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# Autoimmunoreactive IgGs Against Cardiac Lipid Raft-Associated Proteins in Patients with POTS

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## Abstract

Lipid rafts are specialized plasma membrane microdomains that serve as platforms for integrating cellular signal transductions. We have recently reported that autoantibodies against cardiac membrane proteins are present in patients with postural orthostatic tachycardia syndrome (POTS). In this study, we examined the presence of autoimmunoreactive IgGs against lipid raft proteins in these patients. IgGs were purified from the sera of 10 patients and 7 normal controls. Cardiac lipid raft preparations were isolated from normal human heart tissue. The lipid raft-associated proteins were resolved by 2DE and immunoblotted against IgGs from each subject. Protein spots that reacted specifically with patient IgGs were identified by nanoLC-MS/MS. Thirty-four such protein spots, and 72 unique proteins were identified. The targets of autoimmunoreactive IgGs include proteins associated with caveolae structure, adrenergic signaling, calcium signaling, cytostructures, chaperone and energy metabolism. Multiple pathways were involved including those that regulate caveolae-mediated signaling, oxidative phosphorylation, fatty acid metabolism, protein ubiquitination, and cardiac β-adrenergic signaling. Our results suggest that cardiac lipid raft-associated proteins are targets of autoimmunoreactive IgGs from patients with POTS. Autoimmunity may play a role in the pathogenesis of POTS.

Postural orthostatic tachycardia syndrome (POTS) is a common form of orthostatic intolerance, and the etiology of the syndrome is complex and unexplained in most patients. Various causes and mechanisms have been proposed for the disease including autoimmunity and ganglionic acetylcholine receptor (AChR) antibodies[1]. We have recently reported the presence of autoantibodies against cardiac membranes in POTS patients[2]. In this study, we



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examined whether cardiac proteins in lipid raft microdomains constitute targets of autoantibodies in POTS patients.

Subcellular fractionation can reduce sample complexity for proteomic analysis and is most efficient when combined with 2-dimensional gel electrophoresis (2DE) and mass spectrometry (MS) studies[3]. Numerous studies have shown the importance of lipid raft/caveolae microdomains in the regulation of cellular signal transduction, mechanosensing, lipid metabolism, cholesterol homeostasis, and ion channel activities[4–13]. Many critical signaling proteins and their effectors are compartmentalized in lipid rafts and if they become autoimmunoreactive, normal cellular functions may be disturbed. These microdomains are richly and tightly packed with cholesterol, sphingolipids, and glycosyl-phosphatidylinositol (GPI) anchored proteins, and thus have a low buoyant density in sucrose gradient fractionations. Caveolae are particularly abundant in terminally differentiated cells such as adipocytes, squamous epithelial cells, and muscle cells[14].

## METHODS

### Study subjects and IgG isolation

This study was approved by the Mayo Clinic Institutional Review Board and all participants provided written informed consent. Patients were excluded if they had a history of confirmed autoimmune diseases. Seven control subjects (6 females and 1 male, average age 36.1 years) and 10 patients with the diagnosis of POTS (7 females and 3 males, average age 35.1 years) provided 30 ml of venous blood. The detailed clinical and laboratory profiles of the subjects and controls are outlined in Table 1. POTS was diagnosed in the POTS Clinic at the Mayo Clinic under the supervision of Dr. Phillip Low and they satisfied the criteria of such syndrome as previously described[15]. IgGs were purified from each serum sample using the Melon gel IgG isolation kit (Pierce Biotechnology). Normal human heart tissue was obtained from the National Disease Research Interchange (NDRI).

