Leveraging Large, Clinically-Based Datasets to *Classifi* Cancer Predisposition Genes

Introduction

Candidate cancer predisposition genes continue to emerge as exome and genome sequencing have become more accessible. Once a candidate gene is identified, it can take years before there is sufficient evidence to confidently establish gene-disease validity (GDV). This is especially true for rare causes of inherited cancer predisposition. Ambry's Classifi® program powers this process by pairing an ongoing assessment of evidence with active efforts to fill gaps in the evidence.

This white paper briefly introduces Ambry's Classifi program (Figure 1) and provides two examples of how it has impacted Ambry's oncology test menu. The case examples – *RPS20* and *CTNNA1* – demonstrate how Classifi transforms new evidence into clinically-relevant tests for you and your patients.

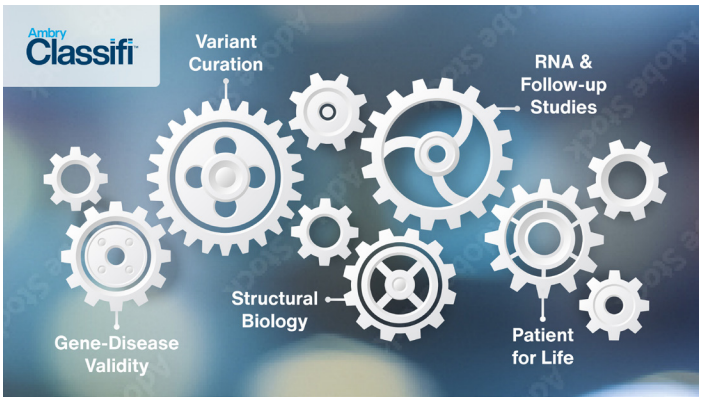


Figure 1. Ambry's Classifi program

Ambry's Classifi Program

Classifi is our commitment to finding answers for patients—operationalized. Powering everything we do, Classifi transforms raw data into meaningful insights through gene classification, variant analysis, and interpretation, leading to high-quality and clinically-useful results. Classifi's fluid design supports reanalysis and updated interpretation as new evidence emerges.

Gene-disease validity	Evaluating the strength of an association between specific genes and diseases
Variant classification	Evaluating the clinical significance of variants within a gene

Spotlight on Ambry's Large Clinical Dataset

The Classifi team leverages Ambry's comprehensive clinical database to generate case-control data that would otherwise be unavailable. Large case-control studies provide one of the most powerful lines of evidence to characterize genes associated with common diseases, such as cancer. Another distinguishing feature of Ambry's clinical database is that it's enriched for phenotypes of interest. This offers an advantage over population databases, especially when validating rare causes of hereditary cancer predisposition and refining penetrance estimates for characterized genes.

Case Example 1: Internal Data Prompts Reclassification of *RPS20* from Limited to Moderate Evidence Gene

Emerging Evidence for *RPS20* in Colorectal Cancer Predisposition

RPS20 was initially linked to colorectal cancer predisposition in 2014[1]. Using whole exome sequencing, Nieminen et al. identified a loss-of-function (LOF) variant in *RPS20* in the family shown in Figure 2[1]. The *RPS20* variant completely segregated with colorectal cancer in this family. Since this initial report, *RPS20* LOF variants have been described in three additional families[2-4].

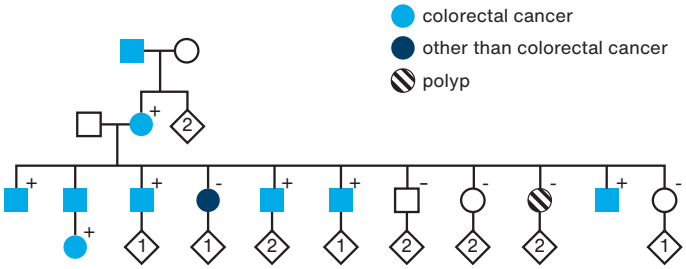


Figure 2. Pedigree for the first reported *RPS20* family

Ambry Classifies *RPS20* as a Limited Evidence Gene

RPS20 was initially classified as a limited evidence gene through Classifi. Although published evidence was compelling at that time, it was insufficient to formally characterize *RPS20* as a colorectal cancer predisposition gene (Figure 3).

