Development and validation of a diagnostic gene expression profile test for ambiguous or difficult-to-diagnose pigmented skin lesions

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BACKGROUND

- A clinical hurdle for dermatopathology is the accurate diagnosis of melanocytic neoplasms. While histopathologic assessment is frequently sufficient, high rates of diagnostic discordance are reported.[1-4]
- Visual assessment of hematoxylin and eosin (H&E) stained lesions is inherently subjective and relies on expert interpretation and integration of a wide spectrum of architectural and cytologic features that are weighted differently based on the presumed subtype of melanocytic neoplasm and heavily influenced by the pathologists' personal experience and training.[5]
- Difficult-to-diagnose lesions are commonly sent for second opinions to expert dermatopathologists who have more experience with challenging cases; however, the nature of many lesions remains ambiguous with discordant rates of diagnoses ranging from 25-43%.[1,6]
- The development and validation of a 35-gene expression profile (35-GEP) test that accurately differentiates benign and malignant melanocytic neoplasms is described.[7]

METHODS

- Clinically diagnosed melanomas that were tested with the 31-GEP were included in this study.
- Benign samples were acquired from 8 centers.
- Benign samples were reviewed by 3-5 independent dermatopathologists and included in the study if 2/3 or 3/3 diagnoses were concordant.
- Samples were randomized into training or validation cohorts (**Table 1**).
- 76 genes were used in a discovery step. Using artificial intelligence techniques (deep learning and neural network modeling), 32 discriminant and 3 control genes were selected. Dual algorithms determine the 35-GEP test result which takes into account unique biology of lesions confined to epidermis and lesions with spitzoid features.
- To reflect the complex biology of melanocytic neoplasms, the 35-GEP test was developed to include an intermediate-risk zone.

RESULTS

Table 1. Demographic information for training and validation cohorts

	Training cohort†		Validation cohort†	
	Melanoma N=216	Benign nevi N=200	Melanoma N=230	Benign nevi N=273
Age, median (range)	66 (18-93)	47 (7-85)	67 (25-98)	48 (2-90)
Sex, % male	55	46	63	39
Breslow thickness, mm (range)	1.22 (0-10)	NA	1.23 (0.1-4.9)	NA
T stage, % (n)				
T1a	29 (56)	-	23 (48)	-
T1b	13 (25)	-	20 (42)	-
T2a	16 (31)	-	16.5 (35)	-
T2b	14 (27)	-	11 (23)	-
T3a	11.5 (23)	-	17.5 (37)	-
T3b	16 (31)	-	11 (23)	-
T4b	0.5 (1)	-	1 (2)	-
Ulceration % (n)				
Present	29.5 (64)	-	23.5 (54)	-
Absent	70.5 (152)	-	76.5 (176)	-
Not addressed	-	100 (200)	-	100 (273)
Body location, % (n)				
Abdomen/Chest	8 (18)	11 (22)	5.5 (13)	11.5 (32)
Acral	3 (6)	1 (2)	2 (5)	1 (2)
Back	27 (58)	36.5 (73)	29 (67)	41 (113)
Extremities	40 (86)	23 (46)	40 (91)	20 (54)
Head/neck	20 (43)	24 (48)	22 (50)	23 (63)
Other	2 (5)	4.5 (9)	1.5 (4)	3.5 (9)

†No statistically significant differences were observed in the training vs. validation cohorts. NA – not addressed.

Table 2. Validation of the 35-GEP in different subtypes of nevi and melanoma

	35-GEP result		
	Benign	Intermediate-risk	Malignant
Melanomas	***2***	***8***	***220***
Acral lentiginous			5
Desmoplastic			14
Lentiginous			3
Lentigo maligna		1	25
In situ	1	1	17
Nevoid			15
Nodular	1		59
Spitzoid		1	2
Superficial spreading		5	72
Not specified			8
Nevi	***248***	***10***	***15***
Blue	42	2	1
Common nevi			
Compound	15		1
Intradermal	40		1
Junctional	10		
Not specified	31		1
Deep penetrating nevus	2		
Dysplastic			
Compound	44a	4b	1c
Junctional	38d	1e	3f
Spitz	26	3	7

Dysplastic nevi had different degrees of atypia: a – mild (n=22), moderate (n=2) and severe (n=3); b – mild (n=1); c - mild (n=1); d - mild (n=21) and moderate (n=14); e – moderate (n=1); f – mild (n=1) and moderate (n=2) atypia.

Table 3. 35-GEP accuracy metrics.

	All Subtypes				Spitz Excluded			
	All ages N=503		>18 years old N=478		All ages N=464		>18 years old N=457	
	35-GEP	*95% CI*	*35-GEP*	*95% CI*	*35-GEP*	*95% CI*	*35-GEP*	*95% CI*
Sensitivity	99.1%	97.9-100	99.1%	97.9-100	99.1%	97.8-100	99.1%	97.8-100
Specificity	94.3%	91.5-97.1	96.2%	93.8-98.6	96.5%	94.2-98.9	96.4%	94.0-98.9
PPV	93.6%	90.5-96.7	96.1%	93.6-98.6	96.5%	94.1-98.9	96.5%	94.1-98.9
NPV	99.2%	98.1-100	99.1%	97.9-100	99.1%	97.9-100	99.1%	97.8-100
Intermediate-risk result	3.6%		3.8%		3.0%		3.1%	

Samples that fall in intermediate-risk zone were excluded from the calculation. PPV – positive predictive value; NPV – negative predictive value; CI – confidence interval.

CONCLUSIONS

- The 35-GEP test was developed to refine diagnoses of melanocytic neoplasms by providing clinicians with an objective ancillary tool with high accuracy.
- The test provides a modest intermediate-risk zone of 3.6%.
- The 35-GEP had a high technical success rate at 96.6%.
- Potential limitations of the test may be performance in pediatric and Spitz lesions.
- A test with these accuracy metrics could alleviate uncertainty in difficult-to-diagnose lesions leading to decreased unnecessary procedures while appropriately identifying at-risk patients.

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