Classifying Variants in the *CHEK2* Gene: The Importance of Collaboration

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BACKGROUND

- Analysis for hereditary cancer has expanded beyond well-known high-risk genes such as *BRCA1* and *BRCA2*, to multi-gene panels, commonly including genes such as *CHEK2*.[1]
- Mutations in *CHEK2* have been linked to an increased risk of several cancers, primarily breast and colorectal cancer. The breast cancer risk is estimated to be approximately 2-fold, but is not well defined.[2-4]
- Estimating cancer risks and providing management recommendations for patients with variants of unknown significance is challenging for clinicians.
- Clinical laboratories must constantly work to classify variants based on evolving data.
- Established recommendations for interpreting genetic variants were developed primarily for highly penetrant genes and may not be appropriate for moderately penetrant genes, like *CHEK2*.[5-7]
- Functional data is often required, but can be difficult to obtain.
- Laboratories may have different internal data and/or may use different lines of evidence to classify variants which may lead to different classifications.[8]
- These discrepancies further complicate the clinical interpretation of test results and may increase anxiety for patients.
- We report on the collaboration of two laboratories to improve *CHEK2* variant classifications using a data sharing approach.

METHODS

- *CHEK2* alterations identified at one U.S. commercial laboratory and one European academic laboratory were compiled and compared for shared alterations.
- When classifications differed between labs, supporting evidence for the classification, including functional evidence, structural analysis, and in some cases, internal frequency data were shared as well as interpretation of in silico and population frequency data.
- Discussions regarding evidence and resolving classification discrepancies are in progress.

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CHEK2 Alterations and Classifications

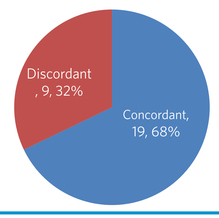
c.	p.	Ambry Classification	Charles University Classification	Concordant?	Primary reason(s) for discrepancy
c.7C>T	p.R3W	VUS	VUS	Yes	
c.190G>A	p.E64K	VUS	VUS	Yes	
c.277delT	p.W93GFS*17	Mutation	VLP	Confidence*	
c.434G>A	p.R145Q	VUS	VLP	No	Functional data weighting
c.444+1G>A	p.E149Ifs*6	Mutation	Mutation	Yes	
c.470T>C	p.I157T	Mutation	Mutation	Yes	
c.503C>T	p.T168I	VUS	VLP	No	Functional data weighting
c.514A>G	p.T172A	VUS	VUS	Yes	
c.528G>C	p.G176	VLB	VLB	Yes	
c.538C>T	p.R180C	VLB	Polymorphism	Confidence*	
c.539G>A	p.R180H	VUS	VUS	Yes	
c.541C>T	p.R181C	VUS	VUS	Yes	
c.542G>A	p.R181H	VUS	VUS	Yes	
c.846+4_846+7delAGTA	p.D265_H282del	VUS	VLP	No	Functional data weighting
c.847_908del (Ex7del)	p.P283Dfs*8	Mutation	Mutation	Yes	
c.909-2028_1095+330del5395 (Ex8_9del)	p.M304Lfs*16	Mutation	Mutation	Yes	
c.917G>C	p.G306A	VLP	VLP	Yes	
c.1037G>A	p.R346H	VUS	VLP	No	Functional data weighting
c.1100delC	p.T367MFS*15	Mutation	Mutation	Yes	
c.1169A>C	p.Y390S	VLP	VLP	Yes	
c.1180G>A	p.E394K	VUS	VLP	No	Functional data weighting
c.1260-8A>G	p.L421Ifs*4	VUS	VLP	No	Unpublished RNA evidence
c.1270T>C	p.Y424H	VUS	VLP	No	Functional data weighting
c.1287G>A	p.E429	VLB	VLB	Yes	
c.1312G>T	p.D438Y	VUS	VLP	No	Functional data and evidence weighting
c.1421G>A	p.R474H	VUS	VLP	No	Functional data and evidence weighting
c.1427C>T	p.T476M	VLP	VLP	Yes	
c.1497G>C	p.L499	VLB	VLB	Yes	

VUS, variant of unknown significance
*Confidence discrepancies are classifications that are discordant but only in confidence, i.e., pathogenic versus likely pathogenic (VLP) or likely benign (VLB) versus polymorphism

RESULTS SUMMARY

- 28 *CHEK2* alterations were seen at both labs
- Classifications were concordant for 67.9% (n=19)
  - Includes 2 alterations with only confidence discrepancies, i.e. pathogenic versus likely pathogenic or likely benign versus polymorphism
- 9 alterations had discordant classifications (32.1%)

CHEK2 Classification Concordance



- Discordant classifications were primarily due to differences in how the following lines of evidence are used in classifying variants:
  - General population frequency
  - Familial segregation data
  - The amount of evidence needed to classify a variant
  - The availability of unpublished functional data

Example Variant Classification Evidence

Evidence in Favor of Pathogenicity

- Confirmed *de novo* alteration with new disease in the family
- Alterations resulting in premature truncation
- Segregation with disease in families
- Significant association with disease in case-control study
- Last nucleotide of exon
- Rare in population databases

Unknown Significance (VUS)

- Insufficient or conflicting evidence

Evidence Against Pathogenicity

- Co-occurrence with mutation in same gene
- No disease association in case-control study(ies)
- General population frequency is too high to be pathogenic based on disease prevalence/penetrance

- The above are examples of the types of evidence that are considered in classifying genetic variants.
- The number of pieces of evidence needed to classify a variant depends on the type of evidence and how strong of evidence it is.
- These examples are based on published criteria from the American College of Medical Genetics and Genomics and International Agency for Research on Cancer.[5,6]
- While there are published criteria for variant classification, laboratories may vary in how strongly they weight various pieces of evidence.

CONCLUSIONS

- 32.1% of classifications were discordant based on differences in how classification criteria and available functional data are weighted at each lab.
- We are currently working to resolve these discrepancies by sharing data and supporting evidence.
- Our study highlights the challenges of interpreting variants in moderate risk cancer genes, and the importance of data sharing and collaboration between laboratories to reduce classification discrepancies, improve variant interpretation, and provide clearer information for clinicians and patients.

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