

OncoGeneDx: Hereditary Pancreatitis Panel

Panel Gene List: *CASR*, *CFTR*, *CTRC*, *PRSS1**, *SPINK1*

*Testing includes sequencing and deletion/duplication analysis for all genes except *PRSS1* (seq only),

Clinical Features:

Pancreatitis is defined as inflammation of the pancreas, with clinical features that can include pain, calcifications, necrosis, fatty replacement, fibrosis, scarring and other complications. Pancreatitis can be divided into three categories: idiopathic sporadic pancreatitis, familial pancreatitis, and hereditary pancreatitis. Idiopathic sporadic pancreatitis is a single incidence in a family of unknown etiology, and is less likely to be genetic. Familial pancreatitis is characterized by an increased incidence of pancreatitis in a family and may have a multifactorial cause including contributions from both genetics and environment. Hereditary pancreatitis refers to pancreatitis that has been proven or appears to be caused by germline variant(s) in one or more pancreatitis predisposition gene(s), such as the appearance of autosomal dominant inheritance within a family. Hereditary pancreatitis is more likely to be related to genetic risk factors; however, inheritance may be complex and the risk can be modified by a combination of genetic variants, family history, environmental factors (such as smoking and alcohol consumption), and unknown risk or protective factors. Variants in the genes included on this panel, *CASR*, *CFTR*, *CTRC*, *PRSS1*, and *SPINK1*, are implicated in hereditary pancreatitis.

Symptoms of hereditary pancreatitis can develop at any age, but many individuals will have their first pancreatic attack before the age of 20. In addition, there is a great deal of variability in the frequency and severity of pancreatic attacks, even within members of the same family. The cause of this variation is unknown. Individuals with hereditary pancreatitis have an increased risk of developing pancreatic cancer. The risk of pancreatic cancer is further increased by environmental factors such as smoking and alcohol.

***CASR*:** The *CASR* gene encodes the calcium sensing receptor protein, which plays an important role in maintaining calcium homeostasis. Pathogenic variants in *CASR* have been associated with disorders including Familial Hypocalciuric Hypercalcemia type 1 (FHH), Familial Isolated Hyperparathyroidism (FIHP), Autosomal Dominant Hypocalcemia (ADH), and Familial Isolated Hypoparathyroidism (FIH), and neonatal severe primary hyperparathyroidism (NSHPT). Variants in *CASR* may also increase the risk of pancreatitis. In the pancreas, *CASR* is expressed in both the acinar and duct cells where it may monitor and regulate calcium concentration. It is thought that *CASR* may influence channel opening and duct flushing, leading to a potential increased risk of pancreatitis when there is a failure to detect elevated calcium in the pancreas.

***CFTR*:** The *CFTR* gene makes a protein, cystic fibrosis transmembrane conductance regulator, that helps control the amount of fluid that enters the pancreas. If the protein is not formed correctly, there may not be enough fluid in the pancreas to flush out the digestive enzymes. The digestive enzymes may then become active in the pancreas and begin to digest the pancreas itself, causing pancreatitis.

Classic cystic fibrosis (CF) is inherited in an autosomal recessive manner, meaning that a person has to inherit two pathogenic *CFTR* variants (one from his or her father and one from his or her mother). Individuals who have inherited two pathogenic variants in *CFTR* may have brothers or sisters with classic cystic fibrosis, pancreatitis, or other *CFTR*-related disorders, but family members in other generations may be unaffected. Individuals who have inherited only one pathogenic variant in *CFTR* are called CF carriers. About 1 in every 28 Caucasians is a carrier of CF. *CFTR* variants are complex and have varying effects. Some *CFTR* variants are risk alleles that may increase the risk of developing pancreatitis, but are not thought to be associated with classic CF.

CTRC: The *CTRC* gene encodes chymotrypsin C, a digestive enzyme that helps regulate activation and degradation of trypsin and other digestive enzymes. Singular risk alleles in *CTRC* have been identified in both individuals with chronic pancreatitis and healthy individuals. Thus, *CTRC* variants are thought to be risk alleles rather than causative alleles for pancreatitis and may not be sufficient to cause pancreatitis on their own.

