



# Antiadrenergic autoimmunity in postural tachycardia syndrome

Artur Fedorowski<sup>1,2\*†</sup>, Hongliang Li<sup>3</sup>, Xichun Yu<sup>3</sup>, Kristi A. Koelsch<sup>3,4</sup>, Valerie M. Harris<sup>3,4</sup>, Campbell Liles<sup>3</sup>, Taylor A. Murphy<sup>3</sup>, Syed M.S. Quadri<sup>3,4</sup>, Robert Hal Scofield<sup>3,4</sup>, Richard Sutton<sup>5</sup>, Olle Melander<sup>1</sup>, and David C. Kem<sup>3†</sup>

<sup>1</sup>Department of Clinical Sciences, Lund University, Lund, Sweden; <sup>2</sup>Department of Cardiology, Skåne University Hospital, Inga Marie Nilssons gata 46, Malmö 20502, Sweden; <sup>3</sup>Department of Medicine, University of Oklahoma Health Sciences Center and VA Medical Center, Oklahoma City, OK, USA; <sup>4</sup>Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; and <sup>5</sup>National Heart & Lung Institute, Imperial College, London, UK

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## Aims

Postural tachycardia syndrome (POTS), a common and debilitating cardiovascular disorder, is characterized by an exaggerated heart rate increase during orthostasis and a wide spectrum of adrenergic-related symptoms. To determine the aetiology of POTS, we examined a possible pathophysiological role for autoantibodies against  $\alpha$ 1-adrenergic ( $\alpha$ 1AR) and  $\beta$ 1/2-adrenergic receptors ( $\beta$ 1/2AR).

## Methods and results

Immunoglobulin G (IgG) derived from 17 POTS patients, 7 with recurrent vasovagal syncope (VVS), and 11 normal controls was analysed for its ability to modulate activity and ligand responsiveness of  $\alpha$ 1AR and  $\beta$ 1/2AR in transfected cells and to alter contractility of isolated rat cremaster arterioles *in vitro*. Immunoglobulin G activation of  $\alpha$ 1AR and  $\beta$ 1/2AR was significantly higher in POTS compared with VVS and controls in cell-based assays. Eight, 11, and 12 of the 17 POTS patients possessed autoantibodies that activated  $\alpha$ 1AR,  $\beta$ 1AR and  $\beta$ 2AR, respectively. Pharmacological blockade suppressed IgG-induced activation of  $\alpha$ 1AR and  $\beta$ 1/2AR. Eight of 17 POTS IgG decreased the  $\alpha$ 1AR responsiveness to phenylephrine and 13 of 17 POTS IgG increased the  $\beta$ 1AR responsiveness to isoproterenol irrespective of their ability to directly activate their receptors. Postural tachycardia syndrome IgG contracted rat cremaster arterioles, which was reversed by  $\alpha$ 1AR blockade. The upright heart rate correlated with IgG-mediated  $\beta$ 1AR and  $\alpha$ 1AR activity but not with  $\beta$ 2AR activity.

## Conclusion

These data confirm a strong relationship between adrenergic autoantibodies and POTS. They support the concept that allosteric-mediated shifts in the  $\alpha$ 1AR and  $\beta$ 1AR responsiveness are important in the pathophysiology of postural tachycardia.

## Keywords

Autoimmunity • Postural tachycardia syndrome • Vasovagal syncope • Adrenergic receptors • Allosteric activation

## Introduction

Postural tachycardia syndrome (POTS) is a functional cardiovascular disorder characterized by excessive heart rate increase and discomfort during orthostasis, usually accompanied by a spectrum of non-specific symptoms such as sporadic syncope, orthostatic intolerance, deconditioning, headache, cognitive impairment, and

gastrointestinal dysfunction.<sup>1,2</sup> The syndrome affects predominantly young women (70–80%) within a range of 15–40 years, first being defined in 1990s as a variant of orthostatic intolerance and dysautonomic syncope, but its aetiology remains unknown.<sup>3–5</sup> Postural tachycardia syndrome is considered to be one of the most common autonomic disorders, with an estimated prevalence of 0.5 million in United States of America alone. Its onset may follow an acute

\* Corresponding author: Tel: +46 40331000; fax: +46 40336225. E-mail address: artur.fedorowski@med.lu.se

† A. Fedorowski and D.C. Kem are co-senior authors.

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### What's new?

- Serum from patients diagnosed with postural tachycardia syndrome contains circulating autoantibodies with a direct stimulatory effect on adrenergic receptors.
- The autoantibodies may also exert an allosterically mediated positive modulatory effect upon  $\beta$ 1AR and a negative modulatory effect on  $\alpha$ 1AR activity.
- These and possibly other yet-to-be identified immunoglobulins with different target epitopes might explain different constellations of symptoms in POTS.
- This concept, if validated, would provide concrete support for novel therapeutic approaches against POTS based on immunotherapy.

infection or vaccination but more commonly appears without antecedent causative events.<sup>2,6</sup> It often leads to debilitating symptoms and uncertain long-term prognosis.<sup>1</sup> A number of treatment options have been proposed in POTS but their efficacy in the more severe forms is limited and often questioned.<sup>2,7</sup> Since the pathophysiological basis for POTS is unclear, therapeutic interventions are empirical.

POTS-related syncope differs from classic vasovagal syncope (VVS), as the underlying symptoms are present constantly with occasional syncope associated with a preceding tachycardia and associated adrenergic symptoms. In contrast, VVS typically features episodic syncope and asymptomatic periods between attacks.<sup>1,4,7</sup>

Li *et al.* recently reported that activating autoantibodies (AAb) to the  $\alpha$ 1-adrenergic ( $\alpha$ 1AR) and  $\beta$ 1/2-adrenergic receptors ( $\beta$ 1/2AR) might play a pathophysiological role in POTS.<sup>8</sup> In that pilot study, the investigators used a limited number of POTS subjects ( $n = 7$ ) and controls from their institution; and added 7 additional POTS subjects and 4 controls submitted in a blinded fashion from an independent institution for a more rigorous confirmation of the discovery. This first study used a functional assay based on immunoglobulin G (IgG)-induced changes in  $\alpha$ 1AR and  $\beta$ 2AR-mediated contractility of small arterioles *in vitro*. While effective, this technique is tedious and does not lend itself to screening larger number of patients. In the present study, we planned to examine the AAb profile of a larger, well-characterized, and representative cohort of patients with POTS from an independent European institution whose selection was completely separate from the originating laboratory. We chose to include two control groups, one being an autonomic control with patients presenting with classic recurrent VVS and the other as normal healthy volunteers without a history of syncope. The study was designed to examine the effect of patient-derived IgG on the activation of adrenergic receptors in transfected cells as well as IgG impact on the adrenergic receptor responsiveness to the well-characterized orthosteric ligands such as phenylephrine and isoproterenol.

