Consultation sponsor (may not be the patient): First LastName
[Patient identity withheld]
Date received by CGI: 2 Sept 2017
Variant Fact Checker™ Report ID: 0000001.5
Date Variant Fact Checker™ issued: 12 Sept 2017

Center For Genomic Interpretation
Driving quality in clinical genetics

Variant FactChecker™

An independent research assessment of your genetic variant.

For Research Use Only. Not for clinical use.

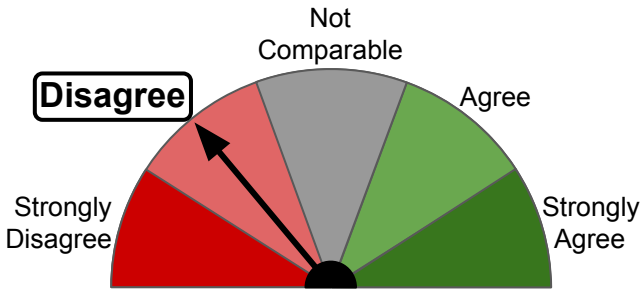
CGI's high stringency research-use-only classification of

PALB2 c.3113+5G>C

is

VARIANT OF UNCERTAIN SIGNIFICANCE (VUS)

CGI's summary of the available evidence is in Appendices A-C.



CGI AGREEMENT METER WITH ORIGINAL REPORT

The original report received by CGI classifies this variant as "Likely Pathogenic" (see Appendix D). CGI's research-use-only, high-stringency classification DISAGREES with the original classification.

CGI recommends that you give your genetics healthcare provider a copy of this CGI research-use-only report. Together you may wish to share it with the originating laboratory.

If you are not already being advised by a clinical genetics professional, in the USA, a Certified Genetic Counselor can be found through insurance provider lists or by using www.findageneticcounselor.com/.

FAQs:

1. Is any genetic variant classification guaranteed accurate?

- No. As stated within the ACMG guidelines[1], "Variant analysis is at present imperfect and the variant category reported does not imply 100% certainty." While CGI prides itself on excellent research-use-only variant classifications, the science remains imperfect. CGI is dedicated to continually improving the science of clinical variant interpretation.

2. Can I use CGI's research-use-only variant classification to guide or influence my clinical care?

- CGI's Variant FactChecker™ is **NOT** intended to alter clinical management. It is a research-use-only tool provided by CGI for patient and clinician research and is not for clinical use.
- The classification or reclassification of a variant issued by the genetic test originating laboratory/company, in connection with other clinical inputs, and the clinician's professional judgement should inform clinical care. The responsibility and liability for accurate clinical variant classification remains with the originating laboratory/company performing the genetic assay and the entity that issues the genetic test report.

3. Why does CGI's research-use-only variant classification sometimes disagree with the classification from the originating laboratory?

- Sometimes CGI may not have access to all of the information that the originating laboratory has.
- However, most often disagreement occurs because laboratories and companies frequently use different variant classification protocols to assess the same publicly available evidence. CGI's assessment protocol is stricter than the ACMG[1] recommended minimal classification standards used by many US laboratories. CGI's variant classification method is designed to minimize false positives compared to most methods. However, in some situations, less stringent approaches to variant classification may be clinically advisable. A qualified healthcare professional can help you judge the associated risks and benefits.

4. Does CGI evaluate all the publicly available evidence for my variant?

- CGI endeavors to evaluate all the publicly available evidence for your variant. CGI uses a strict variant classification protocol to triage out evidence that CGI determines is of weak or no clinical value for variant classification. CGI's Variant FactChecker™ report summarizes all of the evidence CGI used to arrive at its research-use-only classification.

5. Will CGI talk to the originating laboratory/company that issued my original report?

- Yes. If you have questions or concerns about CGI's Variant FactChecker™ findings compared to the findings in your original report, you and your healthcare provider may share it with the originating laboratory. CGI welcomes follow-on discussion with the originating laboratory's scientists. Discussion may lead to a re-assessment of evidence, or the sharing of additional evidence. Sometimes this will result in either or both the laboratory or CGI issuing an amended report. In such situations, if CGI reassesses and makes a meaningful update to its evaluation of the variant, CGI intends to issue you an amended Variant FactChecker™ report within two calendar months of first issuance, at no additional cost to you.