### Purification of lipid raft fractions from human heart tissue

Lipid raft fractions were prepared by the non-detergent method as we have described[16]. Briefly, frozen heart tissue from all four chambers was homogenized and sonicated with 2 ml of 500 mM sodium carbonate (pH11) with protease inhibitors (Roche Diagnostics, Germany). The homogenate was centrifuged at 2,500 × g for 10 min and the supernatant containing 3 mg of proteins was adjusted to 40% sucrose by mixing with 2 ml of 80% sucrose prepared in MBS (0.15 M NaCl, 25 mM 2-[N-Morpholono] ethanesulfonic acid, pH6.5) and placed at the bottom of an ultracentrifuge tube. Discontinuous sucrose gradients of 5% and 30% were layered on top (4 ml of 5% sucrose/4 ml of 30% sucrose, both in MBS containing 250 mM sodium carbonate, pH11). Samples were then centrifuged at 260,000 × g for 20 hours at 4°C. Twelve fractions of 1 ml each were collected sequentially from the top. The lipid raft fractions were identified by immunoblotting against anti-caveolin-3 antibodies (1:1000, BD Transduction labs), dialyzed against ammonium bicarbonate (50 mM, pH7.8) and lyophilized to reduce sample volume.

### 2DE and immunoblotting

The experiments were performed as we have described[2]. Proteins from the human cardiac lipid raft fractions prepared above were resolved by 2DE and transferred electrophoretically onto a PVDF membrane. IgGs isolated from each control and patient were used to develop a separate immunoblot. Antibodies against Desmin (1:1000, Cell Signaling), HSP70 (1:1000, Enzo Life Sciences) and cavin-1 (1:2000, Bethyl Laboratory, Inc) were used for 2DE gel immunoblotting to identify their spot locations in 2DE gels.

### Protein identification and data analysis

Protein spots specifically reacting against patient IgGs were selected and processed for protein identification by in-gel trypsin digestion and nano-LC-electrospray tandem mass spectrometry as previously described[2].

### Protein interaction network analysis

Ingenuity Pathway Analysis (IPA, Ingenuity® Systems) was used to identify signaling pathways and networks as previously described[2]. The composite was laid out graphically using the network visualization algorithm Cytoscape 2.8.2[17].

## RESULTS

We first evaluated the effectiveness of lipid raft/caveolae-rich fraction isolation. Caveolin-3, the marker of cardiomyocyte caveolae, was enriched in the low buoyant density fractions (Figure 1). In contrast, the non-lipid raft membrane marker, clathrin, and the subcellular organelle marker Golgi 58, were differentially distributed (Figure 1). Clathrin appeared predominantly in the heavier fractions of the gradient, suggesting the exclusion of clathrin-coated pits as well as clathrin-associated membranes from our caveolae-rich lipid raft fractions. The Golgi apparatus was also distributed in the heavy density fractions. These results suggest that the lipid raft fraction was relatively free of contamination by intracellular organelles.

After 2DE separation and silver staining, 1159 protein spots were visualized (analyzed by PDQuest, Bio-Rad Laboratories, Inc.) (Figure 2A). 2DE gel immunoblotting analysis demonstrated that 34 spots reacted specifically with patient IgGs (Figure 2B& C, Table 2). These spots were further analyzed by nanoLC-MS/MS and the results identified 72 unique proteins (Table 3). A variety of cardiac lipid-raft associated proteins were targeted by the IgGs from POTS patients. These include proteins associated with caveolae structure (cavin), adrenergic signaling (protein kinase A), calcium signaling (sarcalumenin and S100), cytostructures (desmin, desmoplakin, desmoglein, vimentin, and plakoglobin), chaperone (heat shock 70), and energy metabolism.

To validate the results from nanoLC-MS/MS, specific antibodies against desmin, cavin-1 and HSP70 were used against 2DE blots of cardiac lipid raft/caveolae fractions (Figure 3). The results are in agreement with those from proteomic analysis, confirming that the protein identification results were correct.

To help understand the physiological and pathophysiological relevance of the immunoreactive lipid raft proteins, we performed network and canonical pathway analysis on the data from Table 3 using the IPA. Seventy-two identified proteins were integrated into a composite neighborhood comprised of 113 nodes linked by 389 interactions or edges (Figure 4). Network topology was assessed as an undirected network and the results demonstrated that the network topology was not random. A nonstochastic property was confirmed by examination of the interrelationship between node degree ($k$) and degree distribution (P[$k$]) (Figure 4). The organized assemblage followed a power law distribution falling within the predicted confidence range of biological networks[18].