## Methods

### Study patients

SYSTEMA (Syncope Study of Unselected Population in Malmö) is an ongoing single-centre project aiming at studying syncope epidemiology,

pathophysiology, and prognosis in the general population.<sup>9</sup> Within the SYSTEMA cohort, we randomly selected 17 patients with a confirmed diagnosis, by clinical history and tilt testing, of POTS (16 females; age,  $26 \pm 9$  years), and 7 with recurrent VVS (5 females; age:  $31 \pm 9$  years). These patients were investigated at the Syncope Unit of Skåne University Hospital between February 2011 and March 2014. The POTS patients reported at least 3 months duration of characteristic symptoms such as orthostatic intolerance, tiredness, headache, and were unresponsive to conventional therapy with persistent symptoms of orthostatic intolerance during blood collection. In contrast, VVS patients were selected among those who reported recurrent syncope prior to diagnosis, and were free from symptoms between the episodes of syncope. Eleven normal controls (10 females; age,  $31 \pm 6$  years) were recruited at the study site in Sweden among healthy individuals without any history of syncope, autoimmune disease, and with a normal postural haemodynamics during active standing (i.e. pulse increase  $< 30$  bpm and absence of orthostatic hypotension).<sup>10,11</sup> All the patients and controls were recruited from the same community of Malmö or neighbouring municipalities in Skåne Region, Southern Sweden.

### Examination protocol

The head-up tilt (HUT) test for SYSTEMA patients was performed according to the Italian protocol.<sup>12</sup> The tilt table was raised at constant speed to  $70^\circ$  in  $\sim 18$  s, and had the same lowering time. The patients' haemodynamic parameters and electrocardiogram were monitored using a non-invasive beat-to-beat measurement device (Nexfin, BMEYE, Amsterdam, the Netherlands).<sup>13</sup> Study participants were instructed to fast for 2 h before the test, and they were allowed to drink water *ad libitum*. Participants were also asked to complete a questionnaire, referring to their past medical history, duration, frequency and characteristics of syncope, smoking status, and current medication. The Regional Ethical Review Board in Lund, Sweden approved the study protocol (ref no 82/2008), and all study participants gave their written informed consent including serum analyses.

### Diagnostic criteria of postural tachycardia syndrome and classic vasovagal syncope

The test was diagnostic when patients demonstrated a typical prodrome (such as palpitation, nausea, dizziness, lightheadedness, or intense perspiration) reproducing previous events experienced and/or precipitated syncope, and the haemodynamic parameters met the diagnostic criteria of POTS or VVS, respectively.<sup>4,11,14</sup> Briefly, POTS was defined as characteristic symptoms of orthostatic intolerance (lightheadedness, dizziness, and/or discomfort) with a maximal and persistent heart rate increase of  $> 30$ /min (or  $\geq 40$ /min for those aged  $\leq 19$  years) or tachycardia of  $> 120$ /min in standing position, and the absence of orthostatic hypotension, i.e. max systolic/diastolic BP drop was  $< 20/10$  mmHg on head-up tilt.<sup>2,11</sup> Vasovagal syncope was defined as a reproduction of syncope during HUT associated with a characteristic pattern of pronounced hypotension, bradycardia, or asystole.<sup>4</sup> In order to ascertain the accuracy of diagnoses, the digital test records were subsequently inspected off-line using Nexfin@PC (BMEYE, Amsterdam, the Netherlands), dedicated software provided by the manufacturer.

### Sample preparation

Blood samples for the present study were obtained post-test (i.e. after the diagnostic HUT was performed) following overnight fasting. A trained nurse performed an antecubital venipuncture in a dedicated room after 10 min rest in supine position. Serum was separated by centrifugation and stored at  $-80^\circ\text{C}$  prior to shipping of duplicate aliquots in dry ice to the laboratory in Oklahoma City. The frozen integrity of each

aliquot was confirmed upon arrival and samples were immediately placed in a  $-80^{\circ}\text{C}$  freezer prior to thawing of one aliquot for the first assay. Serum IgG was purified using the NAb Protein A/G Spin Kit (Pierce Biotechnology). Receipt and assay of these de-identified samples was approved by the University of Oklahoma Health Sciences Center Institutional Review Board.

### Cell-based $\alpha 1\text{AR}$ assay

Immunoglobulin G activation of  $\alpha 1\text{AR}$  in  $\alpha 1\text{AR-NFAT-bla CHO-K1}$  cells was assessed using the GeneBLazer FRET-based  $\beta$ -lactamase reporter assay (Invitrogen) according to manufacturer's instructions. Briefly, cells were plated in 96-well plates and incubated overnight. The individual IgG (0.1 mg/mL) samples and positive and negative controls were then added and incubated for 5 h. The  $\alpha 1\text{AR}$  blocker prazosin (10  $\mu\text{M}$ ) was used for specific blockade. The  $\beta$ -lactamase substrate CCF4-AM (LiveBLazer-FRET B/G Loading Kit, Invitrogen) was then added and incubated for 2 h. The plates were read using a fluorescence microplate reader (BioTek Synergy 2 Multi-Detection Microplate Reader). All samples were assayed in triplicate. Negative (buffer) and positive (phenylephrine) controls were included in each assay. Data were expressed as the ratio of the emissions 460/530 nm (blue/green) after subtraction of the background values.

Full dose–response curves for phenylephrine ( $10^{-10}$ – $10^{-5}$  M) were generated in three known positive POTS subjects in the absence and presence of IgG (0.1 mg/mL) to examine the allosteric effect of IgG on the  $\alpha 1\text{AR}$  orthosteric ligand phenylephrine. We then used a simplified one dosage ( $10^{-6}$  M) phenylephrine response in the absence and presence of IgG from the POTS subjects and the normal controls to examine whether an apparent change in sensitivity existed irrespective of whether a direct activation of the receptor by the IgG was present.

