

No correlation between somatostatin 2 receptor expression analyzed by RNA in-situ hybridization and real-time qRT-PCR in clinically non-functioning pituitary adenomas



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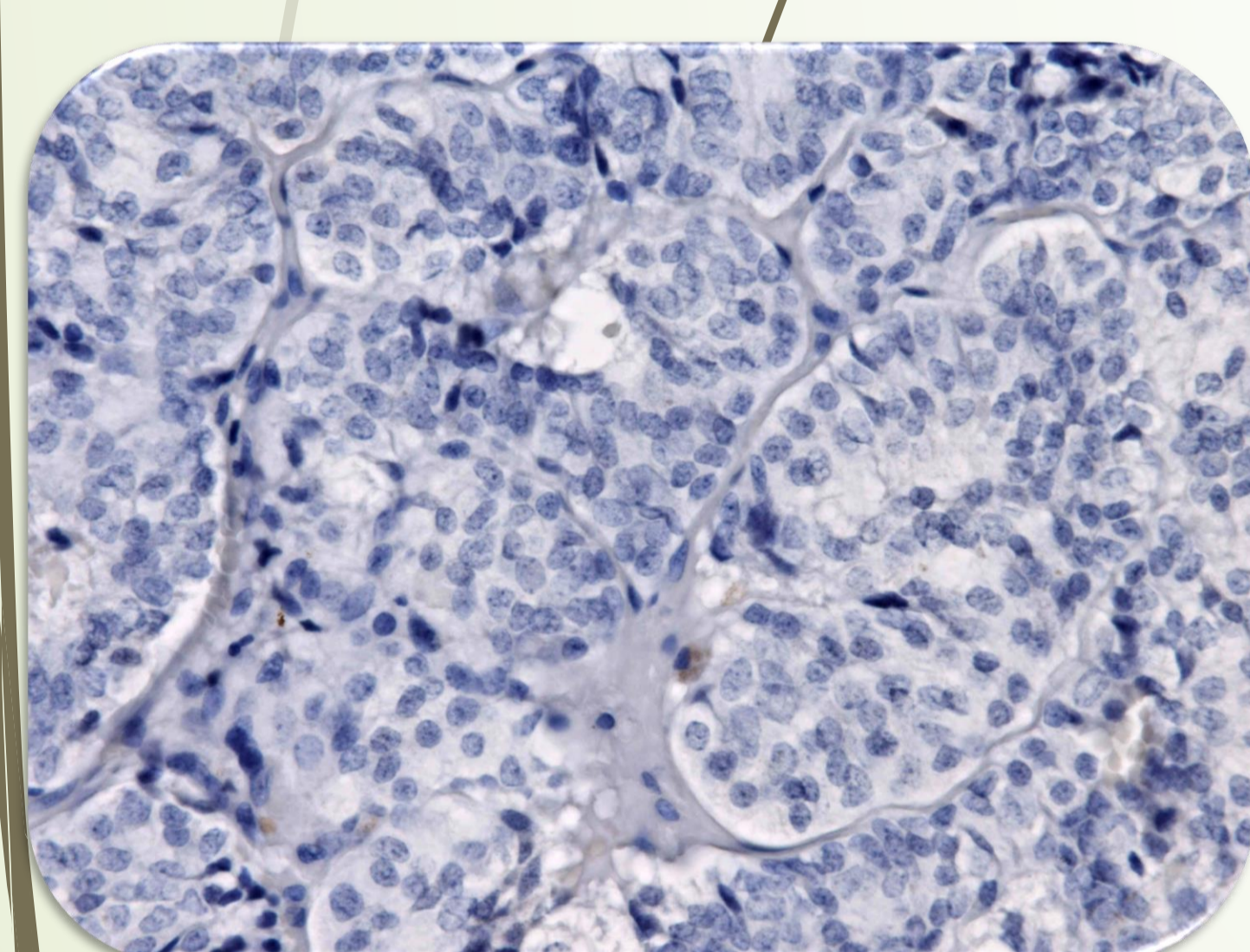
Objective

The aim of this study was to quantitatively estimate somatostatin 2 receptor (SSTR2) in clinically non-functioning pituitary adenomas (CNFAs) with use of RNA in-situ hybridization (ISH) and quantitative real-time RT-PCR and correlate the results of both methods.