PRSS1: The *PRSS1* gene makes the “master” digestive enzyme, trypsin, which activates all of the other digestive enzymes. Most pathogenic variants in *PRSS1* create a form of trypsin that is either prematurely activated or resists degradation. When too much trypsin is active in the pancreas, digestive enzymes begin to digest the pancreas causing pancreatitis.

Pathogenic variants in *PRSS1* are typically inherited in an autosomal dominant manner, meaning that only one copy of the gene needs to have a pathogenic variant in order for an individual to have a high risk of developing pancreatitis. This means that when a parent has a pathogenic variant in the *PRSS1* gene, each of their children has a 1 in 2 or 50% chance of inheriting that variant. Individuals who have the common R122H variant have approximately an 80% chance of developing symptoms of pancreatitis over their lifetime. About 20% of individuals who have the R122H variant do not develop symptoms of hereditary pancreatitis, but each of their children still have a 50% chance of inheriting the variant.

SPINK1: Serine peptidase inhibitor Kazal type 1 (*SPINK1*) normally serves to inhibit or turn off activated trypsinogen. Twenty percent of families with pancreatitis who are negative for a *PRSS1* variant have complex genotypes including variants in *SPINK1*. Risk alleles in the *SPINK1* gene may be associated with an increased risk of pancreatitis, but are not thought to be sufficient to cause pancreatitis alone. Approximately 1% of the general population has the common *SPINK1* variant Asn34Ser and the majority do not develop pancreatitis.

Inheritance Pattern:

The inheritance pattern of hereditary pancreatitis is complex. *PRSS1*-related hereditary pancreatitis is typically inherited in an autosomal dominant manner. However, inheritance of hereditary pancreatitis associated with *CASR*, *SPINK1*, *CFTR*, and *CTRC* is multifactorial. Pancreatitis risk is complex and can be modified by a combination of variants in the genes included in this panel or other pancreatitis associated genes, as well as family history, environmental factors (such as smoking and alcohol consumption), and unknown risk or

protective factors. Consequently, the collection of risk factors can have an unpredictable influence on a person's risk for pancreatitis.

Test Methods:

Genomic DNA is extracted from the submitted specimen. For skin punch biopsies, fibroblasts are cultured and used for DNA extraction. This DNA is enriched for the complete coding regions and splice site junctions of the genes on this panel using a proprietary targeted capture system developed by GeneDx for next generation sequencing with CNV calling (NGS-CNV). For CFTR, the poly-T and poly-TG tracts in intron 9 are also captured. The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons. For *PRSS1* only sequencing is performed. Alternative sequencing or copy number detection methods are used to analyze regions with inadequate sequence or copy number data by next generation sequencing (NGS). Reported clinically significant variants are confirmed by an appropriate method. Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS) or International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

Test Sensitivity:

The clinical sensitivity of sequencing and deletion/duplication analysis of the 5 genes included in the OncoGeneDx Hereditary Pancreatitis Panel depends in part on the patient's clinical phenotype and family history. In general, the sensitivity is highest for individuals with features suggestive of a hereditary predisposition to pancreatitis as outlined above. DNA sequencing will detect nucleotide substitutions and small insertions and deletions, while NGS-CNV analysis, array CGH, or MLPA will detect exon-level deletions and duplications. These methods are expected to be greater than 99% sensitive in detecting pathogenic variants identifiable by sequencing or CNV technology.

