[1]Cristina L. Ronchi, [1]Silviu Sbiera, [1]Sonja Steinhauer, [2]Vanessa Scott-Wild,
[1]Martin Fassnacht, [1]Bruno Allolio

High JAG1 expression in adrenocortical carcinomas is associated with better prognosis

[1]Endocrine and Diabetes Unit, Dpt. of Internal Medicine I, University Hospital of Wuerzburg, Germany
[2]Institute of Pathology, University of Wuerzburg, Germany

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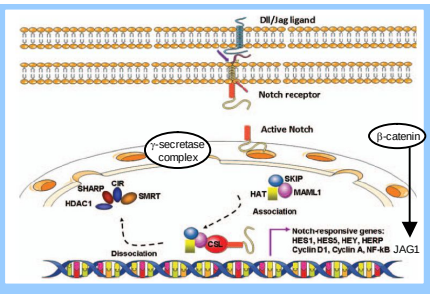
BACKGROUND: Adrenocortical tumors consist of frequent benign adenomas (ACA) and rare highly malignant carcinomas (ACC) with a still incompletely understood pathogenesis.
Dysregulation of the **Notch signalling pathway** is implicated in several cancers with oncogenic or tumor suppressor functions. Up-regulation of **JAG1,** a ligand of Notch receptor and a target gene for Notch and β-catenin pathway (***Fig 1***), has been reported to enhance cell proliferation in ACC (*Ref 1*), but no specific data on Notch1 pathway activation or JAG1 protein expression are available.

Fig. 1 *Schematic representation of the Notch signaling Pathway (Ref 2).*



METHODS:
mRNA expression: *NOTCH1*, *JAG1*, and two specific target genes of Notch pathway (*HES1* and *HEY2*) were evaluated in 49 fresh frozen samples (13 normal adrenal glands=NA, 17 ACA, and 19 ACC) by quantitative real-time PCR.
JAG1 protein expression was investigated in 203 tissues on standard paraffin slides or tissue microarrays (7 NA and 196 adrenocortical tumors, ***Tab 1***) by immunohistochemistry (monoclonal anti-rabbit Ab, Lifespan Bioscience, 1:300). Immunostaining was evaluated according to the H-score. The correlation between JAG1 expression and clinical or histopathological parameters was also investigated in ACC.

	ACA (n=25)	ACC (n=171)	P
F/M	17/8	116/55	NS
Age (yrs) - median	47	50	NS
Tumor size (cm) - median	2.8	9.7	*<0.05*
Steroid secretion (n)			NS
only cortisol/only aldosterone	12/6	30/6	
mixed	-	26	
inactive	7	23	
not known	-	86	

Tab. 1 *Clinical data in the subgroups of ACAs and ACCs.*

RESULTS:
mRNA expression: *NOTCH1* levels were similar in NA and in tumors. *JAG1* and *HES1* were slightly higher in ACC than in ACA, but *HEY2* was significantly higher in ACC (***Fig 2***).

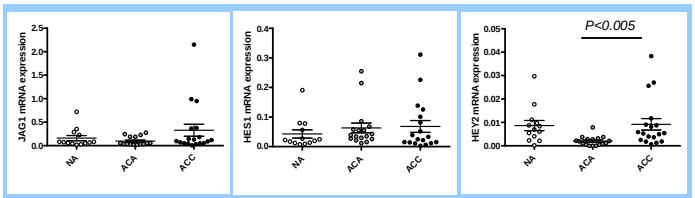


Fig. 2 *Relative mRNA expression of JAG1, HES1, and HEY2 in normal adrenal glands (NA), adenomas (ACA) and carcinomas (ACC)*

No significant correlations were observed between *NOTCH1, JAG1*, *HES1*, and *HEY1* mRNA levels and clinical or histopathological data.

JAG1 protein expression: JAG1 staining was often inhomogeneous (percentage of positive cells ranging from 15% to 90%, ***Fig 3***).

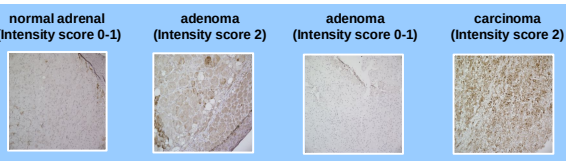


Fig. 3 *Different examples of JAG1 immunostaining in normal and neoplastic adrenal glands.*

JAG1 protein expression was absent or very low (H-score ≤1) in 72% of NA and in 61% of ACAs, but significantly higher in the ACCs (H-score >1 in 73% of ACCs, *P<0.005*, ***Fig 4A***).
In the ACC group (n=126 patients who underwent first surgery), JAG1 expression was higher in patients with early ENSAT tumor stages than in those with metastatic disease (***Fig 4B***). No other significant correlations were observed between JAG1 levels and clinical or histopathological parameters.

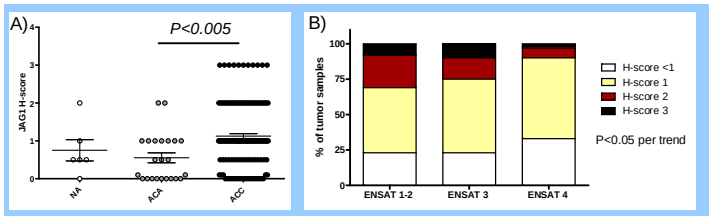


Fig. 4 *JAG1 protein expression in normal and neoplastic adrenal glands evaluated as H-score.*

Interestingly, high JAG1 expression was significantly associated with a longer overall and disease free survival (***Fig 5A*** and ***B***). At multivariate analysis including the ENSAT stage, JAG1 maintained its independent impact on overall survival (*P=0.007*, HR=0.64, 25%CI: 0.46-0.89).

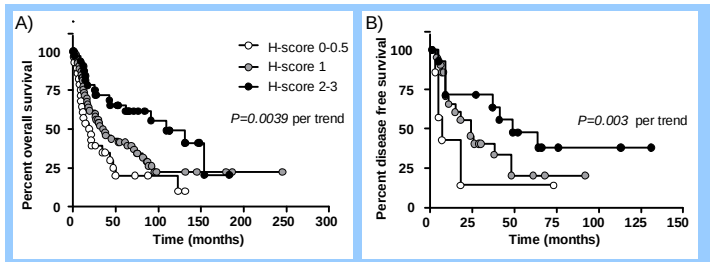


Fig. 5 *Impact of JAG1 protein expression on overall survival (A, n=126) and disease free survival (B, n=45) in patients with ACC.*

CONCLUSION:

- **Notch1 signaling pathway** activation might be involved in adrenocortical tumor progression and needs to be further investigated.
- High **JAG1 expression** is associated with a better clinical outcome in ACC and might represent a new favorable prognostic marker.

References:
-*Simon D, Giordano TJ, Hammer G. Upregulated JAG1 Enhances Cell Proliferation in Adrenocortical Carcinoma. Clin Cancer Res 2012*
- *Yin L, Velazquez OC, Liu ZJ. Notch signaling: Emerging molecular targets for cancer therapy. Biochem Pharmacol. 2010*

This study was supported by a grant of the Wilhelm Sander-Stifftung to B.A.