6. Which healthcare professionals understand CGI's Variant FactChecker™ technical details?

- If complex genetic case assessments are outside the scope of your current healthcare provider, CGI recommends that you seek out a Medical Geneticist or a Certified Genetic Counselor. Within the USA, insurance sponsored provider lists and www.findageneticcounselor.com/ are useful finding resources.

REFERENCE

1. Richards S. et al. 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. *Genetics in Medicine* 2015;17:405-424.

Description of Appendices

Appendix	Description
A	**Variant Classification Evidence Summary**
	A1 CGI's Variant Classification (for research use only)
	A2 CGI's Highest-Value Variant Data Summary
B	**Gene-Disease Association Evidence**
	B1 ClinGen's Information on Gene-Disease Association
	B2 CGI's Gene-Disease Citations Measure
C	**Variant Specific Data Work Up**
	C1 Allele Frequency Data from Population Databases
	C2 Functional Features Analysis
	C3 *in silico* pre-mRNA Splicing Prediction
	C4 Literature & Locus Specific Database Evidence Assessment
D	**Originating Report**
	D1 Originating Report Received by CGI:
E	**CGI Agreement Meter Explanation**
	E1 Matrix for "CGI Agreement Meter With Original Report"

Appendix A - Variant Classification Evidence Summary

A1. CGI's Variant Classification (for research use only)

NM_024675.3(*PALB2*):c.3113+5G>C	
Gene-Disease Association (Appendix B)	*PALB2* gene, and not necessarily this variant, receives a "Definitive" category from ClinGen for its association with Hereditary Breast Cancer. The gene may also be relevant to other diseases. Only a small fraction of the gene's variants will be disease causing.
Unusual points to note	None.
CGI research use only Classification of the Variant (Appendix A-A2)	**Variant of Uncertain Significance (VUS)** Absent strong or moderate evidence. Some conflicting evidence.

A2. CGI's Highest Value Variant Data Summary:

	Biochemistry Findings		**Person, Family or Population Findings**	
	Disease Causality	**Disease Non-Causality**	**Disease Association**	**Disease Non-Association**
Strong Evidence	None	None	None	None
Moderate Evidence	None	None	None	None
Conflicting Evidence	Unresolved in silico splice predictions: NNsplice predicts near abolishment of natural splice site. However, MaxEntScan predicts decreased utilization of the natural splice site, but not abolishment.		None	
Variant Specific Caveats	None		None	

Appendix B - Gene-Disease Association Evidence

CGI's interpretation of a specific genetic variant requires that the gene's association with at least one disease be well defined. Independent of CGI, the ClinGen Gene Curation Workgroup is continually advancing gene level clinical validity methods. When available, CGI defers to the current ClinGen definition of Gene-Disease evidence strength. CGI accepts ClinGen "Definitive" and "Strong" associations.