### Cell-based $\beta 1/2\text{AR}$ assay

Immunoglobulin G-mediated  $\beta 1\text{AR}$  and  $\beta 2\text{AR}$  activation of cAMP production in transfected CHO cells (with either  $\beta 1\text{AR}$  or  $\beta 2\text{AR}$  stimulation) was measured using the cAMP Hunter eXpress GPCR Assay kit (DiscoveRx) as described.<sup>8,15</sup> Briefly,  $\beta 1\text{AR}$ - or  $\beta 2\text{AR}$ -CHO cells were dispensed into 96-well plates and incubated overnight. The medium was then removed and assay buffer containing the cAMP antibody and serum-derived IgG (0.1 mg/mL) was added in the presence and absence of the non-selective  $\beta\text{AR}$  blocker propranolol (1  $\mu\text{M}$ ) and incubated for 30 min. The cAMP standard, negative (buffer), and positive (isoproterenol) controls were included in each assay. All samples were tested in triplicate. Following sample treatment, cAMP detection reagent and solution were added, and chemiluminescent signal was read on a TD-20/20 Luminometer (Turner BioSystems). The cAMP values generated for each assay were expressed as relative luminescence unit (RLU).

Dosage response curves for isoproterenol ( $10^{-10}$ – $10^{-6}$  M) in  $\beta 1\text{AR}$ -CHO cells in the absence and presence of IgG (0.1 mg/mL) from three positive POTS subjects were constructed to examine the allosteric effect of IgG on the  $\beta 1\text{AR}$  orthosteric ligand isoproterenol. Likewise, a simplified single dosage isoproterenol ( $10^{-6}$  M) response was examined using IgG from the POTS subjects and the normal controls to determine if an allosteric-mediated effect on the  $\beta 1\text{AR}$ -CHO cells was present independent of any direct activation from the IgG.

### Isolated cremaster arteriole assay

The vasoconstrictor effect of IgG activation of  $\alpha 1\text{AR}$  on resistance vessels was examined using an isolated rat cremaster arteriole assay as previously described.<sup>8</sup> After equilibration in the presence of propranolol

(1  $\mu\text{M}$ ) and the nitric oxide synthase inhibitor L-NAME (1  $\mu\text{M}$ ) to eliminate any  $\beta 2\text{AR}$ - and M3 muscarinic receptor-mediated vasodilation and to achieve steady-state myogenic tone, the arterioles were perfused with IgG (0.05 mg/mL) and their effects on vessel diameter were recorded. Phentolamine (10  $\mu\text{M}$ ) was used to specifically block  $\alpha 1\text{AR}$  activity. The buffer baseline diameters were normalized to 100% and subsequent IgG-induced contractility was reported as percentage of baseline. This procedure was approved by the Oklahoma University Health Sciences Center Institutional Animal Care and Use Committee.

### Statistical analyses

Data are expressed as mean  $\pm$  SD. Group comparisons were performed using Student's *t*-test for comparison of two groups, or one-way ANOVA followed by *post hoc* Tukey's test for multiple group comparison. Pearson's  $\chi^2$ -analysis was used to compare categorical variables. The positivity of bioactive autoantibodies was defined as values above the mean + 2SD or below the mean – 2SD from the control group. A linear regression analysis was performed to examine the relationship between the haemodynamic parameters resting supine plus during head-up tilt and direct IgG-mediated receptor bioactivity. Analyses were performed using IBM SPSS Statistics version 22 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at  $P < 0.05$ .

## Results

### Clinical characteristics

The demographic and clinical characteristics for each group are shown in Table 1. The mean age for the POTS patients was not significantly different from those with VVS and control subjects. All patients in the POTS group had documented persistent increase in heart rate  $> 30$  bpm vs. supine during head-up-tilt with reproduction of typical orthostatic intolerance symptoms. There was no significant difference in BP response during the first 3 min of head-up-tilt in the POTS compared with VVS and controls and there was no significant difference in catecholamine profile supine and 3-min HUT between POTS and VVS (see Supplementary material online, Table S1). The VVS group reported a longer history of syncopal symptoms ( $P = 0.017$ ), but the number of syncope episodes, proportions of characteristic precipitating symptoms, and self-reported dizziness on standing did not differ between POTS and VVS patients.

### Autoantibody activity

The activities of autoantibodies against the  $\alpha 1\text{AR}$  and  $\beta 1/2\text{AR}$  were examined using IgG (0.1 mg/mL) in cell-based bioassays. These data are shown in Figure 1. Among the 17 patients with POTS, 8, 11, and 12 showed direct activation of  $\alpha 1\text{AR}$  (47%),  $\beta 1\text{AR}$  (65%), and  $\beta 2\text{AR}$  (71%), respectively (Figure 1A–C). Based on these direct assays, 6 of these 17 POTS patients (35%) harboured both  $\alpha 1\text{AR}$ - and  $\beta 1\text{AR}$ -AAb (Table 2). None of these autoantibodies were found in the patients with VVS or healthy controls. The mean activity values of  $\alpha 1\text{AR}$ ,  $\beta 1\text{AR}$  and  $\beta 2\text{AR}$  autoantibodies were all significantly higher in the POTS group than for both VVS and normal controls ( $\alpha 1\text{AR}$ : POTS  $0.31 \pm 0.07(\text{SD})$  vs. VVS  $0.24 \pm 0.04$  and control  $0.23 \pm 0.04$ ,  $P = 0.029$  and  $0.004$ , respectively;  $\beta 1\text{AR}$ : POTS  $1840 \pm 186$  vs. VVS  $1578 \pm 117$  and control  $1608 \pm 127$ ,  $P = 0.002$  and  $0.001$ , respectively;  $\beta 2\text{AR}$ : POTS  $1104 \pm 230$  vs. VVS  $816 \pm 73$  and control  $837 \pm 72$ ,  $P = 0.007$  and  $0.001$ , respectively) (Figure 1A–C). There was no significant difference between the latter two groups.

**Table 1** Characteristics of patients with POTS, VVS and controls

	<b>POTS (n = 17)</b>	<b>VVS (n = 7)</b>	<b>Controls (n = 11)</b>	<b>P-value<sup>a</sup> VVS/ Controls vs. POTS</b>
Age	25 ± 7	31 ± 9	31 ± 6	0.17/0.063
Sex M/F	2/15	2/5	1/10	0.55/0.98
Systolic BP supine	121 ± 14	123 ± 6	112 ± 8	0.95/0.92
Diastolic BP supine	71 ± 8	71 ± 3	70 ± 9	1.0/0.97
Heart rate supine	73 ± 10	73 ± 8	70 ± 9	1.0/0.63
Systolic BP upright 3 min	122 ± 14	125 ± 6	112 ± 8	0.78/0.069
Diastolic BP upright 3 min	80 ± 8	78 ± 5	77 ± 10	0.84/0.63
Heart rate upright 3 min	100 ± 14	82 ± 10	78 ± 9	0.004/0.0002
Heart rate max upright	111 ± 13	90 ± 10	–	0.001
Duration of symptoms (years, median, interquartile range)	3 (1–6)	10 (2–24)	–	0.017
No. of syncope (median, interquartile range)	4 (1–30)	10 (3–10)	–	0.67
Autonomic symptoms (nausea, perspiration) preceding syncope (n, %)	8 (47)	5 (71)	–	0.34
Palpitations preceding syncope (n, %)	4 (24)	3 (43)	–	0.39
Orthostatic dizziness (n, %)	13 (77)	4 (57)	–	0.23

BP, blood pressure.