The decision to include limited evidence genes in testing can be challenging. Variants in limited evidence genes cannot be classified as pathogenic or likely pathogenic. Therefore, including limited evidence genes on a panel can only increase the number of variants of uncertain significance (VUS) without contributing to diagnostic yield[5]. *RPS20* was made available via CustomNext® in 2020 and later as an optional add-on for selected tests to balance flexibility and utility.

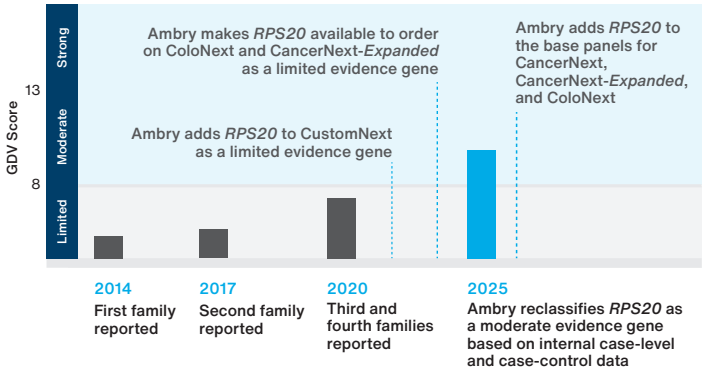


Figure 3. Ambry incorporates evidence into test design

RPS20 GDV Upgraded to Moderate Based on Internal Data

Internal data on *RPS20* has continued to strengthen the association between *RPS20* and colorectal cancer. So far, there are over 30 *RPS20* cases in Ambry's database with putative LOF variants, which is sufficient to reclassify *RPS20* as a moderate evidence gene. Further, our internal data provides much-needed context for the disease trajectory. Comparison of individuals with *RPS20* LOF variants to wild-type individuals (i.e., negative multigene panel testing) yielded odds ratios for colorectal cancer that are in the range of *MLH1*, an established high-penetrance colorectal cancer predisposition gene (Figure 4)[6]. Based on the reclassification and existing medical management guidelines for the gene, *RPS20* was added to added to the base panels of CancerNext®, CancerNext-*Expanded*®, and ColoNext®.

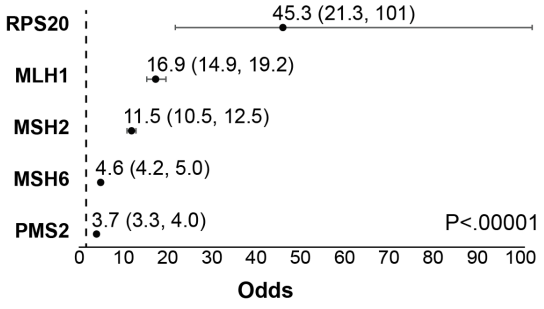


Figure 4. Magnitude of association between selected genes and colorectal cancer compared to wild-type

Case Example 2: Internal Case-Control Data Clarifies Mechanism of Disease for *CTNNA1*

CTNNA1 First Emerged as a Diffuse Gastric Cancer Gene

CTNNA1 first emerged as a diffuse gastric cancer (DGC) predisposition gene in 2013[7], when *CTNNA1* LOF variants were detected in a few families with DGC and no identifiable causative *CDH1* variants. Published literature on *CTNNA1* has come solely from phenotype-first studies, meaning families were ascertained for meeting (or suspicious for) hereditary DGC criteria. Using the highly-selected published families (n<20), penetrance estimates for DGC have been as high as 57% by age 80[8]. This evidence was sufficient to classify *CTNNA1* as a moderate evidence gene for gastric cancer (Figure 5).