Canonical pathway analysis demonstrated that many of the proteins targeted by patient IgGs are interrelated in pathways based on the IPA library (Table 4). Many pathways are involved including those that regulate caveolae-mediated signaling, oxidative phosphorylation, fatty acid metabolism, protein ubiquitination, and cardiac β-adrenergic signaling (Table 4).

## DISCUSSION

In this pilot study, we have made the following observations. First, autoantibodies against cardiac lipid raft microdomain proteins are present in patients with POTS. Second, multiple lipid raft/caveolae proteins are potential targets of the autoantibodies in POTS. Third, the presence of autoantibodies may affect the regulation of multiple cellular processes in patients with POTS.

Proteomic technologies have provided ideal tools in the search of antigens and antibodies in autoimmune diseases. The approach combining 2DE gel immunoblotting and MS has been effective in the identification of new antigens/antibodies in autoimmune and other diseases[19]. While the analytical power of MS-based technologies has become greatly improved in recent years, sample preparation remains a limitation for optimal sensitivity of proteomic analysis. In extremely complex protein preparations, the presence of high abundance proteins reduces the ability to detect low abundance proteins. To reduce sample complexity and augment low abundance proteins, we prepared lipid raft/caveolae fractions which are abundant in cardiomyocytes. Caveolae are specialized lipid rafts enriched with cholesterol, caveolins, and signaling molecules and they provide platforms for integrating specific cellular signal transduction processes[13]. Interaction between autoantibodies and lipid raft/caveolae proteins may trigger alterations in signaling pathways to produce the cardiovascular abnormalities seen in patients with POTS.

Our results showed that proteins regulating multiple cellular processes, such as signaling, metabolism, oxidoreductases, chaperones, catabolic, cytostructure and transcription, could be the targets of autoantibodies in patients with POTS. Many of the proteins have previously been implicated in cardiac dysfunction or cardiac disease, such as S100A8[20], HSP70[21], desmin[22], and desmoplakin[23]. However, involvement of these proteins in the pathogenesis of POTS is unknown. Further studies are needed to substantiate the role of these proteins in POTS. Although it is difficult to predict the prevalence of autoantibodies in the POTS population from the limited number of patients studied, our results indicate that a multitude of cardiac lipid raft-associated proteins could be targeted by autoimmunoreactive IgGs in these patients (Table 2). As shown in Table 2, the putative autoantigen profile of each individual patient was variable. Analyses of large population-based data sets are needed to provide further information on these issues.

Validation of the nanoLC-MS/MS results using affinity purified antibodies of desmin, cavin-1 and HSP70 was performed (Figure 3). The results are consistent with those from MS analyses indicating that this technology is a valuable approach in identifying potential autoimmune targets. Desmin, HSP70 and cavin-1 have been shown to be associated with caveolae[24–26] and cardiovascular diseases[22]. Desmin is a critical component of cardiac myocyte architecture, structure and signaling. Mice without desmin have impaired mitochondrial function with abnormalities in cardiac and skeletal muscles[27]. These animals are weak and easily fatigable, and the lack of desmin renders myofibers more susceptible to damage during contraction[28]. Increased plasma levels of anti-HSP70 were found to be a cardiac risk factor associated with a significant incidence of ECG abnormalities characteristic of chronic myocardial ischemia, conduction abnormality, or heart displacement[29]. Previous study also suggested that higher serum HSP70 antibody level may be a marker for subsequent development of postoperative atrial fibrillation[30]. Our findings suggest that the presence of autoantibodies against HSP70 could be a risk factor for the development of cardiac dysfunction in POTS.

Interestingly, a newly described protein, cavin-1, also known as polymerase I and transcript release factor (PTRF), is one of the targets of the autoantibodies in POTS. Cavin-1 is

required for the formation of caveolae in all mammalian and plays a critical role in regulating caveolae function in endothelial cells[26]. Cavin-1 also facilitates repair of damaged muscle cells[31]. Cavin-1 mutations in human caused a secondary deficiency of caveolins resulting in muscular dystrophy, as well as arrhythmias and atrial fibrillation[32]. Therefore, further studies are warranted to investigate the effect of immune reactions against cavin-1.

