3:c.432 + 2 T > A	p.?	NM_000089.4:c.432 + 2 T > A	.	UNKNOWN	.	NM_000089.3:c.432 + 2 T > A	p.?	intronic	VUS
7	DNAH11	NM_001277115.1:c.10691 + 2 T > C	p.?	NM_001277115.2: c.10691 + 2 T > C	.	UNKNOWN	.	NM_001277115.1:c.10691 + 2 T > C	p.?	intronic	VUS
7	DNAH11	NM_001277115.1: c.13523_13543dup	p.Ala4508_Leu4514 dup	NM_001277115.2: c.13523_13543dup	NP_001264044.1: p.Ala4508_Leu4514dup	NM_001277115:exon82: c.13521_13522 insGCTGGAGTGGCTCTGCTCTTA	p.L4507delins LAGVALLL	NM_001277115.1:c.13523_13543dup	p.Ala4508_Leu4514dup	exonic	VUS
8	VPS13B	NM_017890.4:c.5513_5527del	p.Asp1838_Thr1842del	NM_017890.5: c.5513_5527del	NP_060360.3:p.Asp1838_Thr1842del	NM_017890:exon34: c.5511_5525del	p.1837_1842del	NM_017890.4: c.5513_5527del	p.Asp1838_Thr1842del	exonic	VUS
9	COL5A1	NM_000093.4:c.2153del	p.Gly718 Alafs*86	NM_000093.5:c.2153del	NP_000084.3:p. Gly718AlafsTer86	NM_000093:exon23:c.2152delG	p.G718fs	NM_000093.4:c.2153del	p.Gly718 Alafs*86	exonic	VUS
10	MICU1	NM_006077.3:c.1A > G	p.?	NM_006077.4: c.1A > G	NP_006068.2:p.Met1?	NM_006077:exon11:c.1187-62G > G	.	NM_006077.3:c.1A > G	p.?	exonic	VUS
11	WRAP53	NM_018081.2:c.1566_1567del	p.Pro523 Argfs*6	NM_018081.2: c.1566_1567del	NP_060551.2:p. Pro523ArgfsTer6	NM_018081:exon10: c.1564_1565del	p.A522fs	NM_018081.2: c.1566_1567del	p.Pro523 Argfs*6	exonic	VUS
13	RFXAP	NM_000538.3:c.524_527del	p.Lys175 Argfs*8	NM_000538.4: c.524_527del	NP_000529.1:p. Lys175ArgfsTer8	NM_000538:exon1:c.523_526del	p.K175fs	NM_000538.3: c.524_527del	p.Lys175 Argfs*8	exonic	VUS
16	TSC2	NM_000548.4:c.599 + 5_599 + 7del	p.?	NM_000548.5:c.599 + 5_599 + 7del	.	NM_000548:exon6:r.spl	.	NM_000548.4:c.599 + 5_599 + 7del	p.?	intronic	VUS
17	WRAP53	NM_018081.2:c.1564dup	p.Ala522 Glyfs*8	NM_018081.2:c.1564dup	NP_060551.2:p. Ala522GlyfsTer8	NM_018081:exon10:c.1558dupG	p.C519fs	NM_018081.2:c.1564dup	p.Ala522 Glyfs*8	exonic	VUS
17	FASN	NM_004104.4:c.5113C > T	p.Arg1705Trp	NM_004104.5:c.5113C > T	NP_004095.4:p. Arg1705Trp	NM_004104:exon29:c.5098 + 98C > T	.	NM_004104.4:c.5113C > T	p.Arg1705 Trp	exonic	VUS
17	NF1	NM_000267.3:c.6834del	p.Lys2279 Asnfs*19	NM_000267.3:c.6834del	NP_000258.1:p.Lys2279 AsnfsTer19	NM_000267:exon45:c.6833delC	p.T2278fs	NM_000267.3:c.6834del	p.Lys2279 Asnfs*19	exonic	VUS
17	TNFRSF13B	NM_012452.2:c.204dup	p.Leu69 Thrfs*12	NM_012452.3:c.204dup	NP_036584.1:p. Leu69ThrfsTer12	NM_012452:exon3:c.204dupA	p.L69fs	NM_012452.2:c.204dup	p.Leu69 Thrfs*12	exonic	Pathogenic
19	ICAM1	NM_000201.2:c.1546C > T	p.Gln516*	NM_000201.3:c.1546C > T	NP_000192.2:p.Gln516Ter	NM_000201:exon6:c.1426 + 92C > T	.	NM_000201.2:c.1546C > T	p.Gln516*	exonic	VUS
19	JAK3	NM_000215.3:c.566 + 6_566 + 41del	p.?	NM_000215.4:c.566 + 6_566 + 41del	.	NM_000215:exon5:r.spl	.	NM_000215.3:c.566 + 6_566 + 41del	p.?	intronic	Likely benign
20	KMT2D	NM_003482.3:c.2992C > A	p.Pro998Thr	NM_003482.4:c.2992C > A	NP_003473.3:p.Pro998Thr	NM_003482:exon11:c.2481A > T	p.Q827H	NM_003482.3:c.2992C > A	p.Pro998Thr	exonic	Likely benign
X	DKC1	NM_001363.4:c.1512_1514dup	p.Lys505dup	NM_001363.5: c.1512_1514dup	NP_001354.1:p.Lys505dup	NM_001363:exon15: c.1491_1492insAAG	p.T497delinsTK	NM_001363.4: c.1512_1514dup	p.Lys505dup	exonic	Likely benign
X	MECP2	NM_004992.3:c.806del	p.Gly269 Alafs*20	NM_004992.4:c.806del	NP_004983.1:p. Gly269AlafsTer20	NM_004992:exon4:c.806delG	p.G269fs	NM_004992.3:c.806del	p.Gly269 Alafs*20	exonic	Pathogenic