<sup>a</sup>One-way ANOVA plus Tukey *post hoc* adjustment (for continuous variables) and Pearson's  $\chi^2$  test (for categorical variables) were applied.

The mean activities of  $\alpha$ 1AR,  $\beta$ 1AR, and  $\beta$ 2AR autoantibodies in 13 POTS patients were markedly suppressed by the  $\alpha$ 1AR blocker prazosin (10  $\mu$ M, from  $0.52 \pm 0.06$  to  $0.46 \pm 0.05$ ,  $P = 0.0001$ ) and  $\beta$ -blocker propranolol (1  $\mu$ M,  $\beta$ 1AR: from  $1068 \pm 233$  to  $821 \pm 71$ ,  $P = 0.0013$ ;  $\beta$ 2AR: from  $1221 \pm 164$  to  $988 \pm 37$ ,  $P = 0.0001$ ) (Figure 1D–F). No significant effect of these blockers was observed in the control group.

To examine the effect of autoantibodies on orthosteric ligand activation of  $\alpha$ 1AR and  $\beta$ 1AR, dosage response curves for phenylephrine ( $10^{-10}$ – $10^{-5}$  M) and isoproterenol ( $10^{-10}$ – $10^{-6}$  M) with and without POTS IgG (0.1 mg/mL) were constructed and compared. Immunoglobulin G from three POTS patients who were positive for  $\alpha$ 1AR and  $\beta$ 1AR autoantibodies were used for curve comparisons. In the presence of POTS IgG, the phenylephrine response curve was shifted to the right, demonstrating a decreased  $\alpha$ 1AR response (Figure 2A). In contrast, the isoproterenol response curve was significantly shifted to the left, demonstrating an enhanced  $\beta$ 1AR response with  $\beta$ 1AR autoantibodies (Figure 2B). Neither shift was observed using control IgG. We then tested the effect of IgG (0.1 mg/mL) from all 17 POTS subjects on the responses to phenylephrine (1  $\mu$ M) and isoproterenol (1  $\mu$ M). There was a significant decrease in phenylephrine-induced  $\alpha$ 1AR activation with POTS IgG ( $0.13 \pm 0.02$ ) compared with phenylephrine alone ( $0.18 \pm 0.03$ ,  $P < 0.0036$ ) or IgG from normal controls ( $0.17 \pm 0.02$ ,  $P < 0.0005$ ) (Figure 2C). The isoproterenol-stimulated  $\beta$ 1AR activation, on the other hand, was significantly increased with POTS IgG ( $1835 \pm 309$ ) compared with isoproterenol alone ( $1479 \pm 86$ ,  $P < 0.037$ ) or IgG from normal controls ( $1508 \pm 80$ ,  $P < 0.032$ ) (Figure 2D). Control IgG showed minimal modulating effects.

When each individual data was plotted, 8 of the 17 POTS patients demonstrated an inhibitory effect on phenylephrine-induced  $\alpha$ 1AR

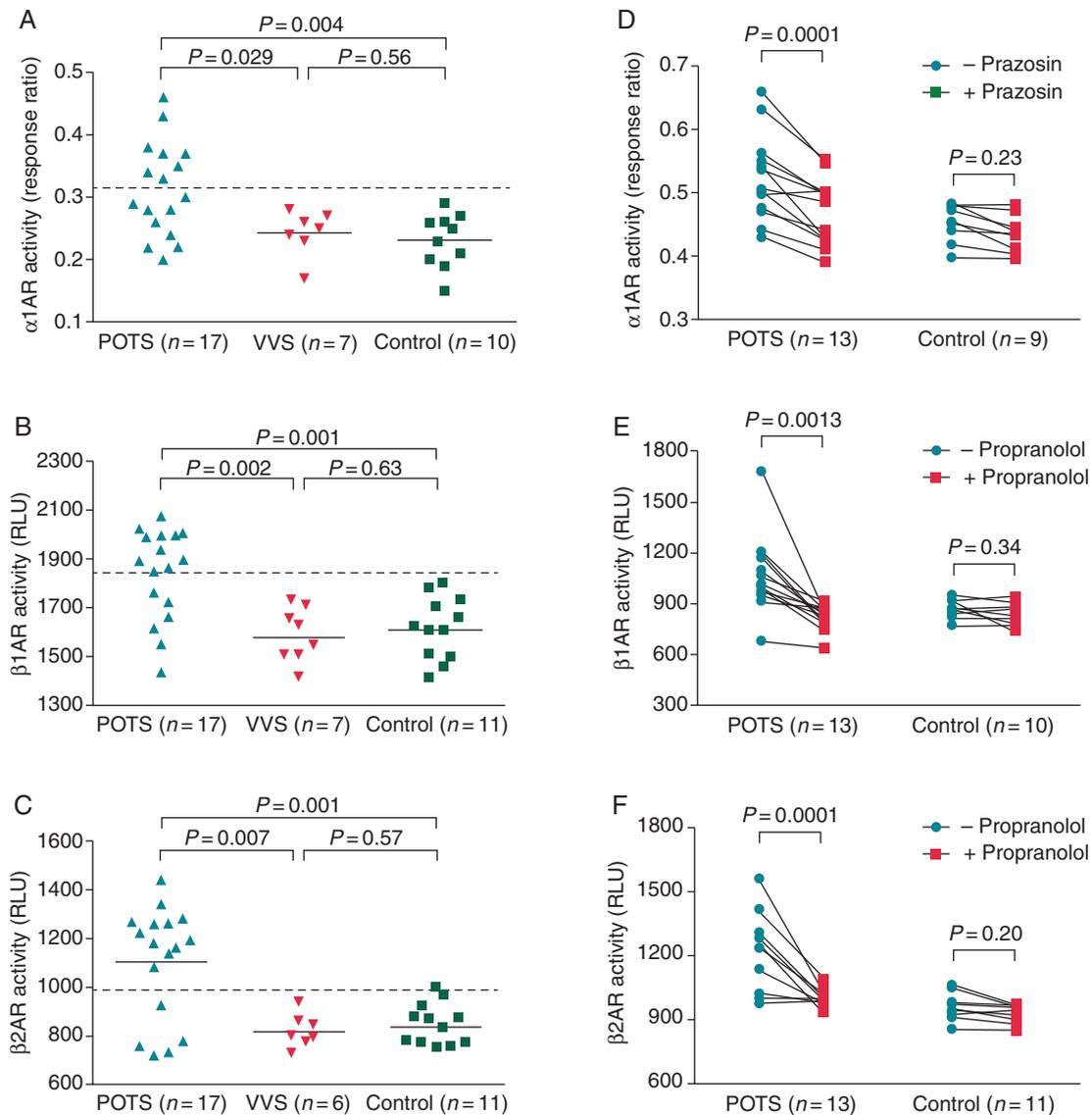
activation using a cut-off value of the mean–2SD from the control group (Figure 2E). Thirteen of the 17 POTS patients were positive for an enhanced effect on isoproterenol-induced  $\beta$ 1AR activation using a cut-off value of the mean + 2SD from the control group (Figure 2F). There was some overlap for the  $\alpha$ 1AR and considerable overlap for the  $\beta$ 1AR with the other tests (Table 2). Overall, all POTS subjects had at least one positive test.