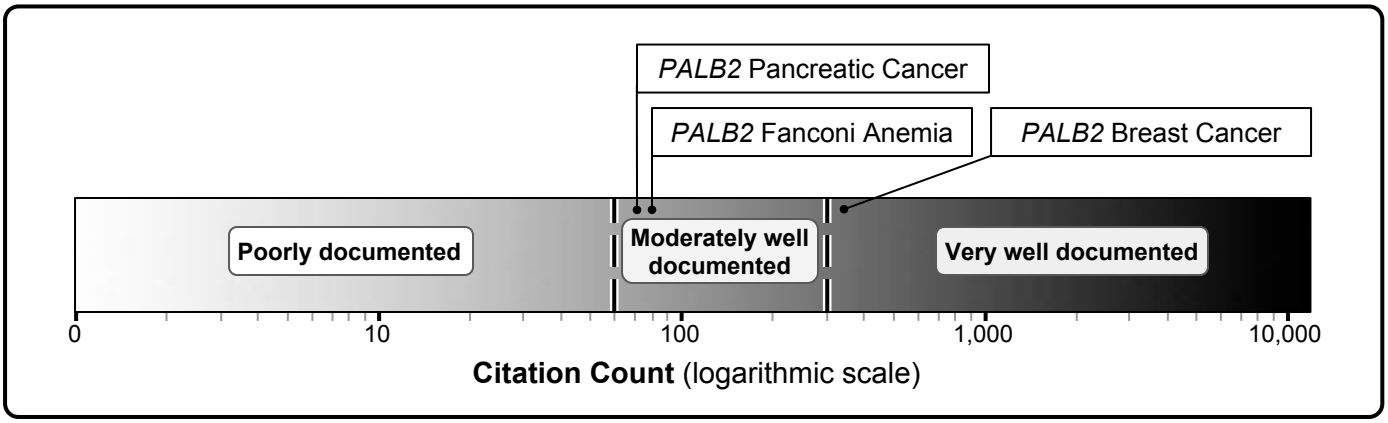
When a qualifying ClinGen gene-disease association is not yet available, CGI estimates the strength of gene-disease association knowledge with CGI's Gene-Disease Citation Measure which has been calibrated against the ClinGen Gene-Disease curation list, and ACMG's secondary findings gene list. CGI searches for citations of the gene with diseases listed in the OMIM resource maintained by Johns Hopkins University. CGI accepts "Very well documented" and "Moderately well documented" from its calibrated citation measure estimate illustrated below.

For gene-disease associations with insufficient evidence, and thus not accepted by CGI, CGI is unable to make a determination as to its research-use-only variant classification. In these situations, unless otherwise specified, CGI defaults its interpretation of the variant to "Variant of Uncertain Significance", as the entire gene is uncertain with regards to human disease risk.

B1. ClinGen's Information on Gene-Disease Association

Gene: *PALB2*	**ClinGen Gene Curation Workgroup Gene-Disease Evidence**					
Disease	Not Yet Available	Contradictory	Limited or None	Moderate	Strong	Definitive
Hereditary Breast Cancer						✓ 1 Dec 2016
Fanconi anemia	✓					
Pancreatic Cancer	✓					

B2. CGI's Gene-Disease Citations Measure



Appendix C - Variant Specific Data Work Up

C1. Allele Frequency Data from Population Databases

	Genome Aggregation Database (gnomAD)	NHLBI Exome Sequencing Project (ESP)
Date accessed	7 Sept 2017	7 Sept 2017
Variant Evaluated	**NM_024675.3(PALB2):c.3113+5G>C**	
Genomic Coordinate	16:23632678 (on Assembly GRCh37)	
Populations & Sample Sizes Populations are derived from a variety of disease and unselected patient cohorts. gnomAD provides only the overall study configuration. ESP provides the representation at the specified genomic coordinate. Up to 3,936 persons in the ESP study may be included in the gnomAD aggregation.	Males & Females combined: African/African American: 7,652 Exomes + 4,368 Genomes. Latino: 16,791 Exomes + 419 Genomes. Ashkenazi Jewish: 4,925 Exomes + 151 Genomes. East Asian: 8,624 Exomes + 811 Genomes. Finnish: 11,150 Exomes + 1,747 Genomes. Non-Finnish European: 55,860 Exomes + 7,509 Genomes. South Asian: 15,391 Exomes + 0 Genomes. Other: 2,743 Exomes + 491 Genomes. Total: 123,136 Exomes + 15,496 Genomes = 138,632 Total persons	Males & Females combined: European American (EA): 4,300.0 ± 0.0 African American (AA): 2,197.0 ± 0.0 Total: 4,300 EA + 2,197 AA = 6,497 Total persons
Depth of Coverage	Sufficient: Ave ~80x Exome, ~34x Genome, ~90% over 20x	Sufficient: Ave EA 81x, Ave AA 88x
Variant frequency in population samples represented	**Absent = Rare** (While pathogenic variants are often rare, most rare variants are benign).	**Absent = Rare** (While pathogenic variants are often rare, most rare variants are benign).

