Modification of the variant classification rules for ornithine transcarbamylase through pilot curation

Presenting author: Kara Simpson, MS, CGC

Children's National Hospital

Rare Disease Institute

7143 13th Pl NW, Washington, DC 20012

Phone: 202-545-2503

Email: Ksimpson@childrensnational.org

*Simpson K[1] , Caldovic L[1,2], Ah Mew N[1], Argueta D[3], Ayyadurai R[4], Kacey Azizi-Namini[5], Bedoyan J[6,7], Berry GT[8], Burrage L[9], Cortes-Fernandez A[10], Crenshaw M[10], Feigenbaum A[11], Groopman E[1], Gropman A[1], Häberle J[12], Haider R[9], Harding CO[14], Konczal L[13], Lichter-Konecki U[6], Lipshutz G[14], McCandless SE[10], Medeiros L[15], Melby S[5], Morizono H[1,2], Naybor M[1], Nguimbous Y[16], Oishi K[17], Osundiji M[18], Rubio V[19], Sako S[17], Spector E[10], St. Jacques M[5], Sudo Y[20], Trainor J[5], Tsunogai T[17], Upadhye A[21], Volpi J[22], Warrier M[23], Weaver M[24], Wong D[16], Zastrow D[23], Thomas-Wilson A[25]

[1] Center for Genetic Medicine Research, Children's National Hospital, Washington, DC, USA

[2] Department of Genomics and Precision Medicine, The George Washington University School of Medicine and Health Sciences, Washington, DC, USA

[3] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

[4] Emory College of Arts and Sciences, Emory University, Atlanta, GA, USA

[5] The George Washington University, Washington DC, USA

[6] University of Pittsburgh Medical Center Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

[7] Division of Genetic and Genomic Medicine, Department of Pediatrics, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

[8] Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, USA

[9] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

[10] University of Colorado Anschutz Medical Center and Children's Hospital Colorado, Aurora, CO, USA

[11] University of California San Diego and Rady Children's Hospital, San Diego, CA, USA

[12] Division of Metabolism and Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland

[13] University Hospitals of Cleveland Medical Center, Cleveland, OH, USA

[14] David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

[15] Hospital de Clínicas de Porto Alegre, Brazil

[16] Howard University, Washington DC, USA

[17] Department of Pediatrics, The Jikei University School of Medicine, Tokyo, Japan

[18] Department of Clinical Genomics, Mayo Clinic, Scottsdale, USA.

[19] Institute for Biomedicine of Valencia-CSIC and CIBERER-ISCIII Group CB06/07/0077, Valencia, Spain

[20] Fujita Health University, Toyoake, Japan

[21] School of Science, Rensselaer Polytechnic Institute, Albany, NY, USA

[22] Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

[23] Invitae Corporation, San Francisco, CA, USA

[24] American College of Medical Genetics & Genomics (ACMG), Bethesda, MD, USA

[25] New York Genome Center, New York, NY, USA

The ClinGen Urea Cycle Disorders Variant Curation Expert Panel (UCD VCEP) was established in 2021 to evaluate evidence and classify genomic variants identified in genes encoding 6 enzymes and 2 transporters of the urea cycle. The UCD VCEP prioritized the development of gene-specific variant classification rules based on disease severity and prevalence. Ornithine transcarbamylase (*OTC*), N-acetylglutamate synthase (*NAGS*) and carbamylphosphate synthetase 1 (*CPS1*) deficiencies typically present with the earliest and most severe symptoms.

ACMG/AMP guideline specifications for *OTC*, *NAGS,* and *CPS1* deficiencies were drafted and were approved by the SVI VCEP Review Committee. Criteria and strength of evidence related to variant type, allele frequency, functional assay(s), and phenotype were adjusted for each disease. The next step was completing pilot specifications for *OTC* to validate the disease-specific guidelines by applying criteria to known variants. Curators applied the criteria to 50 variants of differing classifications and these were reviewed by our expert reviewers and questions that arose were discussed on our biweekly calls. These pilot curations lead to the following modifications of the previously established rules:

PM2/BA1/BS1: Changed from PopMAX frequency to Grpmax filtering allele frequency and changed the number of female homozygotes/male hemizygotes to be in the most current version of gnomAD available at the time of curation.

PP3: Changed for in-frame deletions and insertions to defer to PM4 criteria instead of using PROVEAN and Mutation Taster. Additionally, we added if PP3 and PM1 are utilized for the same variant, the total strength of PP3 + PM1 can be used at a maximum strength level of Strong.

PP4: Adjusted our point table to include 0.25 points for elevated ammonia/hyperammonemia with no additional information. We also separated out into two categories for those with elevated ammonia and normal citrulline for 0.5 points, and added elevated glutamine and low citrulline or elevated ammonia and low citrulline for 0.75 points. Additionally, we added elevated uracil into the elevated urine orotic acid criteria.

BP7: Added further specificity for Strong criteria to include applicable for intronic variants outside of donor and acceptor splice sites as described in Walker et al (PMID 37352859), and intronic variants must be outside +7/-21nt and exonic variants must be outside first and last 3 bases of exon. Removed qualification of only using BP7 if BP4 is met.

These changes will be incorporated and the refined specifications will be submitted to the VCI for approval, and once approved, full curation will begin.