Advanced colorectal adenoma detection based on altered methylation signal in plasma samples

Kristi Kruusmaa[1], Marko Bitenc[1], Primož Knap[2], Marko Chersicola[2], Walter Pulverer[3], Andreas Weinhaeusel[3]

Universal Diagnostics S.L., Seville[1], Spain; Geneplanet d.o.o., Ljubljana, Slovenia[2]; Austrian Institute of Technology, Vienna, Austria[3]

BACKGROUND

- Colorectal cancer (CRC) is one of the major contributors into cancer related mortality in the world [1]
- 80% of sporadic cancers are thought to arise from pre-malignant advanced adenomas (AA) [2]
- Existing screening methods suffer from low accuracy for AA detection or have low compliance rates [3-5]
- Measuring the methylation status of tumor derived cell free DNA in plasma could enable identification of AA
- We report here a plasma targeted methylation panel performance for detection of patients with varied subtypes of AA with good accuracy

METHODS

- Whole genome bisulfite sequencing data of 3 pooled samples consisting of control samples, 3 consisting of AA (classification in Table 1) samples and 4 consisting on CRC samples, was first used for selection of differentially methylated regions in advanced colorectal neoplasia (AA+CRC)
- Candidate regions were further evaluated in the individual pre-colonoscopy plasma samples prospectively collected from 110 participants (Table 2) recruited from 18 endoscopy units in Spain, 2 in USA and 1 in UK and 1 in Australia
- Methylation-sensitive restriction enzyme qPCR method was used for targeting candidate regions in plasma cell-free DNA
- Performance of the methylation marker panel was tested by dividing the sample set into Training and Testing set (Table 2)
- Training set was used for marker ranking and building of support-vector machine (SVM) classification algorithm
- SVM-model was further applied to Testing set
- Prediction accuracies were calculated comparing panel predictions to correct clincal classifications

Advanced adenoma (AA)	
1	Adenoma with high grade dysplasia, any size
2	Adenoma with villous growth pattern (≥25%), any size
3	Adenoma ≥1.0 cm in size
4	Serrated lesion, ≥1.0 cm in size
5	Serrared lesion with dysplasia, any size

Table 1 *Categorization of AA findings*

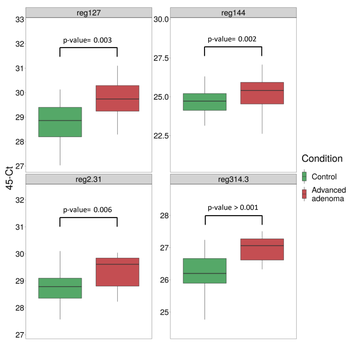

Figure 1 *Detection of methylated markers in plasma. 45 – dCt values plotted for 4 markers in Training set for control (healthy+ HP [green]) and AA samples (red). Higher 45 – dCt values correspond to higher methylation status*

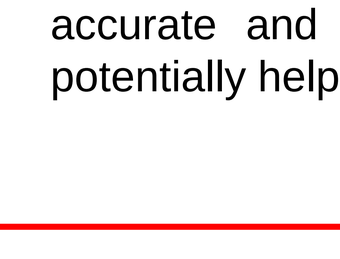

Figure 2 *ROC curve and AUC for 35-marker panel performance on Testing set that includes samples advanced adenoma, healthy, HP and GID patients*

	CNT (healthy+HP+GID)		AA	
	Training	Testing	Training	Testing
Characteristics	(n=30)	(n=40)	(n=24)	(n=16)
Age (years, average (IQR))	62 (51-76)	60 (47-82)	62 (44-78)	58 (47-58)
Gender (n (%))				
Female	15 (50%)	20 (50%)	12 (50%)	6 (37.7%)
Male	15 (50%)	20 (50%)	12 (50%)	10 (62.5%)
Healthy controls	21	18		
Gastrointestinal disease (GID)	/	10		
Hyperplastic polyps (HP)	9	12		
Adenoma characteristics				
High grade dysplasia			2	8
Size > = 10mm			22	8
Location in colon				
Proximal colon			10	8
Distal colon			12	8
Illeocecal junction			2	

Table 2 Sample set. *An initial set of samples was split into a Training set (for marker confirmation and for model building and to Testing set (for model confirmation). CNT- control, health (participant with no findings under colonoscopy), HP- (patients with histologically confirmed hyperplastic polyp findings), GID- (patients found during colonoscopy to have ulcerative colitis or IBD), AA- patients with advanced adenomas*

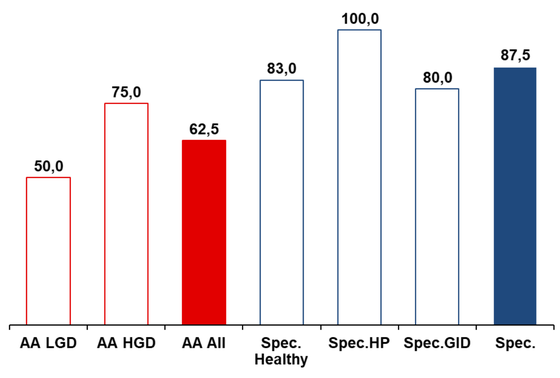

Figure 3 *35-marker panel performance. Prediction accuracy values of 35-marker SVM algorithm on Testing set, where red bar represents the overall sensitivity for AA, red outlined bars represent sensitivity for AA with low grade dysplasia (LGD) and AA with high grade dysplasia (HGD) and blue bar represents specificity (healthy+ HP+GID) and blue outlined bars represents specificity separately for GID group, HP group and Healthy group*

RESULTS

- Individual marker analysis in Training set showed that several markers had strong separation power t-test p-value > 0.01 for distinguishing advanced adenoma patients from controls (Figure 1)
- SVM-model, built on Training set based on 35 methylation markers, showed good prediction on Testing set with area under curve (AUC) of 80% (Figure 2), where sensitivity of detecting advanced adenoma patients was 62.5% (10/16) at overall specificity of 87.5% (35/40) (Figure 3)
- Adenoma sub-class analysis showed very good sensitivity for detection of patients with high grade dysplasia at 75% (6/8) while detection rate of patients with low grade adenomas with size >=10mm was 50% (4/8) (Figure 3)
- Sensitivity for detecting tubulovillous adenoma patients was higher (62.5% [6/8]) than for tubular adenoma (50% [3/6]), while singular serrated and villous adenoma cases were both correctly classified
- 80% (8/10) of patients with gastrointestinal diseases,100% (12/12) of patients with hyperplastic polyps and 83% (15/18) of control patients were correctly classified as controls (Figure 3)

CONCLUSIONS

- We show promising results for using plasma methylation marker panel for detection of patients with advanced adenomas, with especially high sensitivity for adenomas with high grade dysplasia
- This method could serve as the basis for further development of a highly accurate and minimally invasive blood-based screening test and could potentially help to guide downstream clinical evaluation of the patients

REFERENCES

[1] *Bray, F. et al. CA Cancer J Clin. 2018 Nov;68(6):394 424*
[2] *Cunningham, D. El al. Lancet 2010 375: 1030–47*
[3] *Imperiale, T. F., et al. N Engl J Med. 2014 370(14): 1287-1297*
[4] *Potter, N. T., et al. Clin Chem. 2014 60(9): 1183-1191*
[5] *Inadomi, J. M., et al. Arch Intern Med. 2012 172(7): 575-582*

CONTACT

Universal Diagnostics S.L.
kristi.kruusmaa@universaldx.com