Cellular proteins are connected through extensive networks of interacting pathways. Assembly of biologically relevant interactomes may help better understand the effects produced by the binding of autoimmunoreactive IgGs to lipid raft/caveolae proteins. The unbiased gene ontology-based network interrogation further indicates that autoimmune reactions to cardiac proteins are biologically relevant with nonrandom effects on cardiac functions (Figure 4). Some of the targeted proteins are important signaling proteins and they encompass many signaling pathways (Table 4). The potential involvement of the β-adrenergic signaling pathway in POTS is particularly intriguing as a putative mechanism that underlies the clinical manifestation in POTS is an imbalance between sympathetic/parasympathetic regulations. Hence, further studies on the functional relevance of autoantibodies and their potential cardiac membrane protein targets may provide important insights into the pathogenesis of POTS. We hope that this study may serve as an impetus for further investigations in this area.

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## Abbreviations

| | |
|---|---|
| **POTS** | postural orthostatic tachycardia syndrome |
| **IPA** | Ingenuity Pathway Analysis |
| **2DE** | 2-dimensional gel electrophoresis |
| **MS** | mass spectrometry |

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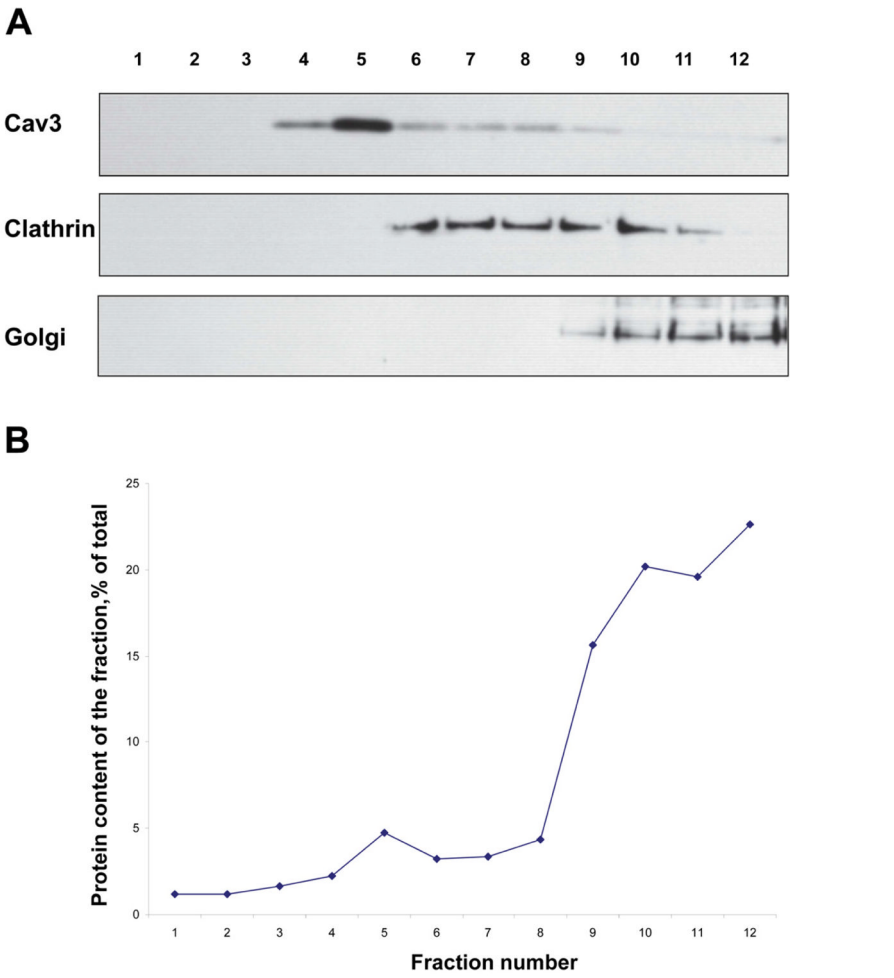


**Figure 1. Immunoblot analysis of human cardiac membrane sucrose density gradient fractions**

(**A**) An equal volume of each sucrose density gradient fraction was loaded onto each lane. Enrichment of caveolin-3 in the low buoyant density fractions indicated the successful separation of caveolae/lipid raft fraction (top panel). The middle and lower panels show clathrin and Golgi 58, the non-lipid raft plasma membrane and intracellular organelle marker proteins, respectively, are predominantly detected in the heavy fractions, but not in the caveolae-rich fraction. (**B**) The distribution of protein content across the gradient fractions was expressed as a percentage of the total amount of protein recovered in the gradient.

