Use of Databases for Establishing the Clinical Relevance of Human Genetic Variants

The information and questions contained in this document are not binding and do not create or propose new requirements or expectations for affected parties, nor is this document meant to convey FDA's proposed or recommended approaches or guidance. Rather, the information contained in this document offers background and considerations regarding use of evidence in genetic databases for discussion at FDA's public workshop on November 13, 2015.

GOAL

As part of the President's Precision Medicine Initiative (PMI), FDA is considering novel ways to optimize its regulation of Next Generation Sequencing (NGS) tests. The ultimate goal of this effort is to develop a flexible, adaptive regulatory approach that ensures that patients receive accurate and meaningful results, while accommodating innovation in test development. FDA posted a paper in December 2014 discussing possible strategies it is considering to accomplish this goal, and obtained stakeholder feedback in a public workshop held on February 20, 2015.

In brief, these strategies involve: 1) identifying and implementing analytical standards that would ensure that NGS tests produce accurate and reliable results; and 2) developing ways to use well-curated databases of genetic variants to guide clinical interpretation of NGS test results. After analysis of public feedback, FDA has further developed more specific concepts for the analytical and clinical strategies. The topic of analytical standards is discussed in a companion paper[1] while this paper discusses factors and principles to identify genetic databases that are of sufficient quality to support a regulatory submission. Defining these factors can facilitate a novel approach toward regulating NGS tests. The concepts developed in this paper will be discussed in a public workshop on Nov 13, 2015[2]; interested parties may provide comment at that time, or submit written comments to an open docket.[3]

SCOPE

This paper discusses data quality and database operations as applied to the clinical interpretation of human genetic variants, i.e., for establishing the link between variants and the risk or diagnosis of disease or other states of health; it also examines the potential use of databases as sources of clinical evidence in support of regulatory submissions for *in vitro* diagnostic tests. While the FDA's PMI activities are specific to NGS tests, the use of databases for clinical evidence may in principle extend to any genetic test that can identify variation in the human genome.

This paper does not address the specific evidence supporting any particular variant's interpretation of pathogenicity or clinical actionability, automated evidence aggregation or variant interpretation, or how healthcare providers evaluate evidence.

[1] http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm459449.htm
[2] http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm459449.htm
[3] http://www.regulations.gov/#!docketDetail;rpp=100;so=DESC;sb=docId;po=0;D=FDA-2015-N-3015

BACKGROUND

The rapid adoption of NGS in both research and clinical practice has led to the identification of an increasing number of genomic variants, including rare variants that may be unique to a single individual or family. Due to the novelty of the variant or the small number of individuals that may possess a particular variant, it is often difficult to obtain evidence linking it to a particular disease or to a specific and timely clinical action. Publicly accessible databases, where data on genetic variants from various sources is aggregated and curated, should enable the collective development of evidence for clinical interpretation of a greater portion of the human genome.

In a February 2015 workshop[4] and a previously published discussion paper[5], FDA discussed the possibility of developing an approach for using high quality, well-curated databases to aid in the clinical interpretation of variants and to support regulatory decision-making for NGS tests. The concept acknowledged the rapid generation of evidence in the clinical arena and reflected FDA's deliberations around its successful use of the CFTR2 database to clear the Illumina MiSeq Cystic Fibrosis 139 Variant Assay (see below). One approach under discussion would permit the use of information contained in curated databases meeting defined quality criteria as an acceptable source of clinical evidence for genetic variants detected by NGS tests. In this model, FDA could certify or otherwise recognize the evidence curation processes of candidate databases based on these criteria. After FDA recognizes databases that meet these criteria (e.g., in whole, in part, or at a particular quality level), test developers could refer, where possible, to the evidence in these databases in premarket submissions to FDA rather than independently deriving the supportive clinical evidence for each variant that the NGS test evaluates. Following an FDA review of the submission, those developing and running NGS tests and reporting back results to patients could provide interpretations based on the evidence within the recognized database in lab reports, with appropriate caveats. This type of approach would allow all parties to contribute to and have access to the latest evidence, coupled with publicly accessible evidence assessments. Over time, interpretation is likely to become more standardized than in current practice. However, the feasibility of this approach depends on FDA's ability to address issues relating to data quality, database operations, and interpretation of evidence.