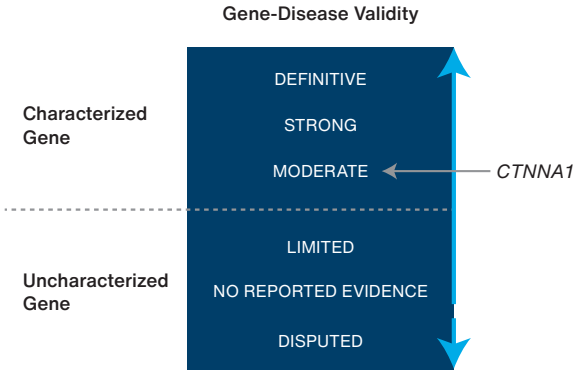


Figure 5. GDV for *CTNNA1* & gastric cancer

Questioning the Mechanism of Disease

Ongoing assessment of *CTNNA1* through Classifi revealed inconsistencies in the phenotypes of internal cases compared to published reports, which likened *CTNNA1* to *CDH1*. Ambry variant scientists were concerned that a substantial proportion of individuals with *CTNNA1* LOF variants lacked the expected phenotype. This called the mechanism of disease into question. To minimize harm to patients, *CTNNA1* LOF variants were classified as VUS until the mechanism of disease could be confirmed. There was still not enough evidence to determine which type(s) of variants were disease-causing (Figure 6).

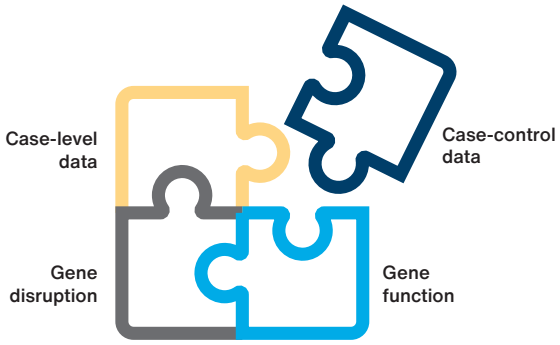
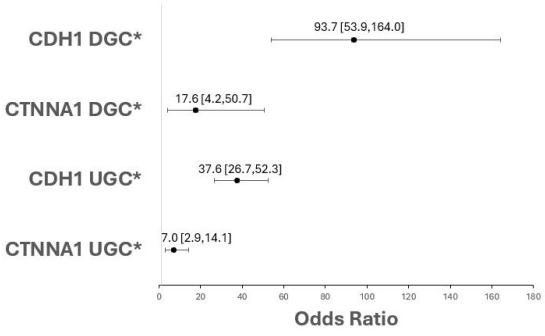


Figure 6. Case-control data completes the *CTNNA1* picture

Confirming the Impact of LOF Variants on Cancer Risk

Ambry's Classifi team continued to collect and evaluate internal data. Results from a case-control analysis between individuals with LOF variants in *CTNNA1* and wild-type controls confirmed a significant association between *CTNNA1* LOF variants and gastric cancer (Figure 6, Figure 7)[9]. Though significant, the risk of gastric cancer was substantially lower for *CTNNA1* carriers than *CDH1* carriers (Figure 7)[9]. This information confirms LOF as the mechanism of disease and enables the Classifi team to confidently report LOF variants as likely pathogenic or pathogenic. Insight into the relative penetrance of *CTNNA1* also provides useful context for clinical management decisions.



* = p-value <0.001
DGC = diffuse gastric cancer
UGC = unspecified gastric cancer

Figure 7. Cancer risks for *CTNNA1* vs *CDH1*

Conclusion

1. **Ambry's Classifi program transforms new evidence and insights into clinically relevant panel updates, in real-time**. *RPS20* and *CTNNA1* demonstrate how the fluid integration of evidence from internal and external data sources yields high-quality and clinically-meaningful results.
2. **The clinical utility of genetic testing hinges upon valid gene-disease relationships and accurate classification of variants as disease-causing**. Ambry's multidisciplinary team actively supports each of these foundational aspects of Classifi.
3. **Internal case-level and case-control data provide powerful evidence for gene disease validity and variant classification.** Ambry is committed to maintaining and leveraging this data within Classifi to independently validate published evidence and provide answers for your patients.

Gene Fact Summary

	CTNNA1	*RPS20*
Mechanism for cancer phenotype	LOF/haploinsufficiency	LOF/haploinsufficiency
GDV	Moderate	Moderate
Disease	*CTNNA1*-related diffuse gastric cancer predisposition	*RPS20*-related colorectal cancer predisposition
Penetrance	Low	High

GDV: gene-disease validity, LOF: loss-of-function

References

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