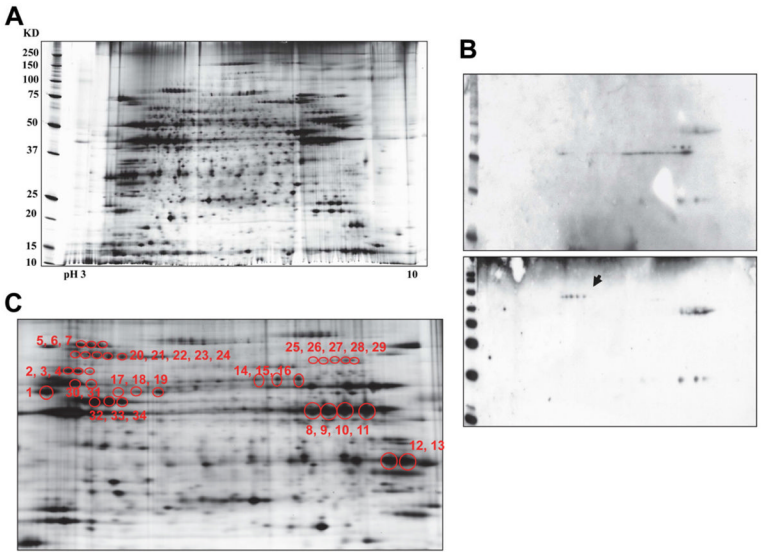


**Figure 2. 2DE gel immunoblotting of human cardiac lipid raft/caveolae fractions**

(**A**) Protein maps resolved by 2DE with isoelectric focusing, SDS-PAGE and silver staining. (**B**) Protein spots in representative 2DE immunoblots reactive against purified IgGs from a healthy control (upper panel) and a patient with POTS (lower panel). Arrow shows patient-specific immunoreactive protein spots. (**C**) Patient-specific immunoreactive spots sampled from silver-stained 2DE gel for protein identification, spots 1 to 34.