Existing FDA Approaches for Assessment of Data

Sponsors generally must provide evidence that supports the claimed intended uses of IVDs in marketing submissions to FDA. Under FDA regulations, the types of valid scientific evidence that may be used in support of an FDA submission vary from well-documented case studies conducted by qualified experts to controlled investigations. FDA determines whether the data provided are accurate, reliable, and relevant; however it is not required that the test developer be the entity that generates the data.

FDA is interested in discussing the value of the following (and any other possible sources that may be identified) types of evidence that may be relevant to interpreting genetic variants:

- Clinical studies such as randomized controlled trials, observational studies, case study reports, and N of 1 trials
- Population and public health information, including frequency data

[4] http://www.fda.gov/MedicalDevices/NewsEvents/WorkshopsConferences/ucm427296.htm
[5] http://www.fda.gov/downloads/MedicalDevices/NewsEvents/WorkshopsConferences/UCM427869.pdf

- Basic science research studies, including functional studies in human cell lines or animal models (e.g., *in vitro* experiments, mouse knockouts)
- In silico assessments of the impact of a variant on protein function, for example, based on the position of the variant within the gene or the effect of the substitution on protein folding or length

In determining whether any particular genetic database can serve as a source of valid scientific evidence, FDA would assess the quality of the database and the quality of the process used to develop assertions.

Genetic Databases: Current Practices

Genetic tests, particularly those that query entire genes, exomes, or nearly the whole genome, are increasingly identifying novel variants and potential disease-causing variants that only a small subset of patients may have in common. This increasing stratification of patients into genetically defined groups is challenging the ability to perform traditional clinical studies because it is often not possible to prospectively identify enough individuals with the same genotype to provide the necessary sample size to demonstrate the clinical significance of particular variants. Even when such studies are possible, identifying and enrolling a sufficient number of individuals with a particular variant may not be feasible.

One alternative to performing a traditional clinical validation study for each variant that is identified by a genetic test is to use well-curated databases that capture information on patients that have been studied individually at various times and locations by different groups. This aggregation of data could relieve the need for each test developer to independently gather the evidence to support a clinical association for the same variant.

A large number of databases that focus on various clinical issues might serve to enable a database-driven approach. Some databases may focus on specific genes, biological pathways, or diseases (locus-specific databases); others may aggregate information from multiples sources and serve as a site for data-sharing (e.g., ClinVar). Currently, a number of such databases have been developed by various organizations, including large public databases, internal databases compiled by commercial entities, and both public and private databases developed by academic medical centers and non-profit organizations. To address the clinical use of such databases, the Royal College of Pathologists of Australasia approved and published in 2014 "Standards for clinical databases for genetic variants".[6]

The evidence residing in these databases has been collected from multiple sources including internally generated data from CLIA-certified laboratories as well as from external data sources, such as from the published literature or case study reports. Regardless of the source of data, the organizations that are developing genomic databases have generally adopted internal protocols and methodologies (e.g., analytic thresholds or quality measures) and/or external guidelines (e.g., ACMG Clinical Laboratory Standards for NGS,[7] ACMG/AMP Standards and Guidelines for the Interpretation of Sequence Variants,[8]

[6] https://www.rcpa.edu.au/Library/College-Policies/Guidelines/RCPA-Standards-for-Clinical-Databases-of-Genetic-V.aspx
[7] http://www.nature.com/gim/journal/v15/n9/pdf/gim201392a.pdf
[8] http://www.nature.com/gim/journal/v17/n5/pdf/gim201530a.pdf

or the Human Variome Project Guideline Gene/Disease Specific Variant Database Quality Parameters[9]) to direct evidence development and curation practices. Some databases also employ version tracking to record the database version, the analytical tools and parameters used, and cutoffs for quality filters used in a particular assay.

