Reporting sequence variants in clinical genetics:
evaluating pathogenicity

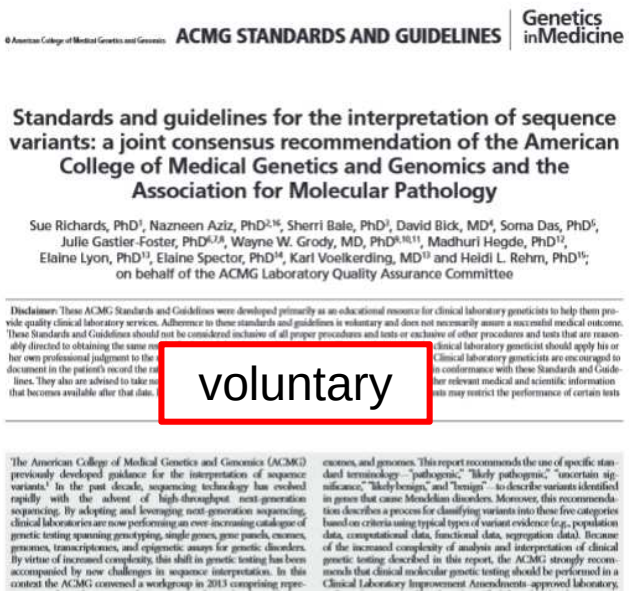
MSc in Genomic Medicine

Lucy Crooks

27/1/2016

Published guidelines

American College of Medical Genetics and Genomics, the Association for Molecular Pathology and the College of American Pathologists 2015



ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[15] and Heidi L. Rehm, PhD[16]; on behalf of the ACMG Laboratory Quality Assurance Committee

Disclaimer: These ACMG Standards and Guidelines were developed primarily as an educational resource for clinical laboratory geneticists to help them provide quality clinical laboratory services. Adherence to these standards and guidelines is voluntary and does not necessarily assure a successful medical outcome. These Standards and Guidelines should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In the ultimate analysis, the clinical laboratory geneticist should apply his or her own professional judgment to the specific circumstances presented by the individual patient or specimen. Clinical laboratory geneticists are encouraged to document in the patient's record the rationale for any significant deviation from, or exception to, these guidelines in conformance with these Standards and Guidelines. They also are advised to take notice of new reports of clinical investigations and other relevant medical and scientific information that becomes available after that date. It also should be noted that laboratories must follow all applicable laws and regulations. Moreover, these Standards and Guidelines may restrict the performance of certain tests

voluntary

The American College of Medical Genetics and Genomics (ACMG) previously developed guidance for the interpretation of sequence variants.[1] In the past decade, sequencing technology has evolved rapidly with the advent of high-throughput next-generation sequencing. By adopting and leveraging next-generation sequencing, clinical laboratories are now performing an ever-increasing catalogue of genetic testing spanning genotyping, single genes, gene panels, exomes, genomes, transcriptomes, and epigenetic assays for genetic disorders. By virtue of increased complexity, this shift in genetic testing has been accompanied by new challenges in sequence interpretation. In this context the ACMG convened a workgroup in 2013 comprising representatives from the ACMG, the Association for Molecular Pathology (AMP), and the College of American Pathologists (CAP) to revisit and revise the standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified in genes that cause Mendelian disorders. Moreover, this recommendation describes a process for classifying variants into these five categories based on criteria using typical types of variant evidence (e.g., population data, computational data, functional data, segregation data). Because of the increased complexity of analysis and interpretation of clinical genetic testing described in this report, the ACMG strongly recommends that clinical molecular genetic testing should be performed in a Clinical Laboratory Improvement Amendments–approved laboratory.

Association for Clinical Genetic Science and the Dutch Society of Clinical Genetic Laboratory Specialists 2013 (UK)

acgs Association for Clinical Genetic Science

VKGL

Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics.

