High-throughput curation and interpretation of inherited disease variants in a CLIA laboratory

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Abstract

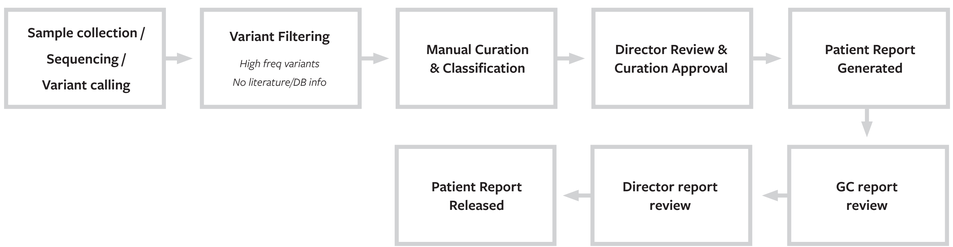
Counsyl is a technology-driven clinical laboratory that offers an expanded carrier screen (aka *Family Prep Screen*: FPS) that includes >175 of the most relevant autosomal/X-linked recessive diseases as well as a test for inherited cancer syndromes (ICS). These tests use next-generation sequencing to analyze all exons for the included genes, excluding those in homologous regions that require custom strategies. Variants discovered by this analysis are sent through a curation workflow to interpret their clinical impact. Real-time interpretation of variants is key to delivering accurate test results to patients and providers, and improves clinical detection of at-risk couples and individuals who are at risk for developing inherited cancers.

Counsyl has developed proprietary software to identify and compile several lines of evidence from public databases and the medical literature, including healthy population frequency, in silico predictions, splicing predictions, conservation, published case reports, and functional studies. A proprietary rule-based system is used to automatically classify variants with high frequency in asymptomatic populations and variants that have not been reported in the medical literature. For the remaining variants, a team of Counsyl's PhD scientists and board-certified genetic counselors perform "real time" curation and review of information from 3rd party databases, in silico sources, and the published literature to determine the variant classification based on ACMG guidelines. To assess pathogenicity, clinical case reports are curated for variant allele frequency among unrelated patients and controls, patient genotype, patient diagnosis, ethnicity, gender, age of disease onset, and disease severity. To assess the molecular impact of variants, functional studies are curated for the effect of variant on protein/mRNA expression, splicing, protein activity, intracellular localization, intracellular processing/maturation and post-translational modifications. The curations are reviewed and classifications are approved by Counsyl's board-certified laboratory directors. In addition, a QC program has been implemented whereby 15-20% of all curations are reviewed for accuracy and completeness of curated content. The data from this program show that ~90% of reviewed curations require no revision, and the remaining ~10% of variants require minor revisions that have not resulted in a reportable classification change. "Edge cases" are reviewed in a weekly meeting and, if recurrent, may motivate clarifying the curation protocol or classification criteria. Edge cases tend to be extreme outliers, and therefore the improvements motivated by the weekly meeting serve the purpose of reducing the turnaround time.