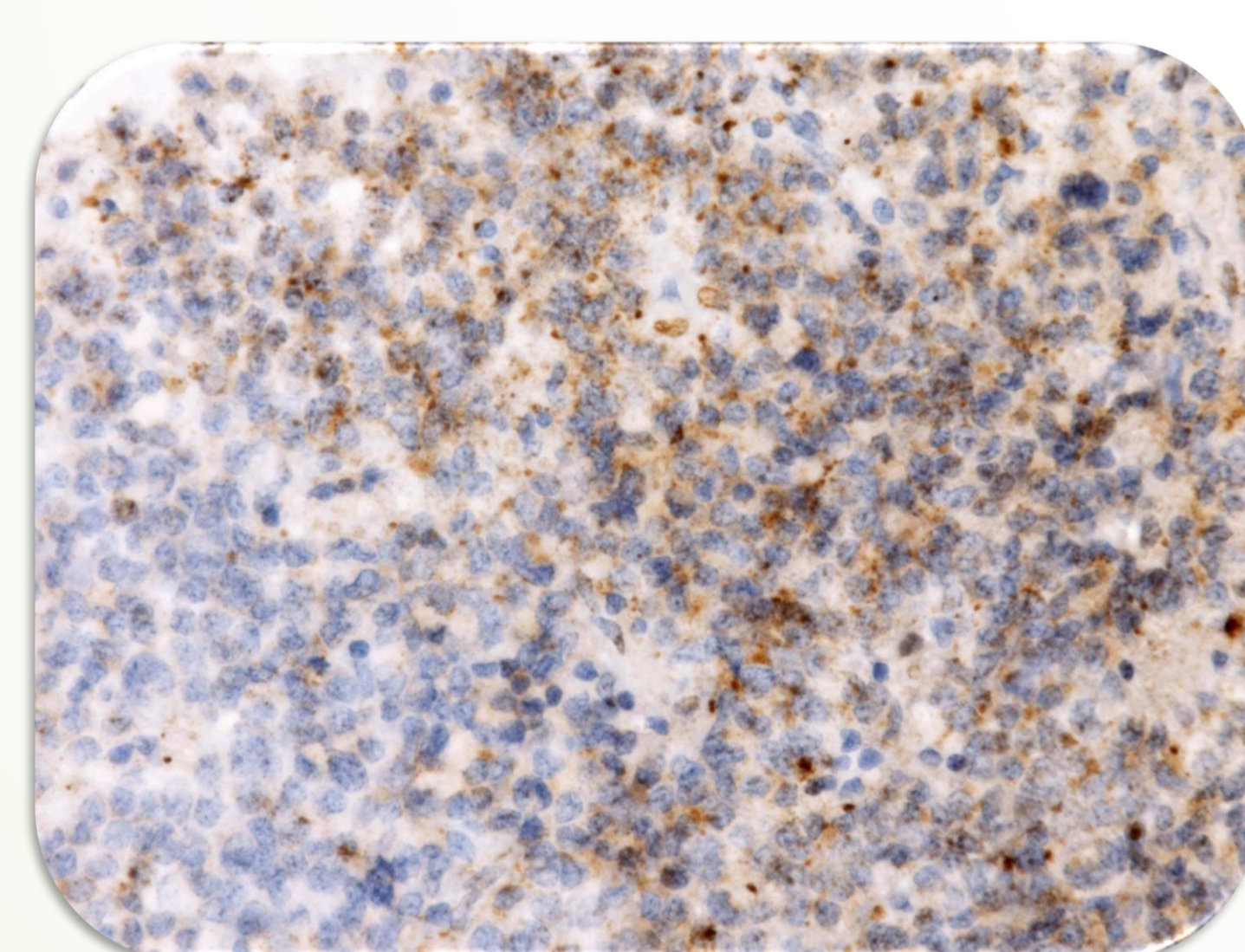
Methods

A standard histological and immunohistochemical examination was performed on the resected pituitary tumour including quantitative real-time RT-PCR as described previously. (Gabalec et al., Pituitary, 2012)