Yvonne Wallis[1], Stewart Payne[2], Ciaron McAnulty[3], Danielle Bodmer[4], Erik Sistermans[5], Kathryn Robertson[6], David Moore[7], Stephen Abbs[8], Zandra Deans[9] and Andrew Devereau[8]

1. West Midlands Regional Genetics Laboratory, Birmingham Women's NHS Foundation Trust, Mindelsohn Way, Edgbaston, Birmingham, B15 2TG, United Kingdom.
2. Kennedy-Galton Centre (NW Thames) Regional Genetics Centre, Level 8V, Northwick Park & St Mark's Hospitals, Watford Road, Harrow, HA1 3UJ, United Kingdom.
3. Northern Genetics Service, Institute of Human Genetics, Central Parkway, Newcastle-upon-Tyne, NE1 3BZ, United Kingdom.
4. Dept of Human Genetics, Radboud University Nijmegen Medical Centre, PO box 9101, 6500 HB, Nijmegen, The Netherlands.
5. De… Amster-
6. Na… s Hospi-
7. So… e Road,
8. Ea… mbridge,
9. UK… Medicine,

Agreed standards but "aspirational"

Original guidelines ratified by the UK Clinical Molecular Genetics Society (11th January, 2008) and the Dutch Society of Clinical Genetic Laboratory Specialists (Vereniging Klinisch Genetische Laboratoriumspecialisten; VKGL) (22nd October, 2007).

Guidelines updated by the Association for Clinical Genetic Science (formally Clinical Molecular Genetics Society and Association of Clinical Cytogenetics) and the Dutch Society of Clinical Genetic Laboratory Specialists (approved September 2013).

Both guidelines recommend 5-class system

UK guidelines

- 5-class system considered essential for standardisation of report wording
- Names not numbers in reports
- It is essential that classes 3,4, 5 are reported

Name	Class
Not pathogenic	1
Unlikely to be pathogenic	2
Uncertain pathogenicity	3
Likely to be pathogenic	4
Predicted to be pathogenic	5

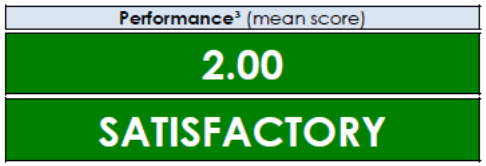
Laboratory certification

- UKAS (United Kingdom accreditation services) accreditation to ISO (International Organization for Standardization) 15189:2012 Medical Laboratories - requirements for quality and competence

- Essential that the interpretation and reporting of sequence variants is carried out by appropriately qualified and experienced staff working within certified laboratories that are working to international quality standards
  - State registered Clinical Scientists (and trainee), Biomedical Scientists and Health Care Scientists, VRC registered and pre-registration Genetic Technologists

Quality standards

- All technologies must be appropriately validated
- Laboratories should have regular independent assessment of the technical performance of their tests to ensure consistency across labs
- SDGS participates in UK NEQAS (National External Quality Assessment Service) and EMQN (European Molecular Genetics Quality Network) schemes



Nomenclature

- Recommend that HGVS (Human Genome Variation Society) guidelines are followed
  - Coordinates should be proceeded by a letter indicating the type of sequence –c for coding and g for genomic
  - Only official gene symbols used
- The reference sequence must be cited including the version number eg NM 004004.3
- It is recommended that variants are submitted to an appropriate database as soon as possible

Types of evidence for pathogenicity

It is essential that a minimum set of standards is clearly defined (recommended to include a literature search)

- Use variant databases including Locus Specific Databases (LSDBs)
- Check SNP databases with caution and datasets from large-scale sequencing projects
- Testing matched controls (eg in routine service)
- Co-occurrence (*in trans*) with known deleterious variant

Types of evidence for pathogenicity

- Segregation with disease in family
- *De novo* variant in patient in strong candidate gene
- For missense variants, conservation of amino acid sequence across species
- For missense variants, *in silico* predictions
  - No one tool is superior or completely accurate
  - Recommend comparative validation of tools for variants of known effect
  - Use at least 3 tools ideally based on different algorithms
  - These predictions **must not** be used alone to designate pathogenicity

Types of evidence for pathogenicity

- *In silico* splice site prediction
  - Changes that disrupt the GT 5' of the intron or AG 3' can be declared pathogenic (consider alternate transcripts)
  - These predictions **must not** be used alone to determine pathogenicity
  - Recommended to use at least 3 tools
- RNA studies essential for definitive interpretation of putative splice site mutations
- Functional studies are recommended if a reliable assay is available
- Loss of heterozygosity can be used to assist prediction of pathogenicity for variants in tumour suppressor genes

Types of evidence for pathogenicity

- Try to engage with projects looking at integrating different types of evidence by Bayesian statistical inference

Standardising the evaluation process

- A checklist is recommended
  - SDGS has a UV (unclassified variant) form with 17 sections
- For each search/tool used the date, version, any changes to default settings, alignments used, database build and scores obtained must be recorded
- It is desirable that variants of uncertain pathogenicity are periodically reviewed
  - UV form is checked each time a new patient with variant is seen