Genetic testing using the methods applied at GeneDx is expected to be highly accurate. Normal findings do not rule out the diagnosis of a genetic disorder since some genetic abnormalities may be undetectable by this test. The methods used cannot reliably detect deletions of 20bp to 250bp in size, or insertions of 10bp to 250 bp in size. Sequencing cannot detect low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect mosaicism and cannot identify balanced chromosome aberrations. Rarely, incidental findings of large chromosomal rearrangements outside the gene of interest may be identified. Regions of certain genes have inherent sequence properties (for example: repeat, homology, or pseudogene regions, high GC content, rare polymorphisms) that yield suboptimal data, potentially impairing accuracy of the results. False negatives may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. In individuals with active or chronic hematologic neoplasms or conditions, there is a possibility

that testing may detect an acquired somatic variant, resulting in a false positive result. As the ability to detect genetic variants and naming conventions can differ among laboratories, rare false negative results may occur when no positive control is provided for testing of a specific variant identified at another laboratory. The chance of a false positive or false negative result due to laboratory errors incurred during any phase of testing cannot be completely excluded. Interpretations are made with the assumption that any clinical information provided, including family relationships, are accurate. Consultation with a genetics professional is recommended for interpretation of results.

Gene	Protein	Inheritance	Disease Associations
<i>CASR</i> ¹⁻⁵	CALCIUM SENSING RECEPTOR	Multifactorial	Pancreatitis risk
		AD	Familial Hypocalciuric Hypercalcemia type 1 (FHH), Familial Isolated Hyperparathyroidism (FIHP), Autosomal Dominant Hypocalcemia (ADH), and Familial Isolated Hypoparathyroidism (FIH)
		AR, AD (Rare)	Neonatal Severe Primary Hyperparathyroidism (NSHPT)
<i>CFTR</i> ⁶⁻¹⁹	CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR	Multifactorial	Pancreatitis risk
		AR	Cystic Fibrosis (CF), Pancreatitis risk, Congenital Absence of the Vas Deferens (CAVD)
<i>CTRC</i> ^{3,20}	CHYMOTRYPSIN C	Multifactorial	Pancreatitis risk
<i>PRSS1</i> ^{3,20-23}	PROTEASE, SERINE 1	AD	Pancreatitis risk
<i>SPINK1</i> ^{20,22,24,25}	SERINE PEPTIDASE INHIBITOR, KAZAL TYPE 1	Multifactorial	Pancreatitis risk

Because of evolving and expanding phenotypes, this list of features is not exhaustive. Gene-specific risk for some of the features listed are not well-defined.

Abbreviations:

AD – Autosomal dominant
AR – Autosomal recessive

CGH – Comparative genomic hybridization
MLPA – Multiplex ligation-dependent probe amplification

References:

1. Vahe, C. *et al.* Diseases associated with calcium-sensing receptor. *Orphanet J. Rare Dis.* **12**, 19 (2017).
2. Hendy, G. N., D'Souza-Li, L., Yang, B., Canaff, L. & Cole, D. E. Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. *Hum. Mutat.* **16**, 281–296 (2000).
3. LaRusch, J., Solomon, S. & Whitcomb, D. C. Pancreatitis Overview. in *GeneReviews®* (eds. Pagon, R. A. *et al.*) (University of Washington, Seattle, 1993).
4. Murugaiyan, E. E., Premkumar, R. M. R., Radhakrishnan, L. & Vallath, B. Novel mutations in the calcium sensing receptor gene in tropical chronic pancreatitis in India. *Scand. J. Gastroenterol.* **43**, 117–121 (2008).
5. Pearce, S. H. *et al.* Calcium-sensing receptor mutations in familial hypocalciuric hypercalcaemia with recurrent pancreatitis. *Clin. Endocrinol. (Oxf.)* **45**, 675–680 (1996).
6. Moskowitz, S. M. *et al.* Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. *Genet. Med. Off. J. Am. Coll. Med. Genet.* **10**, 851–868 (2008).
7. Farrell, P. M. *et al.* Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. *J. Pediatr.* **181S**, S4-S15.e1 (2017).
8. Ren, C. L. *et al.* Cystic Fibrosis Transmembrane Conductance Regulator-Related Metabolic Syndrome and Cystic Fibrosis Screen Positive, Inconclusive Diagnosis. *J. Pediatr.* **181S**, S45-S51.e1 (2017).
9. Cystic Fibrosis Foundation *et al.* Cystic Fibrosis Foundation practice guidelines for the management of infants with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome during the first two years of life and beyond. *J. Pediatr.* **155**, S106-116 (2009).
10. Bombieri, C. *et al.* Recommendations for the classification of diseases as CFTR-related disorders. *J. Cyst. Fibros. Off. J. Eur. Cyst. Fibros. Soc.* **10 Suppl 2**, S86-102 (2011).
11. Clinical and Functional Translation of CFTR. Available at: <https://www.cftr2.org/>. (Accessed: 19th March 2018)
12. Abeliovich, D. *et al.* Screening for five mutations detects 97% of cystic fibrosis (CF) chromosomes and predicts a carrier frequency of 1:29 in the Jewish Ashkenazi population. *Am. J. Hum. Genet.* **51**, 951–956 (1992).
13. Hamosh, A. *et al.* Comparison of the clinical manifestations of cystic fibrosis in black and white patients. *J. Pediatr.* **132**, 255–259 (1998).
14. Ong, T. *et al.* Cystic Fibrosis and Congenital Absence of the Vas Deferens. in *GeneReviews®* (eds. Adam, M. P. *et al.*) (University of Washington, Seattle, 1993).
15. LaRusch, J. *et al.* Mechanisms of CFTR functional variants that impair regulated bicarbonate permeation and increase risk for pancreatitis but not for cystic fibrosis. *PLoS Genet.* **10**, e1004376 (2014).
16. Yu, J., Chen, Z., Ni, Y. & Li, Z. CFTR mutations in men with congenital bilateral absence of the vas deferens (CBAVD): a systemic review and meta-analysis. *Hum. Reprod. Oxf. Engl.* **27**, 25–35 (2012).
17. Watson, M. S. *et al.* Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet. Med. Off. J. Am. Coll. Med. Genet.* **6**, 387–391 (2004).
18. Langfelder-Schwind, E. *et al.* Molecular testing for cystic fibrosis carrier status practice guidelines: recommendations of the National Society of Genetic Counselors. *J. Genet. Couns.* **23**, 5–15 (2014).
19. Grangeia, A. *et al.* Molecular characterization of the cystic fibrosis transmembrane conductance regulator gene in congenital absence of the vas deferens. *Genet. Med. Off. J. Am. Coll. Med. Genet.* **9**, 163–172 (2007).
20. Rosendahl, J. *et al.* CFTR, SPINK1, CTSC and PRSS1 variants in chronic pancreatitis: is the role of mutated CFTR overestimated? *Gut* **62**, 582–592 (2013).
21. LaRusch, J. & Whitcomb, D. C. Genetics of pancreatitis. *Curr. Opin. Gastroenterol.* **27**, 467–474 (2011).
22. Lee, Y. J. *et al.* High incidence of PRSS1 and SPINK1 mutations in Korean children with acute recurrent and chronic pancreatitis. *J. Pediatr. Gastroenterol. Nutr.* **52**, 478–481 (2011).
23. Chen, J. M. & Ferec, C. Gene conversion-like missense mutations in the human cationic trypsinogen gene and insights into the molecular evolution of the human trypsinogen family. *Mol. Genet. Metab.* **71**, 463–469 (2000).
24. Aoun, E. *et al.* Pathways to injury in chronic pancreatitis: decoding the role of the high-risk SPINK1 N34S haplotype using meta-analysis. *PLoS One* **3**, e2003 (2008).
25. Pfützer, R. H. *et al.* SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. *Gastroenterology* **119**, 615–623 (2000).
26. LaRusch, J., Barmada, M. M., Solomon, S. & Whitcomb, D. C. Whole exome sequencing identifies multiple, complex etiologies in an idiopathic hereditary pancreatitis kindred. *JOP J. Pancreas* **13**, 258–262 (2012).
27. Whitcomb, D. C. & Lowe, M. E. Hereditary, familial and genetic disorders of the pancreas and pancreatic disorders in childhood. in *Sleisenger and Fordtran's Gastrointestinal and Liver Disease*. 931–57 (WB Saunders Company, 2010).