Immunoglobulin G (0.05 mg/mL) from each subject with POTS and 8 controls were tested for  $\alpha$ 1AR autoantibody-mediated contractile activity using an isolated rat cremaster arteriole assay. A total of 13 among the 17 POTS patients demonstrated significantly increased contractility (Figure 3A). There was a significant difference in the mean contractile activity between the POTS group and control group ( $91.0 \pm 3.1\%$  vs.  $96.5 \pm 1.3\%$ ,  $P < 0.0001$ ). The control subjects failed to produce any significant vasoactivity. Six POTS subjects with positive  $\alpha$ 1AR autoantibodies and 6 controls were then selected to test the effect of specific  $\alpha$ 1AR blockade. As shown in Figure 3B, POTS IgG-induced contractility was largely returned to baseline level by addition of the  $\alpha$ 1AR blocker phentolamine (10  $\mu$ M, from  $88.9 \pm 3.0\%$  to  $96.5 \pm 2.1\%$ ,  $P = 0.0006$ ). Phentolamine had no significant impact on control IgG activity.

In the linear regression analysis, IgG-mediated  $\beta$ 1AR activity was strongly correlated with both 3 min ( $P = 0.0009$ ) and maximal upright heart rate ( $P = 0.0006$ ), whereas the latter was also correlated with  $\alpha$ 1AR activity ( $P = 0.039$ ) but not with  $\beta$ 2AR activity ( $P = 0.11$ ). Neither SBP nor DBP were correlated with adrenergic receptor stimulation.

## Discussion

We have demonstrated that serum from subjects with POTS contains circulating autoantibodies, some with a direct stimulatory



**Figure 1** Effects of serum IgG (0.1 mg/mL) from POTS and VVS patients and healthy control subjects on direct activation of  $\alpha$ 1AR,  $\beta$ 1AR, and  $\beta$ 2AR in cell-based bioassays. The  $\alpha$ 1AR activity is expressed as the FRET blue/green response ratio, and the  $\beta$ 1/2AR activity is expressed as RLU. There was a significant increase in  $\alpha$ 1AR (A),  $\beta$ 1AR (B), and  $\beta$ 2AR (C) activity in the POTS group compared with the VVS and control groups. The addition of the  $\alpha$ 1 blocker prazosin (10  $\mu$ M) or non-selective  $\beta$ -blocker propranolol (1  $\mu$ M), respectively, suppressed the  $\alpha$ 1AR (D),  $\beta$ 1AR (E), and  $\beta$ 2AR (F) activity values to control levels. The dashed line is the threshold derived from the mean activity values + 2SD of the healthy controls.

effect on adrenergic receptors. A majority also exerted an allosterically mediated positive modulatory effect upon  $\beta$ 1AR and a negative modulatory effect on  $\alpha$ 1AR activity. These and possibly other yet-to-be identified immunoglobulins with different target epitopes may exert varying biological effects in particular patients, which may explain different constellations of symptoms in POTS. It is of interest that IgG-mediated  $\beta$ 1AR and  $\alpha$ 1AR activation were correlated with the heart rate increase on standing. The relatively high correlation with the  $\beta$ 1AR autoantibody activity would appear to be clinically relevant. The lower but still significant relationship of the allosterically mediated inhibitory action of the  $\alpha$ 1AR response would

secondarily lead to the increase in heart rate by reflexly increasing circulating catecholamines.

The primary objective of this study was to determine if autoantibodies with the capacity to modify the activity of autonomic adrenergic receptors, previously reported in POTS patients in the USA,<sup>8</sup> were likewise present in a cohort of POTS subjects from a European location. A secondary goal included usage of an 'autonomic' control group characterized by subjects with recurrent VVS. This permitted us to determine if such autoantibodies were different between normal subjects and POTS; and as well between POTS and a group with recurrent VVS. These latter two groups shared somewhat broadly

**Table 2** Test positivity (direct-activating and/or ligand-modulating activity) among patients diagnosed with POTS

Patient no.	$\alpha$ 1AR Ab		$\beta$ 1AR Ab		$\beta$ 2AR Ab
	Activating	Modulating	Activating	Modulating	Activating
1		x			x
2			x	x	
3		x	x	x	x
4	x		x	x	x
5	x		x	x	
6			x	x	x
7	x		x	x	
8	x		x	x	
9				x	x
10			x	x	x
11	x			x	x
12	x	x	x	x	x
13		x			x
14		x			x
15	x	x			
16		x	x	x	x
17	x	x	x	x	x
Total	8/17	8/17	11/17	13/17	12/17

based autonomic disorders but clearly were different from each other and from normal controls. A third goal was to develop bioactivity assays that might permit larger scale testing for pathophysiological and therapeutic study.

