A novel cell-based assay with α3 nicotinic receptor detects antibodies selectively in autoimmune autonomic ganglionopathy

by Karagiorgou et al.,

Supplementary Tables

eTable 1. Search for the optimum conditions for α3β4-nAChR CBA

	CBA with HEK293 cells transfected with α3β4-nAChR, RIC3 and:			
Patient (numbers as in Table 1)	-	NACHO	Nicotine in the culture	NACHO (+ Nicotine in the culture)
Healthy control	Negative	Negative	Negative	Negative
5	3+	3+	3+	3+
9	1+	3+	1+	3+
10	Negative	1+	1+	2+
12	Negative	2+	1+	2+
18	Negative	Negative	Negative	1+
Total:	**4+**	**9+**	**6+**	**11+**

1+: weakly positive , 3+: strongly positive. Sera were tested at dilution 1/100, except of serum 18 which was tested at dilution 1/10.

eTable 2. Clinical information of the AAG-suspected patients, positive for α3-nAChR antibodies

								AUTONOMIC MANIFESTATION							EXTRA-AUTONOMIC MANIFESTATIONS			
Patient no.	Country	F/M	α3-nAChR Abs	Final diagnosis	Age at onset	Months with autonomic dysautonomia symptoms up to enrollment		Orthostatic hypotension without heart rate changes	Severe constipation	Bladder retention	Loss of sweating	Lightheadedness	Dry mouth and/or dry eyes		Any specific CNS findings	Any specific PNS findings	Presence of any specific cancer?	Other autoimmune diseases?
1	IT	F	RIA+/CBA+	AAG	67	180		Yes	yes	yes	yes	yes	yes		no	no	no	no
2	IT	M	RIA+/CBA+	AAG	56	48		Yes	no	no	no	no	yes		no	no	no	No
3	IT	M	RIA+/CBA+	AAG	77	108		Yes	yes	no	no	no	yes		no	no	no	yes[a]
4	IT	M	RIA+/CBA+	AAG	65	192		Yes	yes	no	no	yes	yes		no	no	no	no
5	IT	F	RIA+/CBA+	AAG	64	3		Yes	yes	no	yes	yes	yes		no	no	no	no
6	GR	F	RIA+/CBA+	AAG	70	10		Yes	yes	no	no	yes	yes		no	no	no	no
7	IT	F	RIA+/CBA+	AAG	59	180		Yes	yes	yes	no	yes	yes		no	no	no	no
8	GR	F	RIA+/CBA+	AAG	67	48		Yes	yes	no	yes	yes	no		no	no	no	no
9	IT	F	RIA+/CBA+	AAG	71	30		Yes	yes	yes	yes	yes	yes		no	no	no	no
10	IT	M	RIA+/CBA+	AAG	65	18		Yes	yes	yes	yes	yes	yes		no	no	no	no
11	IT	M	RIA+/CBA+	AAG	53	96		Yes	yes	Bladder incontinence	yes	no	yes		no	no	no	Hashimoto
12	IT	F	RIA+/CBA+	AAG	66	156		Yes	yes	no	yes	no	yes		no	no	no	yes[b]
13	GR	M	RIA+/CBA+	AAG	54	2		Yes	yes	yes	yes	yes	yes		no	no	no	no
14	IT	M	RIA+/CBA+	AAG	49	180		Yes	yes	no	no	yes	yes		no	no	no	no
15	IT	M	RIA+/CBA-	POTS	24	18		No	no	no	no	yes	no		no	no	no	no

16	GR	F	RIA+/CBA-	POTS	35	48		No	no	no	no	Yes	no		Yes[c]	No	no	no
17	GR	M	RIA+/CBA-	IMG*	47	-		No	yes	no	no	no	no		no	no	no	no
18	IT	M	RIA+/CBA+	AAG[#]	60	12		Yes	no	no	yes	yes	yes		no	no	no	no
19	GR	F	RIA+/CBA-	ASG*	44	12		No	no	no	yes	no	no		no	no	no	Yes[d]

*IMG: Inflammatory myenteric ganglionopathy; ASG: autoimmune sensory ganglionopathy

[#]Limited symptoms of AAG (with transient episode of hypotension)

[a]Anti-parietal gastric cells. This patient is described in ref. 22.

[b]Autoimmune thyroiditis – Sjogren

[c]Visual positive disturbances in the form of palinopsia, flickering lights and blurred vision - PET showed focal hypometabolism in visual cortex bilaterally and right temporal cortex.

[d]Presence of anti-ANA, SS-A (Ro), and SS-B (La) antibodies

eTable 3. Immunoglobulin class and subclass of the CBA-positive sera[a]

Patient no. (as in Table 1)	Ig class, subclass*				
	IgG1	IgG2	IgG3	IgG4	IgM
	CBA for α3β4 nAChR				
1	+	-	-	-	-
2	+	-	-	-	-
3	+	+	+	-	-
4	+	-	-	-	+
5	+	-	-	-	-
6	+	-	-	-	-
7	+	-	-	-	-
8	+	-	+	NT	-
9	+	-	-	-	-
10	+	-	-	-	-
11	+	-	-	-	-
12	+	-	-	-	-
13	+	-	-	-	-
14	+	+	-	-	-

[a] Sera were incubated with α3β4 nAChR transfected cells at 1/10 dilution. After fixation, cells were incubated with mouse anti-human IgG1, IgG2, IgG3 or IgG4 (Invitrogen) at 1/500 dilution or IgM at 1/200 dilution. Then Alexa Fluor-488 goat anti-mouse IgG at 1/750 dilution was added as 3rd antibody.

* +: positive, -: negative, NT: not tested