## Results

In this study, we evaluated the performance of 3 variant annotation software packages: the commercial Alamut® Batch, and the open source Ensembl Variant Effect Predictor (VEP), and ANNOVAR, against a manually curated ground-truth set of 298 variants from the medical exome database at the Molecular Diagnostics Laboratory at Lurie Children's Hospital. All 3 platforms generate transcript and protein-based variant nomenclature from genomic coordinates according to the guidelines by the HGVS. Our analysis of the 298 variants revealed that a vast majority of them were single nucleotide variants (SNVs, 92.6%) in the ground-truth set; the rest comprise a smaller number of deletions, duplications, complex, and insertions [Fig. 2a]. SNVs are the most prevalent form of genetic variants and have been shown to play a potential role in the predisposition of disease.<sup>34</sup>

Although the input transcript alignments for VEP, ANNOVAR, and Alamut® Batch were identical, the tools produced a different number of transcripts and annotations. As shown in Fig. 2b, individually, all 3 tools - Alamut® Batch, VEP, and ANNOVAR - exhibited similar annotation profiles with regards to distribution of variant types, although VEP was unable to identify one of the variants, and ANNOVAR was unable to annotate 4 changes.

To investigate the degree of concordance between the 3 software packages, we compared the variant annotation calls with reference to the ground-truth set. When the annotations from all 3 software tools are exactly equivalent at the coding level, it is referred to as 100% concordance. A total 278 of the 298 variants were found to be concordant between the 3 software packages (Fig. 2c). While Alamut® Batch and VEP exhibited comparable accuracy and precision with the ground truth with >99% concordance, ANNOVAR exhibited the greatest number of discrepancies (20 of the 298 variants, 93.3% concordance), a majority of which were non-SNVs (13/20) [Fig. 2c and d].

The functional annotation of the discrepancies between the 3 software packages are further characterized in Table 1. As observed for the 2 discrepancies in the CSF3R and the ERCC6L2 gene, the NM transcript versions between Alamut® Batch and VEP are different, resulting in varying genomic coordinates, and thus, differing nomenclature based on the locations in the gene transcript versions. In the case of the CSF3R gene, while Alamut® Batch called the variant as exonic, VEP was in agreement with the ground truth in calling the alteration as intronic. In some cases, the implications of annotating on a different region of the gene can be severe and result in an incorrect diagnosis of the patient's condition.<sup>35</sup> For the third discrepancy between Alamut® Batch and VEP, present on the BTK gene, the former correctly identified it as a 5' UTR variant, while the latter was unable to annotate it.

Of the 20 discrepancies between ANNOVAR and the ground truth, 16 were exonic, while 4 were intronic. The transcript version for ANNOVAR was not provided by the software, so the cause for these discrepancies is unknown. For the 4 variants that it was unable to call, 2 were splicing variants and 2 were intronic variants. These 20 discrepancies additionally exhibit a spectrum of pathogenicity, ranging from likely benign to pathogenic. An overwhelming majority of the variants (14/20) were classified as Variants of Unknown Significance (VUS), so their phenotypic effect has not been discovered yet. Despite substantial progress in variant detection genome-wide, a significant majority of annotated genes have yet to be assigned function in the context of human disease traits.<sup>36</sup>

## Discussion

Based on this data, it is evident that with respect to the HGVS nomenclature standards and clinical integrity, VEP has produced most accurate variant annotations. The inconsistencies in the data were observed in SNVs, and with the exception of the upstream gene variant, VEP was able to correctly identify and produce the HGVS nomenclature for 297 out of 298 variants. On the other hand, Alamut® Batch called 296 out of the 298 variants correctly. Performing significantly worse than the former 2 softwares, ANNOVAR was only able to correctly annotate 278 out of the 298 variants.

Similar observations have been reported by other studies evaluating the performance of other variant annotation tools. McCarthy et al. (2014) recorded 551 983 concordant variants out of the 637 841 (86.5%) between ANNOVAR and VEP; the discrepancies arising due to a difference in transcript versions.<sup>24</sup> A 92.6% concordance (100/108 variants) between SnpEff and VEP was reported by Yen et al. (2017).<sup>25</sup>

To our knowledge, our study is the first relative performance evaluation of all 3 tools - ANNOVAR, Alamut® Batch, and VEP. Due to the advantage of the VEP algorithm to default the usage of the latest transcript version and unlimited licensing requirements, the Lurie Molecular Diagnostics Laboratory has decided to incorporate VEP as the variant annotator replacing Alamut® Batch.

While the chemistries of NGS library generation have matured and costs of sequencing have greatly declined over the last decade, the primary challenges facing a clinical genetic testing laboratory are in the expeditious analysis of large amounts of genetic data and interpretation of their clinical significance, as the scope of testing has approached exome and genome scales. While extensive cataloging of the human genetic variation is happening at a rapid pace, accurate detection and annotation of genetic variants are crucial to ensuring pediatric patients are receiving an accurate molecular diagnosis for their genetic conditions. Accurate identification and annotation of genetic variants will enable the establishment of substantial literature and information on the genetics of specific disease areas. Without accurate genetic variant identification and annotation to form the basis of a strong scientific literature, clinical interpretation of Variants of Unknown Significance (VUS) will continue to be a challenge for clinical diagnostics. Therefore, adoption of appropriate and validated methods of variant annotations is critical in the post analytical phases of clinical testing.

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## Conflict of interest

None.

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