While curation processes may vary across organizations, they typically involve the use of experts who make informed conclusions about the presence of a genetic variant and its meaning for a particular disease (pathogenicity) or recommended clinical decision (actionability).[10] FDA's internal assessment suggests that expert reviewers tend to be full-time Ph.D.-level scientists with biological sciences backgrounds and prior experience in curation. In some cases, organizations use panels of experts to perform variant assessments and interpretations. Initial expert assessments may be quality checked by a second reviewer. In some cases, organizations use internal training methodologies for reviewers, such as template-practice curation or spot checks.

Literature is generally manually reviewed and assessed by expert reviewers. A variety of internal and external resources, such as other public databases, functional data, evidence-based rules, associated clinical characteristics, population frequency, or *in silico* assessments, may be used to provide corroborating evidence, especially when a new, previously unseen variant is encountered. Some organizations are also considering the use of computer algorithms to aid in variant interpretation.

Previous Use of a Databases to Support FDA Regulatory Decisions

FDA has previously accepted the CFTR2 database as source of clinical evidence in clearing the Illumina MiSeq Cystic Fibrosis 139 Variant Assay. This targeted NGS assay reports on variants in the CFTR gene that are known to be pathogenic variants for cystic fibrosis. Instead of requiring the independent demonstration that each variant detected by the test causes disease, FDA was able to accept the evidence within the CFTR2 database because it contains nearly all known variants observed in cystic fibrosis patients, combined with evidence from clinical and functional studies demonstrating the level of pathogenicity for cystic fibrosis. The Agency found that the data and the evidence evaluation process for curating the database were of sufficient quality to provide assurance of the clinical relevance of the variants reported by the test.

POTENTIAL PRINCIPLES AND FACTORS FOR ASSURING DATABASE QUALITY

FDA is considering and seeks comment on the following general principles for assurance of the quality of the evidence and assertions contained within a genetic database.

General Principles for Databases

- Operation in a manner which provides high confidence in the quality of original data and the evidence, review, and interpretation processes;

[9] http://www.humanvariomeproject.org/assets/hvp-guidelines/HVP-GL-001-01-EN-GDSDB_Quality_Parameters.pdf

[10] For the purposes of this discussion paper, FDA is defining actionability as leading to a clinical decision, such as a treatment course or medical procedure, as there is not currently an agreed upon definition of "actionability" within the genetics community.

- Transparency regarding data sources and processes for evaluation, review, and interpretation of evidence;

- Long-term sustainability;

- Collection of data in compliance with all applicable requirements regarding protected health information, patient privacy, research involving human subjects, and data security.

Factors for Assessing Database Quality

Evidence needs to be of sufficient quality to meet regulatory requirements for clearance or approval of a NGS-based test. FDA is considering and seeks comment on the following factors that can be used to assess the quality of ***database operations***:

A. Whether the purpose of the database is clearly stated and freely available for public review, such as in a mission statement along with both short- and long-term goals;
B. Whether a database used in support of a regulatory submission has a plan in place for long-term sustainability, and a plan to ensure that database content and processes are preserved and made publicly accessible in the event a database becomes unsustainable;
C. Whether overall operations and standard operating procedures (SOPs) are reviewed on at least an annual basis;
D. Whether policies and SOP's for reassessment of classifications and interpretations over time are in place and made available to the public, and whether versioning of variant interpretation and classification are recorded;[11]
E. Whether a database has adequate security measures in place, as well as protections to ensure patient privacy and protection of patient health information;
F. Whether a database employs common and accepted data exchange formats that support interoperability;
G. Whether SOPs are in place for assessing overall database stability and architecture and ensuring that data linkages are properly maintained;
H. If previously curated data are to be integrated into the database, whether an audit of curation processes and data quality are performed on a regular basis.

