Carriers of the Fragile X Mental Retardation 1 (FMR1) premutation allele present with increased levels of cytokine IL-10

UNIL | Université de Lausanne
Département de génétique médicale
Centre hospitalier universitaire vaudois
SIB Swiss Institute of Bioinformatics

Diana Marek[+], Stephanie Papin[+], Kim Ellefsen, Julien Niederhauser, Nathalie Isidor, Adriana Ransijn, Lucienne Poupon, Francois Spertini, Giuseppe Pantaleo, Sven Bergmann, Jacques S.Beckmann, Sebastien Jacquemont, Goranka Tanackovic

ABSTRACT

Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) is an inherited late onset neurodegenerative disorder, caused by premutation expansions (CGG repeats) in the *FMR1* gene. Since abnormal immunological patterns are often associated with neurodegenerative disorders and implicated in their etiology, interleukins 6, 8 and 10, from 15 FMR1 premutation carriers and 20 age-matched controls, were measured in peripheral blood mononuclear cells supernatants.

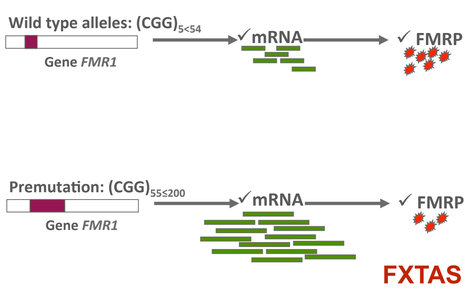
We found that interleukin-10 levels significantly and postively correlated with CGG repeats (*p*-value = 0.002) and that the mean interleukin-10 concentration was significantly higher in the premutation group (*p*-value = 0.019).

Therefore, interleukin-10 may be the first biomarker to follow the onset and progression of FXTAS.

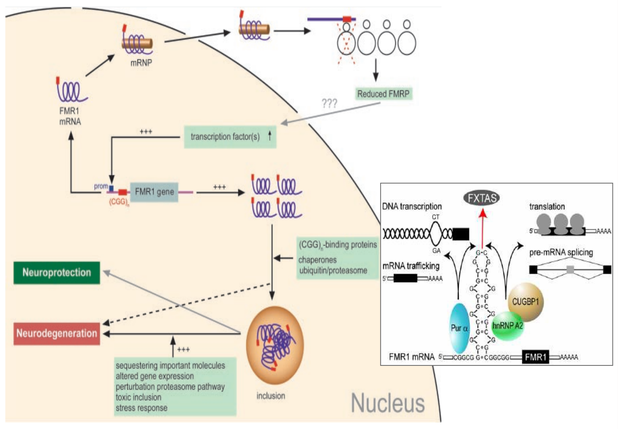
BACKGROUND

- FXTAS is characterized both by neurological and cognitive deficits.
- The severity of both the clinical and the neuropathological phenotypes correlates positively both with:
  - the extent of the CGG-repeat expansion
  - and the increase in the amount of expanded mRNA.
- The neuropathology of FXTAS includes significant white matter disease, prominent sub-cortical astroglial activation, Purkinje cell loss and the presence of intranuclear inclusions in neurons and astrocytes throughout the cortex.
- Excessive inflammatory response correlated with neuronal loss in various disorders (Parkinson, Alzheimer and Huntington's diseases)

Cell biology of FXTAS



FXTAS : RNA gain-of-function model



Adapted from Brouwer et al. (2009)

HYPOTHESIS

As neuro-inflammation plays a major role in neurodegeneration, could uncontrolled inflammation, either as an initiator or as a secondary reaction, drive chronic and progressive neurodegenerative processes in FXTAS?

DATA & METHODS

1. **Participants and study design**: 35 asymptomatic individuals (15 premutation carriers (CGG≥55) and 20 age-matched controls (CGG<55) were recruited. Several biological and clinical features were recorded in both groups (age, blood pressure, Diabetes, smoking status, treatment status, FXTAS Rating Scale (neurological assessment).
2. **Isolation of peripheral blood mononuclear cells** (PBMC) and **measurements of three cytokines** (IL-6,IL-8 and IL-10) with ELISA kits
3. **Statistical analyses** were performed:
   - To assess **the group effect** on a set of clinical feature means/medians (one-way ANOVA, Kruskal-Wallis test, generalized linear models)
   - To assess **the influence of CGG repeats on log-transformed cytokine concentrations** (linear regression)
   - To identify **differences in IL-10 mean concentrations between the premutation carrier and control groups** (one-way ANOVA)