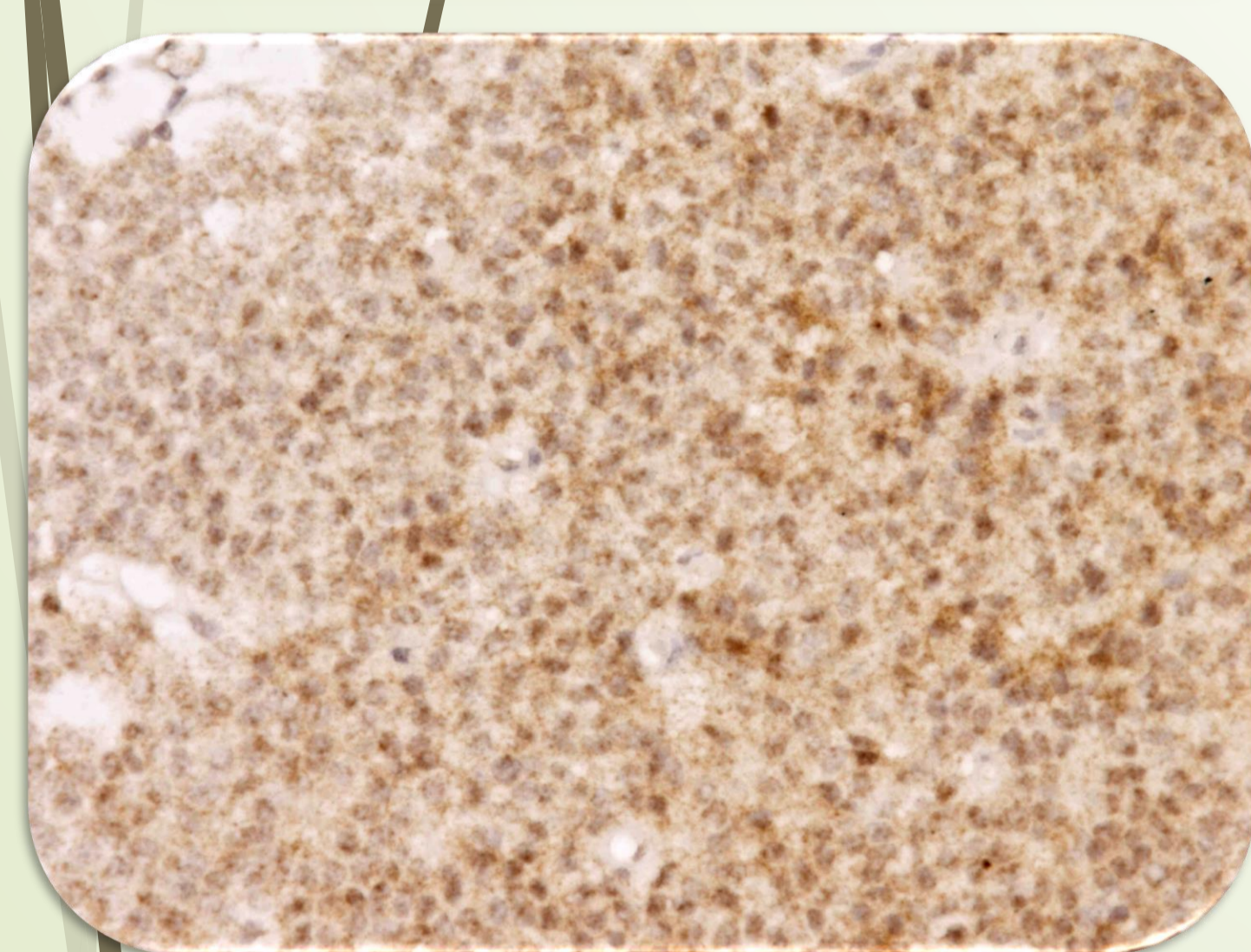
ISH for SSTR2 mRNA was performed using the RNAscope® 2.0 FFPE Assay (Advanced Cell Diagnostics, Inc., Hayward, CA, USA) on 4-µm formalin fixed and paraffin embedded tissue sections. After deparaffinization they were pretreated with heat and protease prior to hybridization with a target probe to the SSTR2. A horseradish peroxidase-based signal amplification system was then hybridized to the target probes followed by color development with 3,3'-diaminobenzidine. Control probes for the bacterial gene DapB (negative control) and for the POLR2A gene (positive control – evidence of adequate RNA) were also included in each case. The study slides (and corresponding controls) were read independently by two study pathologists (A.K. and J.L) and classified in a binary manner as either positive or negative. Positive cases had to have granular cytoplasmic and/or nuclear brown staining that was higher than the signal on the DapB negative control slide. Positive cases were subsequently semi-quantitatively assessed according to the manufacturer's scoring guideline as follows:



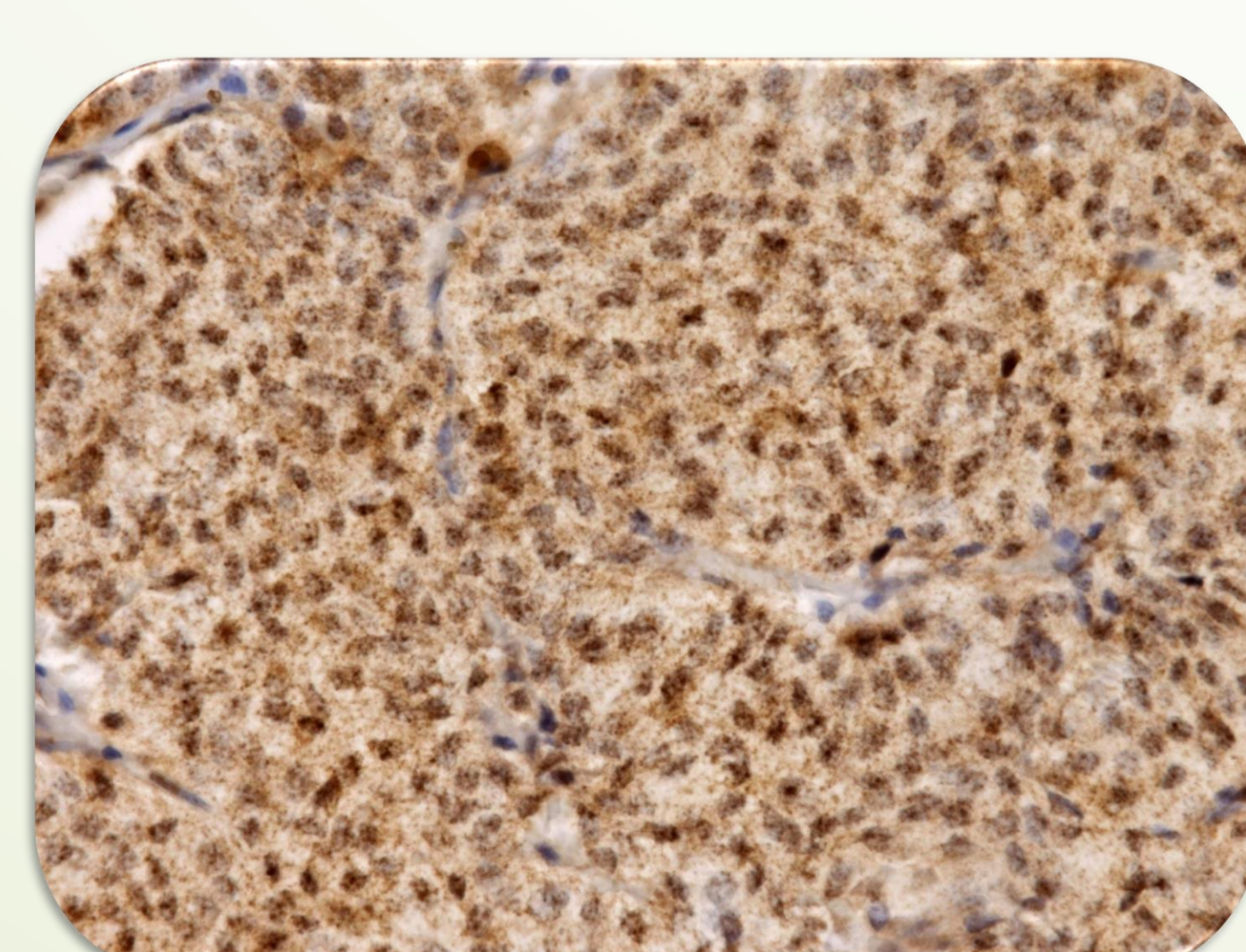
0, no staining or less than 1 dot/cell (at magnification (m.) 40x)



1+, 1-3 dots/cell (visible at m. 20-40x)



2+, 4-10 dots/cell and no or very few dot clusters (visible at m. 20-40x)



3+, more than 10 dots/cell and less than 10 % positive cells have dot clusters (visible at m. 20x)

Conclusion

Use of somatostatin analogues or dopastatins remains controversial in CNFAs. Verification of SSTR presence before use of drug treatment should be useful. Both RNA ISH and qRT-PCR have their pitfalls.