For the primary objective, we found that antiadrenergic  $\alpha$ 1AR autoantibody activity was evident in POTS, while the VVS subjects were not different from normal subjects. In contrast to our initial publication,<sup>8</sup> nearly half of the POTS subjects had *direct* activation of the  $\alpha$ 1AR autoantibody activity. The  $\alpha$ 1AR activity in our previous study was assayed using IgG in an arteriolar assay and not the present cell-based assay. For this reason, we re-assayed IgG from each POTS patient and a representative group of the controls in the rat cremaster arteriole assay. These data showed a heterogeneous response in which the majority with increased  $\alpha$ 1AR activity from the cell-based assay also demonstrated arteriolar contractile activity. Eight subjects with no  $\alpha$ 1AR direct activity from the cell-based assay also demonstrated positive contractile activity, while three subjects with positive  $\alpha$ 1AR activity from the cell-based assay demonstrated no arteriolar contractile activity.

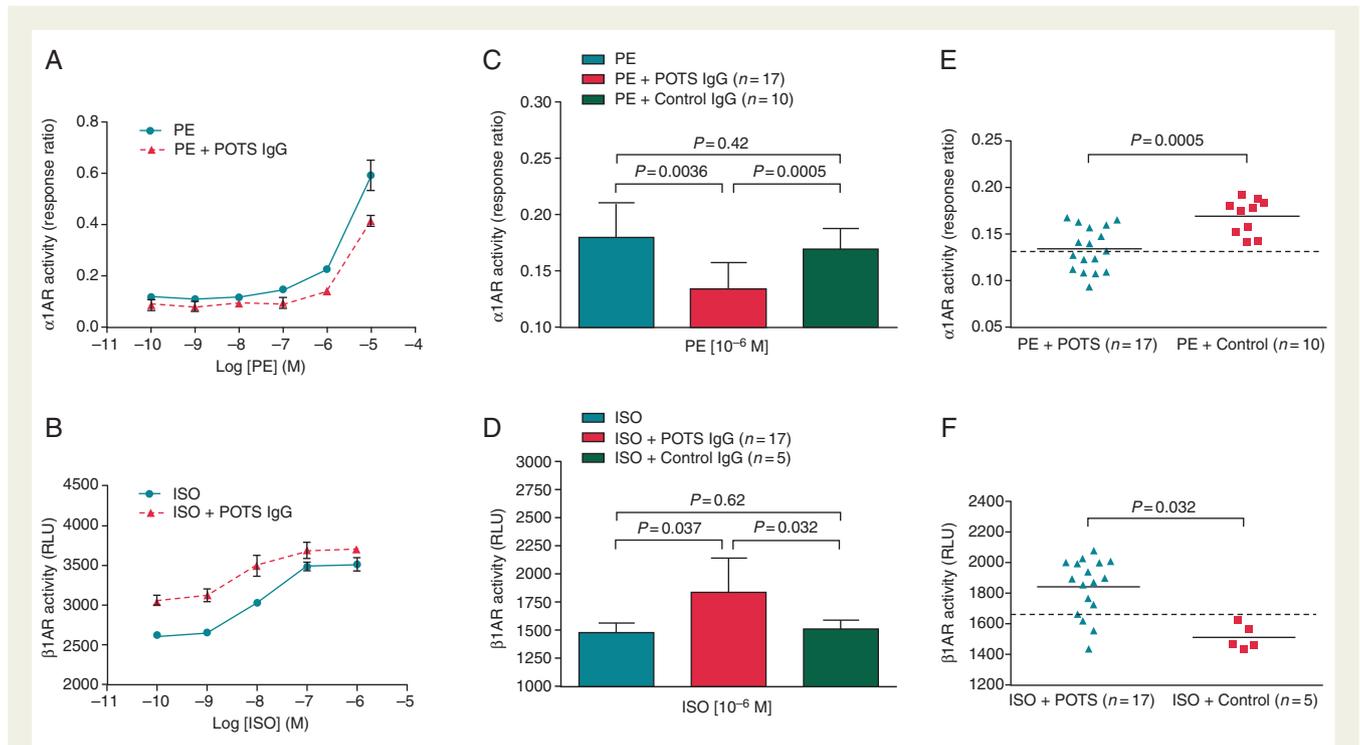
We then examined the impact of representative IgG from POTS and normal controls on phenylephrine dosage response curves. As previously reported,<sup>8</sup> these data confirmed the active  $\alpha$ 1AR autoantibodies inhibited the phenylephrine response and shifted the phenylephrine dosage response curve to the right. These autoantibodies appear to function as partial agonists and the absence of measurable direct activation of the transfected receptors *in vitro* in the absence of their normal ligand does not exclude their presence. It is possible this allosteric impact on the activity of the  $\alpha$ 1AR ligand norepinephrine represents the distinct pathophysiological feature characteristic of POTS. Consequently, identification of the antiadrenergic autoantibodies may additionally require testing for altered

dosage response curves using established receptor orthosteric agonists.

$\beta$ 1/2AR autoantibody activity was increased in POTS but not in VVS. In the present study, approximately two-thirds activated  $\beta$ 1/2AR in cell-based assays. Moreover,  $\beta$ 1AR autoantibodies enhanced the isoproterenol response and most importantly shifted the isoproterenol dosage response curves to the left, confirming our previous observations.<sup>8</sup>

Postural tachycardia syndrome continues to be a vexing dysautonomia<sup>2</sup> that has long challenged afflicted patients, their families, and caregivers. The variable presentation and seemingly associated autonomic disorders, and other non-specific symptoms including gastroparesis, migraine headaches, chest pain, chronic fatigue, and mental confusion (brain-fog) have perplexed medical personnel. Thus, it is important to define the aetiology and develop a rationale for specific interventions for controlling the underlying pathophysiology and thereby providing symptomatic relief. There has long been speculation that the predilection for POTS in younger females and occasional association with a viral/bacterial infection, vaccination or a stress event would support an autoimmune component in its aetiology.<sup>6,16,17</sup>

Recognition that circulating autoantibodies interact with G protein-coupled receptor targets and modify the autonomic system has led our group to identify AAb to the  $\beta$ 2AR and M3 muscarinic acetylcholine receptor in a group of subjects with idiopathic orthostatic hypotension.<sup>15</sup> The present study focused on the *activity* expected with these receptors; but identified a unique property of the studied IgG directed toward the  $\alpha$ 1AR which exerted *allosteric* effects characteristic of a partial agonist since its presence shifted the phenylephrine dosage response curve to the right. In contrast, the  $\beta$ 1AR effect was just the opposite and shifted the  $\beta$ 1AR agonist



