The presence of pseudogenes has the potential to confound the interpretation of genetic testing results. The focus of this analysis was to investigate the presence of processed pseudogenes in the SMAD4 and NBN genes not present in the reference genome in patients who underwent genetic testing with a pan-cancer panel. Genetic testing for SMAD4 is performed to identify patients with Juvenile Polyposis Syndrome (JPS) and Hereditary Hemorrhagic Telangiectasia. Patients with JPS have a high risk for cancer as a result of hamartomatous polyposis in the gastrointestinal system, particularly in the colon, rectum and stomach, as well as an elevated risk for small bowel and pancreatic cancer. Individuals with mutations in the NBN gene carry an increased risk for breast cancer in women, and prostate cancer in men.

The collective evidence from MLPA, microarray, and NGS are consistent with the presence of a novel pseudogene showing high sequence homology to the exonic regions of the SMAD4 gene. This pseudogene appears to be processed because:
- All elevated probes on MLPA are completely exonic (Figure 1).
- Only exonic probes show an increase in dosage on microarray (Figure 2, left).
- Elevated amplicons on NGS LR analysis utilize primer pairs that are completely exonic (Figure 3, top).

NGS sequencing was designed to detect a 95bp deletion, reflecting the absence of intron 5 sequence in the pseudogene (Figure 4).

200 patients tested showed evidence of the putative SMAD4 pseudogene.
- 7 reported a colon cancer diagnosis, but none reported a diagnosis of JPS.
- 12 carried pathogenic mutations in genes other than SMAD4.
- There was no common ancestry among these 200 patients.

We also detected aberrant gene dosage patterns in the NBN gene for 2 patients, which is suggestive of a putative NBN pseudogene by the same criteria as SMAD4.

Microarray and NGS LR results for NBN (Figure 2, right and Figure 3, bottom) support the presence of a processed pseudogene, since only exonic regions are affected. This is similar to the evidence observed for SMAD4. Available evidence strongly supports the presence of pseudogenes in SMAD4 and NBN.

The presence of such pseudogenes has the potential to confound the interpretation of genetic testing results, as only mutations in the native gene are clinically significant.

Studies are ongoing to confirm the structure and locations of these putative pseudogenes.