C2. Functional Features Analysis

Variant:	**NM_024675.3(PALB2):c.3113+5G>C** Alternate name(s): IVS10+5G>C
Genomic location:	Chr16: 23621357 (on Assembly GRCh38) Chr16: 23632678 (on Assembly GRCh37)
Known mechanism of disease:	Loss of Function. Null variants are considered disease causal.
RNA Transcripts:	More than one transcript is described by GENCODE v 26.
Functional location of the variant across all RNA transcripts (GENCODE v 26):	All transcripts maintain the same functional position within this gene: Intronic and proximal to a splice donor. The intron is NOT the final intron.
Predicted functional impact:	This variant is intronic and has the potential to disrupt RNA splicing in a way that could lead to RNA nonsense mediated decay resulting in loss of function of the gene. However, in silico RNA splice predictions are INCONCLUSIVE (see Appendix C-C3).

C3. *in silico* pre-mRNA Splicing Prediction

Variant evaluated in context of reference sequence: NM_024675.3(PALB2):c.3113+5G>C

Does the variant qualify for pre-mRNA splicing analysis? YES
The gene has a known Loss of Function mechanism for disease, and the variant is proximal to an RNA donor site.

in silico NNSplice predictions:

NNSplice **reference** coding sequence input (5′ to 3′, UPPER CASE = Natural exon):
AGAAGCTCTGCTTGGTACTACTATTATGAACAACATTGTTATTTGgtaagctttccctctaggtcctcagttccctcatctgtagtatgaggatatac

NNSplice **reference** seq prediction #1. (Underlined=predicted exon. No underline=predicted intron):
ctgcttggtactact
Not considered a natural donor site. Predicted score = 0.05 (predicted extremely weak)

NNSplice **reference** seq prediction #2. (Underlined=predicted exon. No underline=predicted intron):
ttatttggtaagctt
Considered the natural donor site. Predicted score = 0.98 (predicted very strong)

NNSplice **variant** coding sequence input (5′ to 3′, UPPER CASE = Natural exon):
AGAAGCTCTGCTTGGTACTACTATTATGAACAACATTGTTATTTGgtaacctttccctctaggtcctcagttccctcatctgtagtatgaggatatac

NNSplice **variant** seq prediction #1. (Underlined=predicted exon. No underline=predicted intron):
ctgcttggtactact
Not considered a natural donor site. Predicted score = 0.05 (predicted extremely weak)

NNSplice **variant** seq prediction #2. (Underlined=predicted exon. No underline=predicted intron):
ttatttggtaacctt
Considered the natural donor site. Predicted score = 0.08 (predicted extremely weak)

in silico MaxEntScan predictions:

MaxEntScan **reference** coding sequence input (5′ to 3′, UPPER CASE = Natural exon):
TTGgtaagc

MaxEntScan **reference** seq prediction #1. (Underlined=predicted exon. No underline=predicted intron):
ttggtaagc
Considered natural donor site. Predicted score = 8.72 (predicted strong)

MaxEntScan **variant** coding sequence input (5′ to 3′, UPPER CASE = Natural exon):
TTGgtaacc

MaxEntScan **variant** seq prediction #1. (Underlined=predicted exon. No underline=predicted intron):
ttggtaacc
Considered natural donor site. Predicted score = 3.53 (predicted moderate)

in silico pre-mRNA splicing prediction summary: INCONCLUSIVE
The variant was evaluated in the context of the natural RNA splice donor site.
NNsplice predicts near abolishment of natural splice site.
MaxEntScan predicts decreased utilization of the natural splice site, but not abolishment.