**Figure 2** Effects of IgG (0.1 mg/mL) from POTS patients on PE and ISO responses in cell-based assays. IgG from 3 POTS patients with  $\alpha$ 1AR-activating activity shifted the PE dosage response curve to the right indicating an inhibitory allosteric effect (A). In contrast, IgG from 3  $\beta$ 1AR antibody-positive POTS patients shifted the ISO dosage response curve to the left compatible with a positive allosteric effect. Control IgG failed to alter these curves. When IgG from all 17 POTS patients was tested in the presence of PE (1  $\mu$ M) or isoproterenol (1  $\mu$ M), there was a significant decrease in the PE response compared with PE alone and control IgG (C) and a significant increase in the ISO response compared with ISO alone and control IgG (D). Control IgG showed minimal modulating effects. Individual data from (C) and (D) for the POTS and control subjects are shown in (E) and (F), respectively. The dashed line is the threshold derived from the control mean values—2SD for the  $\alpha$ 1AR-modulating effect or control mean values + 2SD for the  $\beta$ 1AR-modulating effect.

dosage response curve to the left. It is likely that under these conditions, the primary action of the autoantibodies is not an orthosteric direct activation or inhibition of the receptor but rather modulation of the activity and action of the ligand norepinephrine/epinephrine that normally is involved in the particular receptor transduction. These contrasting effects provide an appealing explanation for the cardiovascular effects associated with upright posture in those so afflicted.<sup>1,8</sup>

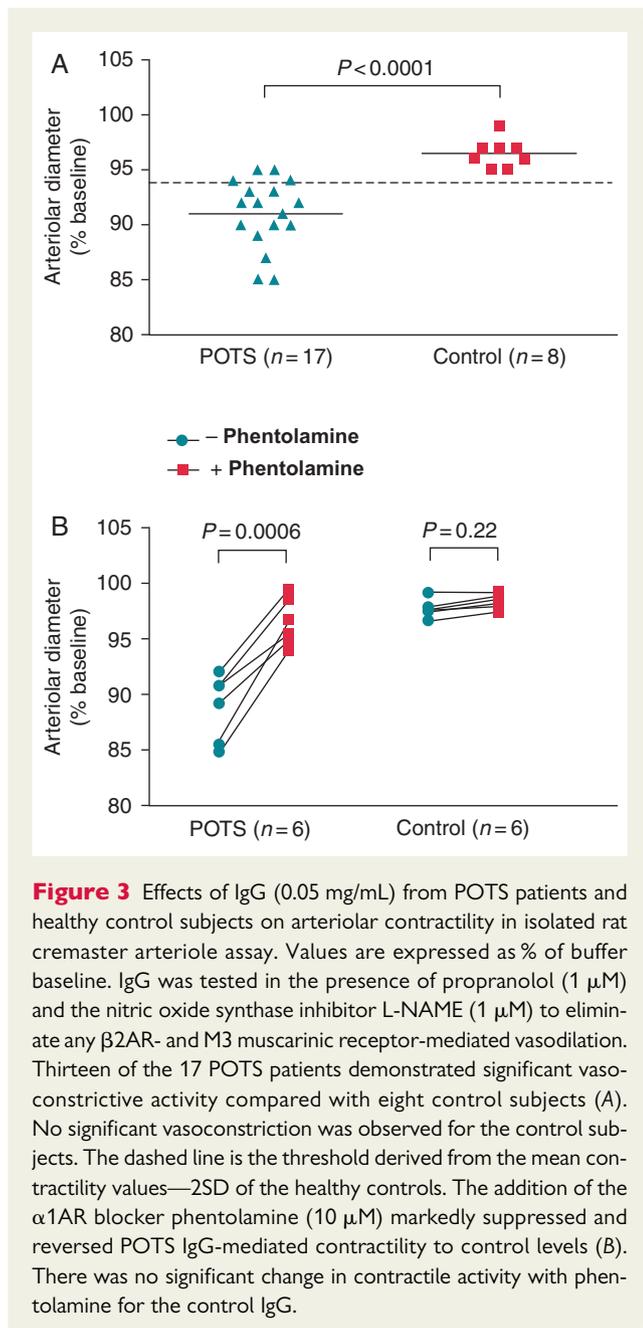
We believe that the present study helps affirm our concept that common cardiovascular dysautonomias may express a spectrum of autoantibodies, which contribute to a variety of clinical manifestations. This spectrum would certainly range from subclinical to overt expressions that may overlap with other entities such as inappropriate sinus tachycardia (IST), where circulating antibodies against cardiac  $\beta$ -receptors have been previously reported.<sup>18</sup> Both POTS and IST share abnormal sinus tachycardia tendency, and differ in regard to resting heart rate control that is usually preserved in POTS but not in IST.<sup>2</sup> Thus, some subjects who do not formally meet the diagnostic criteria of POTS may have partial presentation of such autoantibodies. From our studies, subjects with recurrent VVS and normal orthostatic tolerance would not be likely candidates for inclusion in this grouping. We have recently demonstrated an exaggerated catecholamine response in postural tachycardia among patients with syncope and dysautonomic cardiovascular response

to orthostasis.<sup>19</sup> The catecholamine surge may be seen as a compensatory mechanism to override the  $\alpha$ 1AR malfunction associated with the proposed autoimmune blockade as present in POTS patients but this explanation is unlikely in those with recurrent VVS.

Although it is likely that POTS has multiple causes, there has been a paucity of concrete evidence for other pathophysiological bases for an underlying pathophysiology to date for the vast majority of patients. It seems apparent that the high percentage of POTS subjects examined by our group present with an apparent autoimmune diathesis that supports the concept that these autoantibodies play important role in the pathophysiology of this entity.

