Serum immunoassays identify Cartilage Acidic Protein 1 (CRTAC1) as a biomarker of Osteoarthritis

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BACKGROUND

Cartilage acidic protein 1(CRTAC1), a glycosylated extracellular matrix protein, is primarily produced by chondrocytes in articular cartilage. Recent studies have indicated that circulating CRTAC1 levels are associated with the severity and progression of osteoarthritis (OA). However, CRTAC1 levels were measured using proteomic platforms, highlighting the need for easily quantifiable and reliable assays.

METHODS

Two chemiluminescence immunoassays, totalCRTAC1 and neoCRTAC1, were developed to quantify total and a neo-epitope fragment of CRTAC1 levels, respectively. The totalCRTAC1 assay used a monoclonal antibody targeting the N-terminus of CRTAC1. In contrast, the neoCRTAC1 assay employed a monoclonal antibody specific to a neo-epitope derived from the serine protease high-temperature requirement A1(HtrA1). Serum samples were collected from commercially available healthy controls and patients with mild, moderate, and advanced OA (n=20 per group), all recruited from a GSK clinical unit (Cambridge, UK, Registry GSK ADM114261). The Shapiro-Wilk test was applied to assess the normality of the distribution. Data were shown as the mean with 95% confidence intervals (CI). A T-test was used to analyze differences between controls and OA patients, while One-way ANOVA was used to compare differences among controls and OA subgroups. A p-value of <0.05 was considered statistically significant and is marked with an asterisk (*).

RESULTS

Neo-epitope fragments identified in the *In vitro* cleavage products of CRTAC1 generated by HtrA1

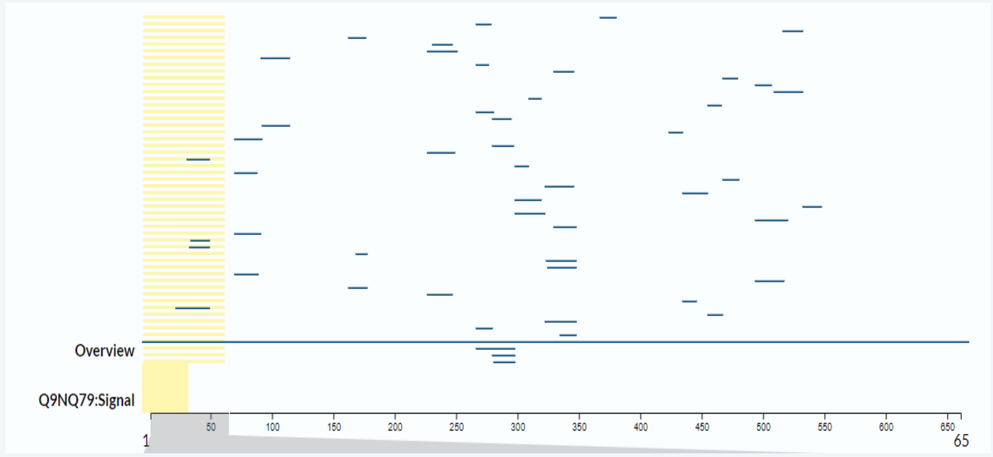


Fig1. The locations of HtrA1-derived CRTAC1 fragments with a PS M score above 10. The higher the PSM score, the more confident that the given peptide identification is correct. PSM score=10 is normally considered as a cut-off value.

Silver staining of cleaved or non-cleaved CRTAC1

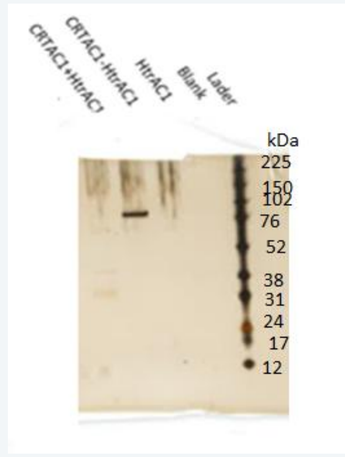
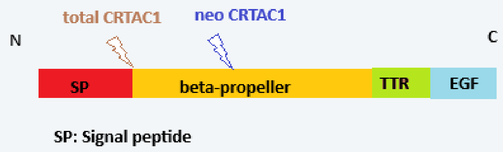


Fig. 2 Silver staining of CRTAC1 with or without CRTAC1 proteolysis.