C4. Literature and Locus Specific Database Evidence Assessment

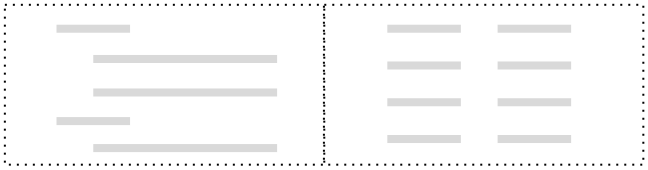
Variant evaluated:	**NM_024675.3(PALB2):c.3113+5G>C Alternate name(s): IVS10+5G>C**	
Reference	**Data Description**	**Evidence Evaluation**
PMID: 24415441 Fernandes 2014	Ascertained case report.	DISQUALIFIED Phenotype ascertained case.
PMID: 24870022 Pauty 2014	References PMID: 24415441 Fernandes 2014	DISQUALIFIED Unique evidence absent.
PMID: 23824750 Wong-Brown 2013	Ascertained case report.	DISQUALIFIED Phenotype ascertained case.
PMID: 21165770 Bogdanova 2011	References case in PMID: 17200671	DISQUALIFIED Unique evidence absent.
PMID: 17200671 Reid 2007	Supposed biallelic Fanconi Anemia case.	DISQUALIFIED Fanconi Anemia patient was never DNA sequenced. Only parent samples were tested.
ClinVar Database, Variant ID 231961	Cases with some references listed above. Ambry - Likely Path. (May 27, 2015) Invitae - VUS (Sep 3, 2016) GeneDx - Likely Path. (June 17, 2016)	DISQUALIFIED Unique evidence absent other than phenotype ascertained cases.
Public Database: https://databases.lovd.nl/shared/genes/PALB2 , DB-ID PALB2_000004	Cases with some references listed above. Database curators concluded Pathogenic in five cases, and Likely Benign in one case.	DISQUALIFIED Unique evidence absent other than phenotype ascertained cases.
Evidence obtained from Literature or Locus Specific Databases that can be input for CGI's variant classification:		**NONE**

Evidence is "DISQUALIFIED" when it is redundant, or of no, low, or insignificant quality or quantity to be applicable in a clinical setting. Most "Disqualified" evidence represents data in the earliest, and most error and biased prone, stages of basic research.

Appendix D - Originating Report

D1. Originating Report Received by CGI:

Fictional Genetics Lab Final Report



31 Hereditary Cancer Genes Analysis

PALB2 c.3113+5G>C: Likely Pathogenic

POSITIVE Variant

This variant is denoted PALB2 c.3113+5G>C. It has also been previously described as IVS10+5G>C. It consists of a G>C nucleotide substitution at the +5 position of intron 10 of the PALB2 gene. Several in silico models predict this variant to destroy the nearby natural RNA splice donor site and to result in abnormal splicing. Reid et al (2007, PMID 17200671) suggested that this variant results in a deletion of exons 9 and 10. Loss of these exons is expected to be significant as this region contains the WD3/4 repeat region and is required for interaction with multiple proteins (UniProt).This variant has been observed in an individual with Fanconi Anemia who was found to harbor a second pathogenic variant in this gene, presumably on opposite alleles, as well as at least two individuals with personal and/or family histories of breast cancer (PMIDs 23824750, 17200671 and 24415441). PALB2 c.3113+5G>C was not observed in approximately 6,500 individuals of European and African American ancestry in the ESP database, suggesting it is not a common benign variant in these populations. Also, the guanine (G) nucleotide that is altered is conserved across species. Our assessment using the currently available information leads our scientists to consider PALB2 c.3113+5G>C to be a likely pathogenic variant.

Appendix E - CGI Agreement Meter Explanation

E1. Matrix for "CGI Agreement Meter With Original Report"

		Variant Classification from the originating report				
		Pathogenic	Likely Pathogenic	VUS	Likely Benign	Benign
CGI's research-use -only variant classification	Pathogenic	Strongly Agree	Agree	Strongly Disagree	Strongly Disagree	Strongly Disagree
	Likely Pathogenic	Agree	Strongly Agree	Disagree	Strongly Disagree	Strongly Disagree
	VUS	Strongly Disagree	Disagree	Strongly Agree	Disagree	Strongly Disagree
	Likely Benign	Strongly Disagree	Strongly Disagree	Disagree	Strongly Agree	Agree
	Benign	Strongly Disagree	Strongly Disagree	Strongly Disagree	Agree	Strongly Agree