## Strengths and limitations

This report more than doubles the number of our previous observations, and focuses on a tightly characterized group of patients and controls. We have introduced a new direct assay to measure  $\alpha$ 1AR activity, which although similar in principle to the assay for  $\beta$ 1/2AR in transfected cells was only positive in half of the POTS patients compared with the more demanding previous bioassay that measured contractility in the cremaster arterioles *in vitro*. This cremaster arteriolar bioassay is not suitable for use in large numbers of samples and the specific cell-based assays are an improvement with regard to numbers. Diagnostic criteria based on measuring the autoantibodies' impact on an allosteric-mediated receptor response to the



**Figure 3** Effects of IgG (0.05 mg/mL) from POTS patients and healthy control subjects on arteriolar contractility in isolated rat cremaster arteriole assay. Values are expressed as % of buffer baseline. IgG was tested in the presence of propranolol (1  $\mu$ M) and the nitric oxide synthase inhibitor L-NAME (1  $\mu$ M) to eliminate any  $\beta$ 2AR- and M3 muscarinic receptor-mediated vasodilation. Thirteen of the 17 POTS patients demonstrated significant vasoconstrictive activity compared with eight control subjects (A). No significant vasoconstriction was observed for the control subjects. The dashed line is the threshold derived from the mean contractility values—2SD of the healthy controls. The addition of the  $\alpha$ 1AR blocker phentolamine (10  $\mu$ M) markedly suppressed and reversed POTS IgG-mediated contractility to control levels (B). There was no significant change in contractile activity with phentolamine for the control IgG.

natural ligand may provide a more favourable estimate of autoantibody involvement in the complex pathophysiology of POTS. This study has an important limitation as the parasympathetic tonus was not assessed during HUT, and possible autoantibodies against muscarinic receptors and/or acetylcholine system were not investigated. The vagal drive may have an important implication in related POTS symptomatology.<sup>20</sup> Finally, the study groups were not strictly age matched, although the age difference was not significant.

## Conclusion

The marked presence of either or both  $\alpha$ 1AR and  $\beta$ 1/2AR autoantibodies with their potent allosteric impact on the relevant

orthosteric ligands provides an appealing framework to support an autoimmune pathophysiology of POTS. This concept, if validated, would provide concrete support for novel therapeutic approaches against POTS based on immunotherapy. These could currently include suppression of autoantibody production using immunoglobulin infusions and in the foreseeable future by development of decoy peptides that are specific for autoantibodies that target the identified receptor epitopes.

## Supplementary material

Supplementary material is available at *Europace* online.

**Conflict of interest:** none declared.

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## References

- Benarroch EE. Postural tachycardia syndrome: a heterogeneous and multifactorial disorder. *Mayo Clin Proc* 2012;**87**:1214–25.
- Sheldon RS, Grubb BP 2nd, Olshansky B, Shen WK, Calkins H, Brignole M et al. 2015 Heart Rhythm Society Expert Consensus Statement on the diagnosis and treatment of postural tachycardia syndrome, inappropriate sinus tachycardia, and vasovagal syncope. *Heart Rhythm* 2015;**12**:e41–63.
- Low PA, Opfer-Gehrking TL, Textor SC, Benarroch EE, Shen WK, Schondorf R et al. Postural tachycardia syndrome (POTS). *Neurology* 1995;**45**:S19–25.
- Moya A, Sutton R, Ammirati F, Blanc JJ, Brignole M, Dahm JB et al. Guidelines for the diagnosis and management of syncope (version 2009). *Eur Heart J* 2009;**30**:2631–71.
- Brignole M, Menozzi C, Del Rosso A, Costa S, Gaggioli G, Bottoni N et al. New classification of haemodynamics of vasovagal syncope: beyond the VASIS classification. Analysis of the pre-syncope phase of the tilt test without and with nitroglycerin challenge. Vasovagal Syncope International Study. *Europace* 2000;**2**:66–76.
- Brinth LS, Pors K, Theibel AC, Mehlsen J. Orthostatic intolerance and postural tachycardia syndrome as suspected adverse effects of vaccination against human papilloma virus. *Vaccine* 2015;**33**:2602–5.
- Fedorowski A, Melander O. Syndromes of orthostatic intolerance: a hidden danger. *J Intern Med* 2013;**273**:322–35.
- Li H, Yu X, Liles C, Khan M, Vanderlinde-Wood M, Galloway A et al. Autoimmune basis for postural tachycardia syndrome. *J Am Heart Assoc* 2014;**3**:e000755.
- Fedorowski A, Burri P, Juul-Møller S, Melander O. A dedicated investigation unit improves management of syncopal attacks (Syncope Study of Unselected Population in Malmö – SYSTEMA I). *Europace* 2010;**12**:1322–8.
- Smith JJ, Porth CM, Erickson M. Hemodynamic response to the upright posture. *J Clin Pharmacol* 1994;**34**:375–86.
- Freeman R, Wieling W, Axelrod FB, Benditt DG, Benarroch E, Biaggioni I et al. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. *Auton Neurosci* 2011;**161**:46–8.
- Bartolletti A, Alboni P, Ammirati F, Brignole M, Del Rosso A, Foglia Manzillo G et al. 'The Italian Protocol': a simplified head-up tilt testing potentiated with oral nitroglycerin to assess patients with unexplained syncope. *Europace* 2000;**2**:339–42.

13. Eeftink Schattenkerk DW, van Lieshout JJ, van den Meiracker AH, Wesseling KR, Blanc S, Wieling W *et al*. Nexfin noninvasive continuous blood pressure validated against Riva-Rocci/Korotkoff. *Am J Hypertens* 2009;**22**:378–83.
14. Parry SW, Reeve P, Lawson J, Shaw FE, Davison J, Norton M *et al*. The Newcastle protocols 2008: an update on head-up tilt table testing and the management of vasovagal syncope and related disorders. *Heart* 2009;**95**:416–20.
15. Li H, Kem DC, Reim S, Khan M, Vanderlinde-Wood M, Zillner C *et al*. Agonistic autoantibodies as vasodilators in orthostatic hypotension: a new mechanism. *Hypertension* 2012;**59**:402–8.
16. Kimpinski K, Figueroa JJ, Singer W, Sletten DM, Iodice V, Sandroni P *et al*. A prospective, 1-year follow-up study of postural tachycardia syndrome. *Mayo Clin Proc* 2012;**87**:746–52.
17. Mathias CJ, Low DA, Iodice V, Owens AP, Kirbis M, Grahame R. Postural tachycardia syndrome – current experience and concepts. *Nat Rev Neurol* 2012;**8**:22–34.
18. Chiale PA, Garro HA, Schmidberg J, Sanchez RA, Acunzo RS, Lago M *et al*. Inappropriate sinus tachycardia may be related to an immunologic disorder involving cardiac beta adrenergic receptors. *Heart Rhythm* 2006;**3**:1182–6.
19. Nilsson D, Sutton R, Tas W, Burri P, Melander O, Fedorowski A. Orthostatic changes in hemodynamics and cardiovascular biomarkers in dysautonomic patients. *PLoS ONE* 2015;**10**:e0128962.
20. Yoshida S, Tanaka H, Nakao R, Okamoto N, Kajiura M, Kanbara Y *et al*. Variant cardiovascular regulation in children with postural tachycardia syndrome. *Pediatr Int* 2014;**56**:328–35.