totalCRTAC1 vs. neoCRTAC1



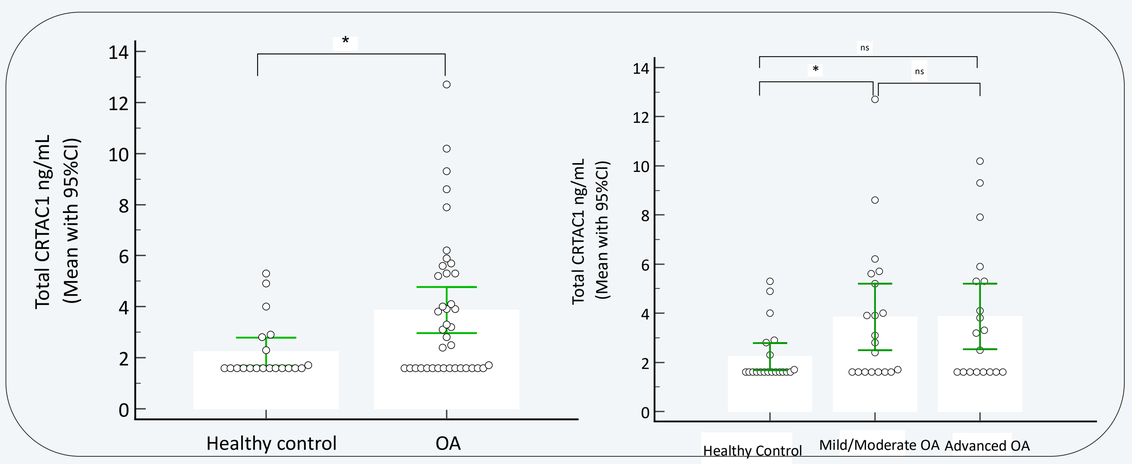
SP: Signal peptide
TTR: Transthyretin-like domain
EGF: Epidermal growth factor-like

Fig. 3. Schematic presentation of the CRTAC1 domains and the locations of total CRTAC1 and neo CRTAC1 assays within the protein.

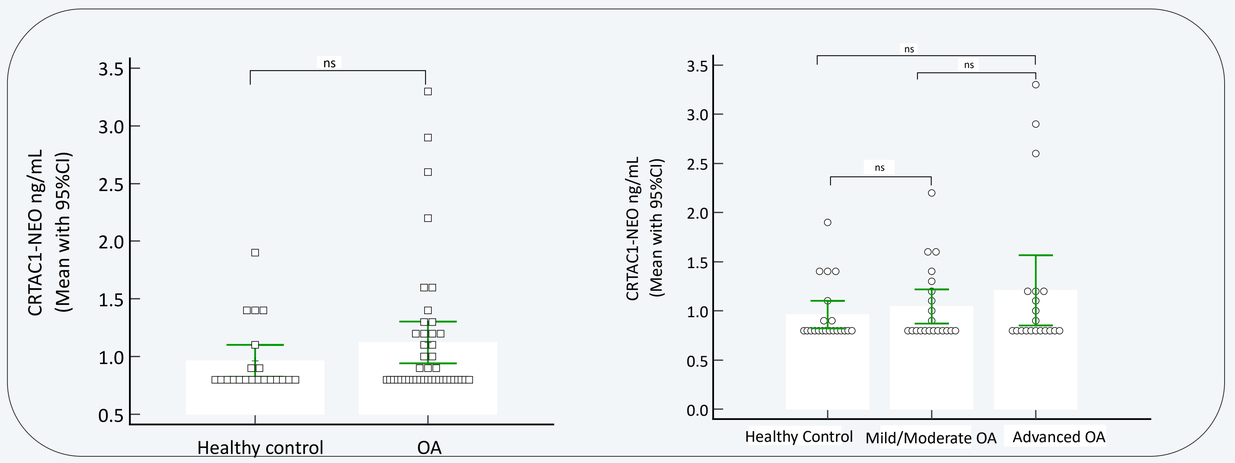
Table 1. Demographics of the OA study

	Healthy controls	Mild/Moderate OA	Advanced OA (TKR)
Subject number	20	20	20
Age, yrs *	64.1±1.3	66.5±1.6	68.7±2.5
Gender (F:M)	12:8	12:8	12:8
Samples	Serum, Urine	Serum, Urine	Serum, Urine

Note: yrs: years; * : mean±SEM; F: female; M: male



Total CRTAC1



Neo CRTAC1

Fig.4 A T-test was used to analyze differences between controls and OA patients, while One-way ANOVA was used to compare differences among controls and OA subgroups. A p-value of <0.05 was considered statistically significant and is marked with an asterisk (*)

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CONCLUSION

This study validated CRTAC1 as a promising new biomarker for OA, utilizing easily quantifiable serum immunoassays. Differences in diagnostic performance between the total and neo-epitope CRTAC1 assays were observed. This study offers valuable insights into the role of CRTAC1 and its degradation process in Osteoarthritis.

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