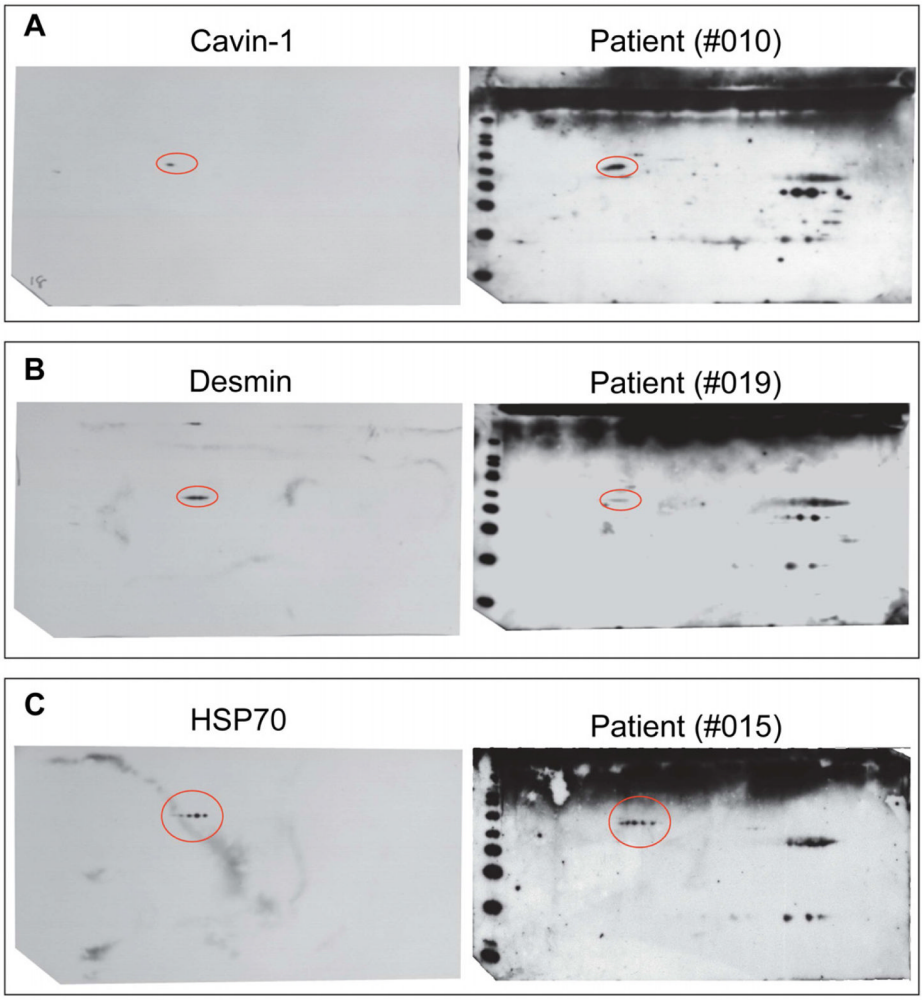


**Figure 3. 2DE gel immunoblot of human heart lipid raft/caveolae fractions against anti-cavin-1, -desmin and -HSP70 antibodies**

Immunoblot analyses of 2DE of lipid raft/caveolae fractions against commercially available antibodies (left panel) and against patient serum IgGs (right panel). The results show agreement for anti-cavin-1 (**A**), anti-desmin (**B**), and anti-HSP70 (**C**).

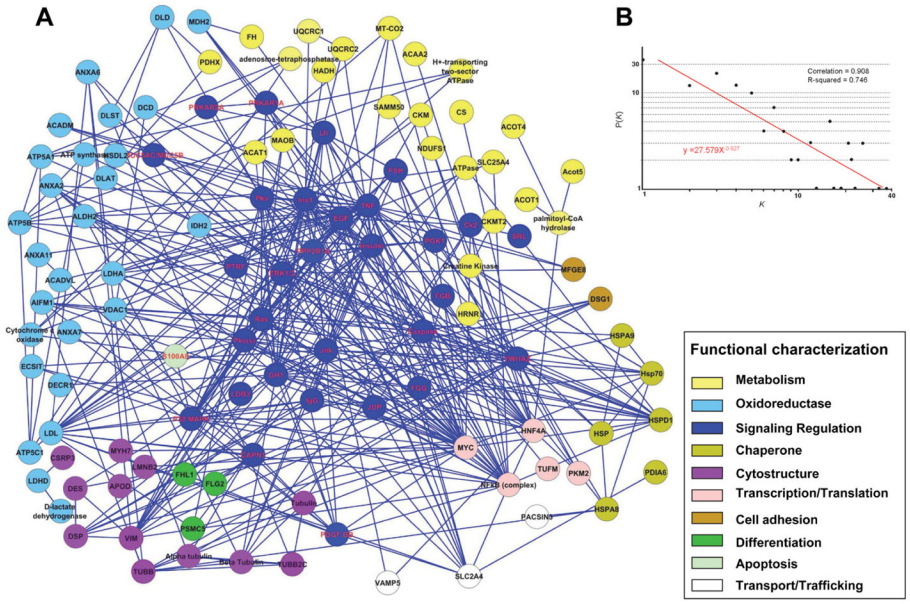


**Figure 4. Network analysis of the proteins identified by MS from patient-specific protein spots**

(**A**) Seventy-two unique proteins identified by MS submitted to Ingenuity Pathways Analysis as focus nodes were integrated into a composite neighborhood comprised of 113 protein interaction network linked by 389 interactions or edges. Nodes are listed by Swiss-Prot gene designations and the color corresponds to ontological function. (**B**) Network degree distribution, (P[k]) versus degree (k), followed a power law distribution indicating nonstochastic scale-free network architecture a typical biological networks.