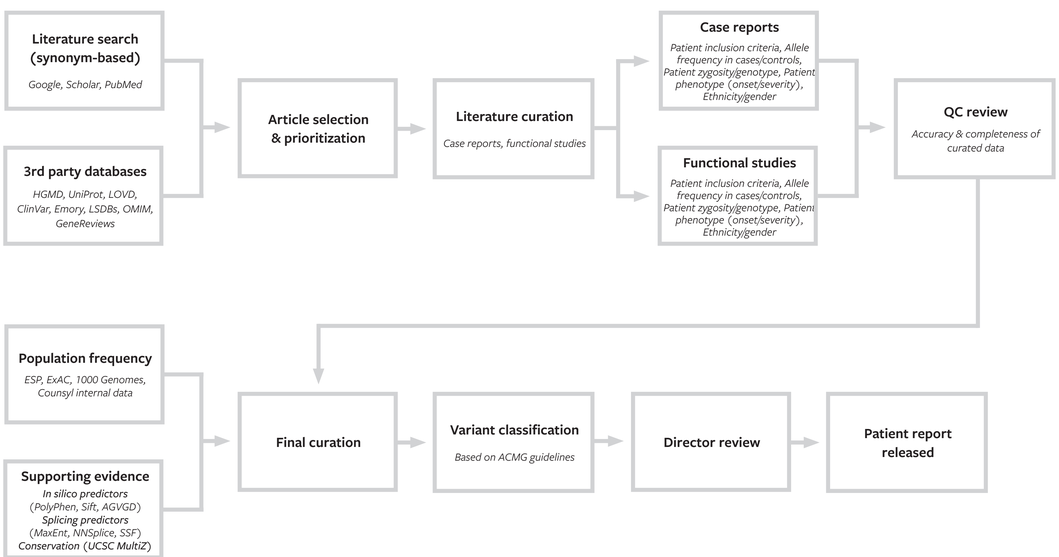
A worklog integrated into the curation software is used to record the length of time spent on each step of the curation process. There is a tight distribution of curation times per variant and a small percentage of outliers, with a median curation time of ~50 minutes per recessive variant and ~90 minutes per cancer syndrome variant. The time spent on manual curation depends on the level of available evidence (~0.80 literature references per recessive variant, ~1.75 references per cancer syndrome variant). Approximately 50% of the total curation time per variant is spent on extraction of information from literature references, with the remaining 50% spent on database searches, literature searches, and summarization of evidence. Monitoring the curation workflow enables the identification of steps that would benefit from efficiency improvements and hence reduced turnaround time, as well as allowing for measurement of the impact of such improvements. For example, we have recently implemented a third-party solution (Qiagen Clinical Insight) that reduces time spent on manual literature searches by ~50%.

As new published evidence becomes available, or when there is discordance in classification among laboratories, variants are periodically re-examined to determine whether a change in classification is warranted. To date, >300,000 variants have been classified at Counsyl, including >17,000 manually curated variants. All variant classifications are periodically submitted to ClinVar to enable peer review and data sharing with the clinical community.

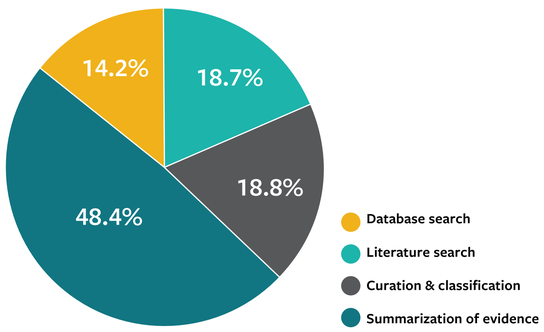
Genetic screening workflow



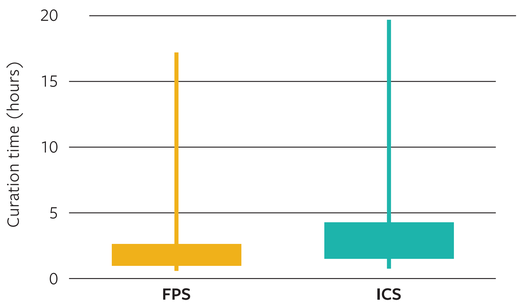
Curation workflow



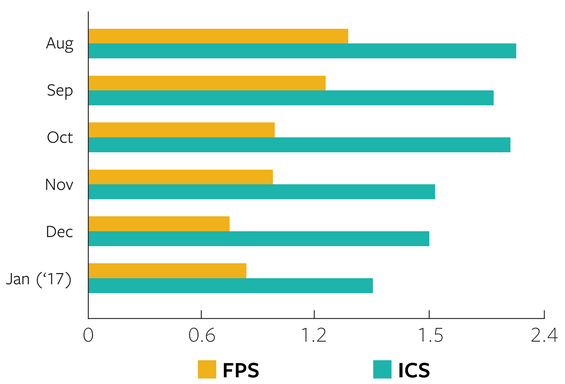
Time distribution of curation workflow steps



Curation time per variant distribution



Impact of workflow improvements on efficiency



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