RESULTS

A

Characteristics	Mean +/- SD or %		One-way Anova	Kruskal-Wallis test	Generalized linear model regression
	Controls	Premutation	*p*-value		
Age (years)	45.70 +/- 11.36	52.20 +/- 11.60	0.11	NA	NA
DBP (mmHg)	78.35 +/- 8.95	83.15 +/- 7.12	NA	0.10	NA
SBP (mmHg)	120.30 +/-12.74	124.36 +/- 11.74	NA	0.21	NA
HbA1c (% total Hb)	5.42 +/- 0.50	5.86 +/- 1.50	NA	0.21	NA
Smoking (%)	25	26.67	NA	NA	0.91
Drugs (%)	15	46.67	NA	NA	0.048
FXTAS score	12 +/- 11.07	23.73 +/- 10.40	0.003	NA	NA

B

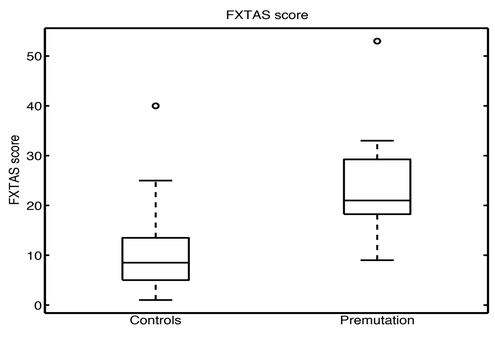


Fig 1 : Effect of the FXTAS premutation status on the various phenotypes studied

A

Log-cytokine	beta	se	*p*-value	R^2
mIL-6	0.0063	0.0037	0.0982	0.0784
mIL-8	0.0036	0.0022	0.1066	0.0748
mIL-10	0.0100	0.0030	0.0021	0.2451

B

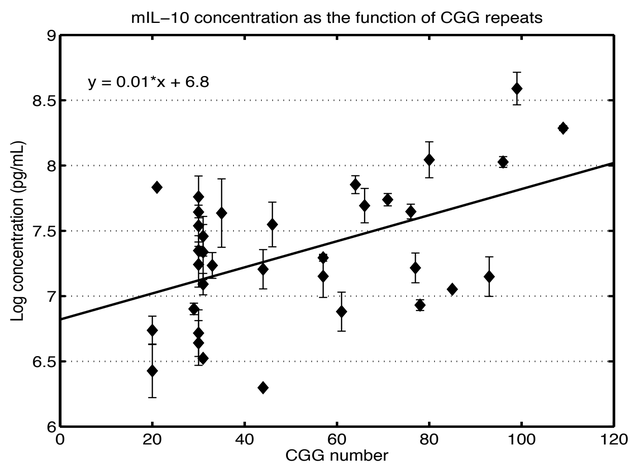


Fig 2 : Effect of CGG repeat length on the mean cytokine concentrations

A

	Mean +/- SD		One-way Anova
	Controls	Premutation	*p*-value
mIL-10 (pg/mL)	7.16 +/- 0.15	7.57 +/- 0.09	0.019

B

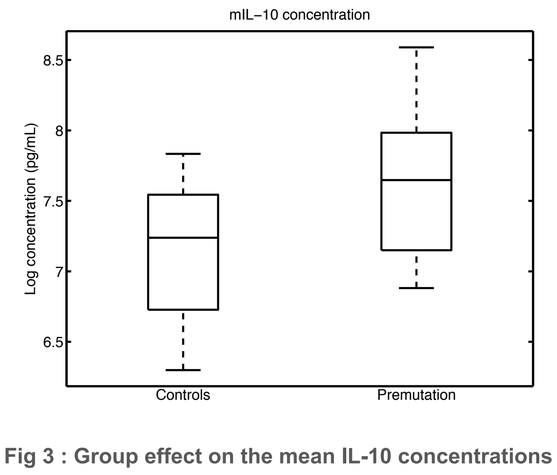


Fig 3 : Group effect on the mean IL-10 concentrations

- Neurological assessment of the participants showed that the FXTAS Rating Scale scores were low to normal. **The scores were significantly higher in carriers**, when compared to controls (*p*-value = 0.003) (Fig 1A) .The boxplot also showed differences in median between both groups (Fig 1B).
- We observed **a positive linear relationship between the concentration of the anti-inflammatory cytokine IL-10 and the number of CGG repeats** (Figs 2A and 2B). The number of CGG repeats was able to explain 24% (R^2) of the observed variations of the averaged log-transformed IL-10 levels, with a *p*-value = 0.002 (Figs 2A and 2B).
- **A significant increase of IL-10** (*p* = 0.019**) was observed in premutation carriers** when compared to controls (Figs 3A and 3B).

CONCLUSIONS & DISCUSSION

Levels of IL-10 discriminate premutation carriers from controls and may represent a valuable early biomarker. IL-10 is a key orchestrator of the immune system with potent anti-inflammatory effects. Further studies will be needed to elucidate the precise mechanism of IL-10 production. The relationship between increased concentration of IL-10 in the peripheral blood of FXTAS patients and central nervous system pathology remains unknown. Longitudinal studies including carriers affected with FXTAS will be required to study the evolution of their immune system.

Contact: diana.marek@unil.ch & www2.unil.ch/cbg