Development of a novel assay to quantify circulating full-length endotrophin and validation as a risk marker of complications in type 2 diabetes.

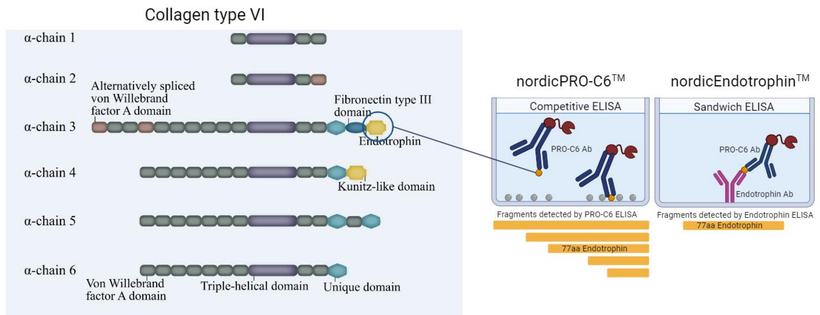
Federica Genovese[1], Elisavet Angeli[1], Solveig S. Groen[1], Alexandra L. Møller[1], Tine Hansen[2,3], Peter Rossing[2,3]

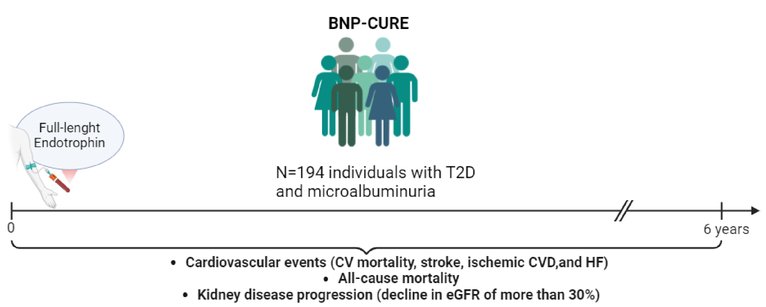
[1]Nordic Bioscience, Herlev, Denmark, [2]Steno Diabetes Center Copenhagen, Herlev, Capital Region of Denmark, Denmark; [3]Copenhagen University, Department of Clinical Medicine, Copenhagen, Hovedstaden, Denmark.

BACKGROUND

Endotrophin (ETP), a bioactive fragment of type VI collagen (COL6), has been widely evaluated as a biomarker of risk of outcome in type 2 diabetes (T2D).
The most used assay to quantify ETP is a competitive ELISA employing an antibody targeting the C-terminal of the α3 chain of COL6, encompassing part of the ETP sequence (nordicPRO-C6).
We developed a sandwich ELISA targeting full-lenght ETP (nordicEndotrophin), which employes an antibody targeting the N-terminal end of the ETP molecule and the antibody targeting the C-terminal end of COL6A3 (PRO-C6). We evaluated the potential of the two assays as risk markers of T2D adverse outcomes.

METHODS





RESULTS

Technical evaluation summary for Full-length Endotrophin	
ELISA format	Sandwich ELISA
Intended matrix (MRD)	Human serum (1+1) Human EDTA (1+1)
Incubation buffer	50 mM PBS-BTB, 8g/L NaCl + 5% liquid II, pH 7.4
Measurement range, LLOQ-ULOQ	Human serum: 5.93 – 200 (ng/ml) uncorrected Human EDTA: 6.82 – 200 (ng/ml) uncorrected
LOB	2.48 ng/ml
Mean slope (±20%)	1.27 (1.06-1.58)
Mean IC25 (±20%) **Mean IC50 (±20%)** **Mean IC75 (±20%)**	22.07 (17.66-26.48) 52.51 (42.01-63.01) 125.66 (100.53-150.79)
Intra-assay CV%	2.5% – 4.3%
Inter assay CV%	9.7% – 12.9%
Dilution recovery (REC%)	Human serum (1+1): 111.9 Human EDTA (1+1): 107.6
Spiking peptide in matrix	Human serum: Accepted Human EDTA: Accepted
Spiking matrix in matrix	Human serum: Accepted Human EDTA: Accepted
Sample freeze-thaw (mean REC% in 5 cycles)	Human serum: 94.2 Human EDTA: 94.3
Sample stability	Human serum: 48h at 36-38° (2/3) Human EDTA: 48h at 36-38° (2/3)
Interference hemoglobin (mean REC%), low/high	Human serum: 94.7/95.5 Human EDTA: 103.4/94.5
Interference lipid (mean REC%), low/high	Human serum: 96.9/93.8 Human EDTA: 100.4/100.1
Interference biotin (mean REC%), 5ng/ml/40ng/ml/100 ng/ml	Human serum: 98.9/ 104.9/103.6 Human EDTA: 104.7/109.2/111.5

Table 1: Technical evaluation of the full-length endotrophin assay

Figure 1: signaling roles of endotrophin, a degradation product of collagen type VI (α3 chain)

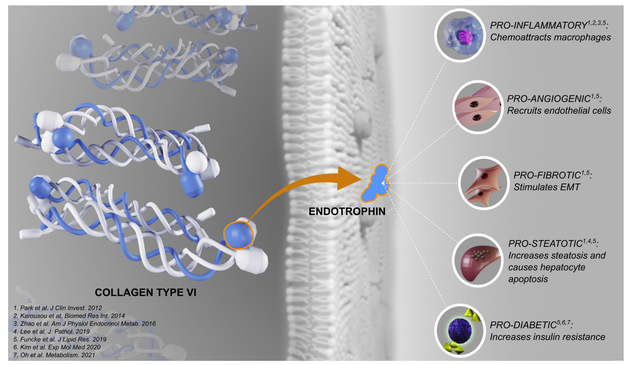


Table 2: Association of endotrophin measured with the PRO-C6 and full-length endotrophin assays and outcome in the BNP-CURE cohort

	PRO-C6		Endotrophin	
Outcome	**HR (95% CI)***	**P**	**HR (95% CI)***	**P**
All cause mortality (n=25/187)	2.99 (1.60-5.62)	0.0006	4.97 (2.11-11.70)	0.0002
CV events (n=37/187)	1.61 (0.82-3.18)	0.17	3.51 (1.69-7.27)	0.0007
CKD progression(n=40/166)	3.22 (1.75-5.92)	0.0002	2.42 (1.22-4.80)	0.01

*Adjusted for baseline age, sex, BMI, HbA1c, systolic BP, LDL cholesterol, UAER, eGFR, current smoking. Adjusted hazard ratios (HR's) are reported as doubling of baseline biomarker

CONCLUSION

The nordicEndotrophin assay is a technically robust sandwich ELISA quantifying the full-lenght endotrophin molecule in circulation. This biomarker presents a similar, or possibly higher prognostic power for complications of T2D than the competitive ELISA nordicPRO-C6, used so far to quantify endotrophin in circulation.

Contact: Federica Genovese, PhD, Director CVR at Nordic Bioscience
fge@nordicbio.com; linkedin.com/in/genovesefederica/

NORDIC BIOSCIENCE