Supplementary Methods online

Pancreatitis families and controls

This study was approved by the university’s medical ethical review committee. All participants gave informed consent for genetic analysis. A total of 34 unrelated Caucasian families with hereditary pancreatitis were recruited from different regions of France. All families were defined as having at least three cases of recurrent acute pancreatitis or chronic pancreatitis from whom known precipitating factors such as alcohol abuse and gallstones were excluded. In addition, these families had not been found to carry any known point mutations in the \textit{PRSS1}, \textit{PRSS2}, \textit{SPINK1} and \textit{CFTR} genes after screening the coding regions by our previously established methods.\textsuperscript{1-4} 200 unrelated healthy French individuals were used as controls.

qHPLC

We did qHPLC analysis without a reference amplicon in accordance with ref 5, which gives only a rough estimation of the copy number. All five exons of the \textit{PRSS1} gene were analyzed in one patient from each of the 34 unrelated families with hereditary pancreatitis. Sequences of primers used for qHPLC analysis and PCR conditions are available upon request. See also \textit{Supplementary Fig. 1} online.

QFM-PCR

The principle of the method has been described in detail elsewhere\textsuperscript{6}. DNA samples, if possible, were extracted with the same procedure (home made salt precipitation) and quantified with picogreen fluorescence measurement. In brief, co-amplified PCR products targeting different genomic regions under quantitative conditions were separated by capillary electrophoresis with fluorescence detection. Peak heights were normalized against a reference amplicon located outside the region of interest and compared between samples. All analyses were repeated twice. Sequences of primers used for QFM-PCR analysis and PCR conditions are available upon request. Normalized ratios above 1.4 were suspected to bear more than 2 copies (wild-type). For example, the CI for the amplicons at the limits of the triplicated segment are: for \textit{MGAM} (wt(0.94-1.20)), \textit{TRY1} (wt(0.94-1.14)/TRI(1.81-2.38)), \textit{PRSS2} (wt(0.99-1.14)/TRI(1.90-2.50)) and \textit{EPHB6} (wt(0.92-1.13)).
Walking QFM-PCR

We termed “walking-PCR” our strategy for refining the limits of the rearranged sequences. We started QFM-PCR with the “screening panel” (refer to Fig. 1) that included one amplicon located in each of the 5 genes of the trypsinogen locus (PRSSI to PRSS2 genes) and amplicons in the genes adjacent in the centromeric (MGAM and TRY1 genes) and telomeric (EPHB6 gene) directions. Triplicated samples (Fig. 1) indicated that the centromeric limit was located between amplicons MGAM and TRY1 (233,052 bp gap) and the telomeric one between amplicons PRSS2 and EPHB6 (97,385 bp gap). Two rounds of new QFM-PCR amplicons were designed in these two gaps to delineate the 605kb triplicated sequences with 3kb at both ends that were unsuccessfully characterized (Fig. 1b).

FISH

FISH analysis was performed on peripheral lymphocytes on interphase cells and metaphase chromosomes using the BAC clone RP11-771O19 (California Institute of technology, Pasadena, CA) and a chromosome 7 centromere probe (D7Z1, spectrum aqua, Vysis, Inc., Downers Grove, IL) in accordance with standard protocols. For each analyzed patient, 20 metaphase and 100 interphase cells were analyzed.

References