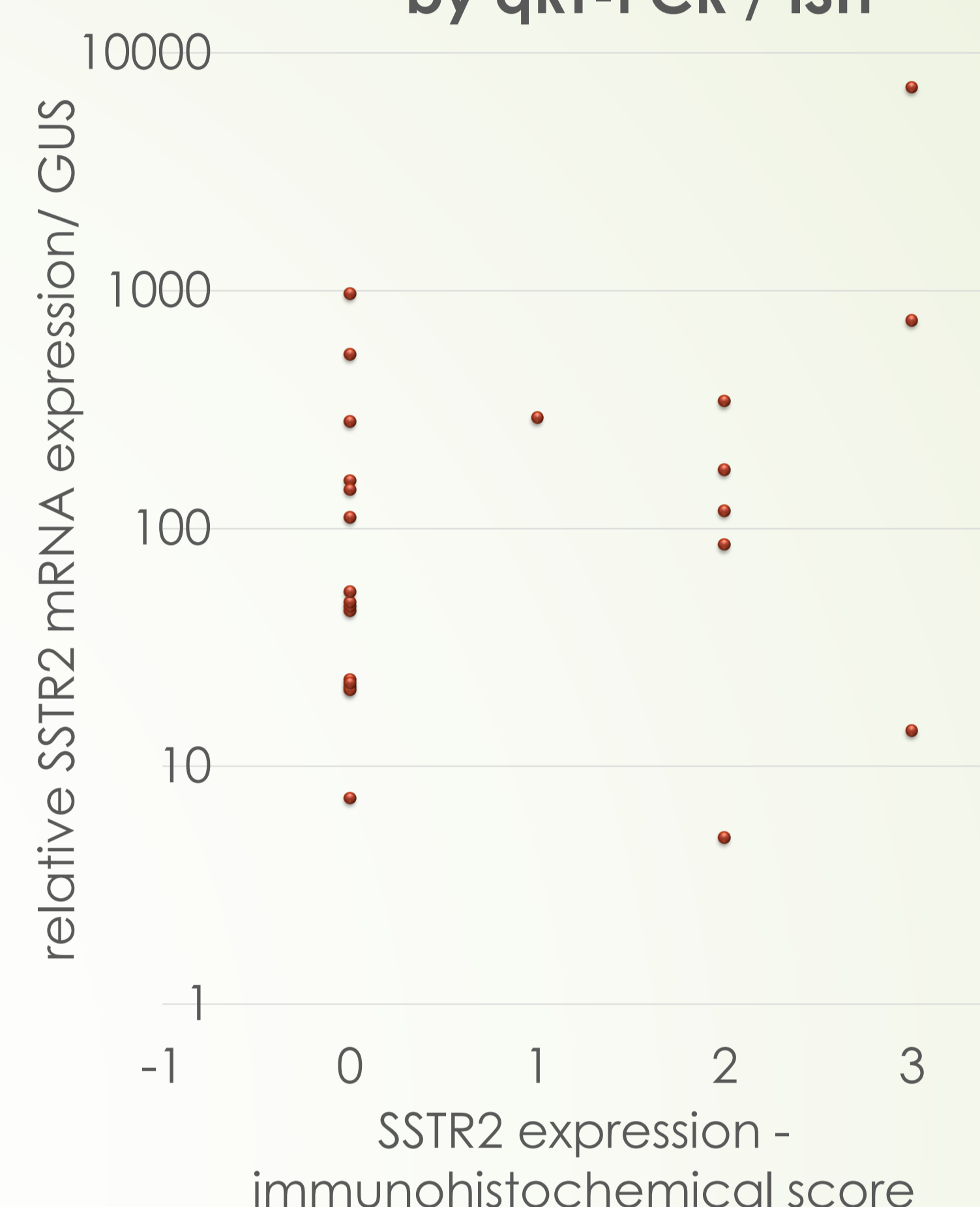
In our case:

No correlation between these methods.

RNA-ISH is definitely less sensitive and specific.

We think that results for SSTR2 expression in RNA-ISH and qRT-PCR should be interpreted carefully.

SSTR2 expression evaluated by qRT-PCR / ISH



Results

25 adenomas with positive SSTR2 mRNA expression in qRT-PCR were chosen for further evaluation with RNA-ISH to compare both methods.

SSTR2 mRNA was expressed in all adenomas from 1413-148680 copies/5µl cDNA; the median of relative quantity (after normalization to housekeeping gene GUS) for SSTR2 was 111 %.

In contrast to qRT-PCR immunostaining was positive only in 9 adenomas with the use of RNA-ISH.

Positive cases were subsequently semi-quantitatively assessed according to the manufacturer's scoring guideline as follows: 1 adenoma with 1+, 5 with 2+ and 3 with 3+. No adenoma scored 4+ although high expression of SSTR2 mRNA was present.

We did not find any correlation between data (Spearman's rank correlation coefficient 0,243).