It is essential that the genotypes and phenotypes placed into the database are of sufficient quality to assure that the assertions between specific genetic variants and disease are as accurate as possible based on current knowledge. FDA is considering and seeks comment on the following factors for assuring ***data quality***:

A. Whether a database uses a consistent and accepted nomenclature for gene names and/or symbols, genomic coordinates, variants, described clinical and functional characteristics, and classifications;
B. Whether a database employs complete provenance tracking, including a full recording of database versions and tools that are used in the analytical pipeline at the time of any assessment, parameters in those pipelines, and cutoffs used for quality filters for the particular

[11] Decisions on whether to recontact patients after a reclassification of a particular variant are outside the scope of this discussion.

assay, as well as any changes in versioning of annotation, whether tracking includes documentation of the methodology and instrument used to detect genetic variants, and whether the particular source of the evidence is recorded, including links to relevant literature sources;

C. Whether software analytical tools are validated, with limitations in abilities to detect specific genetic alterations noted and recorded as part of the provenance tracking system;
D. Whether the reference genome used at the time of calling a particular variant is recorded, and whether the organization assesses the impact of updating the reference sequence on its processes and ability to make comparisons to previous variant calls;
E. Whether variant characteristics such as zygosity, phasing, cis/trans relationships, and segregation are annotated, if known, including the evidence source, whether clinical and phenotypic characteristics are included if available or known, and whether the database clearly specifies if any of this information is not available.

Proper training and expertise of personnel are critical to assuring the quality of variant review and interpretation. FDA is considering and seeks comment on the following factors regarding ***curation*** and the ***personnel*** involved in the review and classification of variants:

A. Whether each organization operating a database has a written SOP for curation that includes a validated decision matrix, and whether guidelines, when available and used, are accepted by one or more professional societies, or otherwise are supported by evidence that is publicly available for independent evaluation;
B. Whether a separate written SOP for evaluation of literature findings is available that clearly defines the classification of evidence and criteria to be used in its evaluation and includes a validated decision matrix, and whether a pre-curated literature knowledgebase, when practical, is developed;
C. Whether curation procedures ensure that data from all sources has been collected in compliance with all applicable legal and regulatory requirements for protected health information and research involving human subjects;
D. Whether curation is performed by full-time qualified experts possessing a doctorate-level degree (Ph.D., M.D., or equivalent) in a relevant biological science discipline, and whether a secondary evaluation is performed by a different doctorate-level expert with a biological science background or a master's-level genetic counselor;
E. Whether curators receive adequate training in the curation process, and whether an SOP for training is available, including on template practice curation, spot checks, or other methodologies determined to be appropriate;
F. Whether methodologies are in place to ensure that curators maintain high quality standards over time, and whether proficiency testing is performed regularly to assess the curators' ability to develop acceptable interpretations for known variants;
G. Whether methodologies are in place to ensure that individual evidence points are not represented more than once and that data inconsistencies are identifiable, and whether these methodologies ensure, for instance, that an individual patients' information is only represented a single time within a database and that comparisons between databases can identify when information from the same patient is replicated.

QUESTIONS

1. Since differences in nomenclature can cause difficulty in comparing evidence, reviews, and interpretations of gene variants, should there be a single standard nomenclature adopted by all certified databases? If so, is there a preferred nomenclature that should be used and what are the benefits of using it over others?
2. As reference genomes are updated, what processes should be employed by database holders to assess whether and when to update the reference genome used for sequence alignment?
3. What requirements should be in place for curators? What training should they receive and how should it be maintained? Should curators be accredited and if so, by what organization? Should well-trained MS-level genetic counselors or scientists be able to perform first-level curation?
4. What criteria should curators use to evaluate evidence from clinical studies? From basic research? From literature sources? From other databases? How can data quality be assured long-term?
5. How often should previous variant classifications be reviewed? How should variant interpretation changes be handled? Should discrepancies between databases be looked for and resolved? If so, how?
6. What information should databases include on each variant?
7. How can databases ensure sustainability? How should databases be supported? In the event a database becomes unsustainable, where should the content be housed? If test developers will refer to the database version that was extant at the time of device approval, will these be archived? Should the test developer state the version that was used?
8. How often should FDA re-review certified databases?