**Table 1**

Clinical profiles and laboratory findings of controls and POTS patients

| | Age | Sex | Allergies | PMH | Medications | Symptoms & Study Findings | ECG HR BPM | Holter HR BPM Avg (range) | Tilt Study Baseline HR (BP) | Tilt Study 10 min HR (BP) | ECHO (LVEF %) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Controls | | | | | | |
| 1 | 43 | F | Codeine, procaine | Benign thyroid nodule | Levapro | | | | | | |
| 2 | 23 | F | | | | | | | | | |
| 3 | 45 | F | | Lipids, LBP, Herpes zoster | | | | | | | |
| 4 | 37 | F | Azithromycin, Pseudoephed rine | Lipids | | | | | | | |
| 5 | 44 | F | PCN, shellfish | Sinus rhinitis | Advil, fish oil, Imitrex | | | | | | |
| 6 | 24 | F | | Asthma | Alavert | | | | | | |
| 7 | 37 | M | | HBP, GERD | Lisinopril, Prilosec | | | | | | |
| | | | | | POTS Subjects | | | | | | |
| 1 | 44 | F | Sulfur, Levofloxacin | Melanoma, meningitis, lipids, fibromyalgia, eczema | RetinA cream | ortho intolerance | 126 | 101 (70–169) | 99 (122/80) | 122 (110/84) | Normal (60–65%) |
| 2 | 51 | F | | Bipolar, HBP | Catapres, Metoprolol, Lipitor, Clonidine, Provera | ortho Intolerance, mild auto neuropathy | 117 | 114 (89–165 | 92 (148/92) | 134 (125/76) | Normal (62%) |
| 3 | 48 | M | | Fibromyalgia, chronic fatigue, PTSD | Vit D, Lyrica, Imitrex, Inderal, Florinef | ortho intolerance, mild postgang sudomoter impair, mild multifocal anhidrosis | 63 | 73 (50–145) | 74 (122/78) | 125 (110/70) | Normal (60%) |
| 4 | 56 | M | | GI dysmotility | Florinef, Neurontin, K, Midodrine, Mestinon | severe autonomic neuropathy, severe cardiovagal and adrenergic failure, distal anhidrosis, ortho hypotension | 67 | | 64 (164/94) | 64 (50/−) (1.5 min) | |
| 5 | 35 | F | | Asthma, lipids, depression | Klonopin, Midodrine, Nadolol, BCP, inhaler | mild vascular adrenergic impairment, minor hypohidrosis | 88 | 93 (64–152) | 88 (122/74) | 165 (104/82) | Normal (63%) |
| 6 | 18 | F | Hydrocodone | Irritable bowel, hypovitaminosis D | Metoprolol, Citalopram, vit D | ortho tachycardia | 88 | | 76 (108/64) | 120 (100/80) | |
| 7 | 20 | F | Amoxacillin | | Allegra, Microgestin | ↓ortho tolerance, hyperadrenergic state | 92 | | 82 (110/56) | 131 (100/70) | Normal (61%) |
| 8 | 21 | F | Tramadol, Sprintec | Mononucleosis, irritable bowel | Florinef, Neurontin, Lexapro, Metoprolol, Prilosec, Mestinon | focal sudomotor failure, ortho intolerance | 67 | 90 (64–137) | 72 (92/64) | 101 (86/64) | |
| 9 | 26 | F | Amoxacillin | Eczema. GERD, sinus, periurethral cyst | Klonopin, Microgestin, Zyrtec, Norco, Cymbalta | ortho intolerance | 82 | 84 (67–137) | 83 (114/70) | 137 (108/84) | |
| 10 | 32 | M | | HBP, lipids, arthritis, asthma. GI dysmotility | Flexeril, Florinef, Neurontin, Klonopin, Mestinon, Propranolol | ortho intolerance, mild adrenergic vasoconstr failure | 71 | 87 (54–136) | 80 (110/76) | 131 (112/78) | |

**Table 2**

POTS patient specific immunoreactive spots

| Patient # | Patient specific spots* |
|---|---|
| 009 | 2, 3, 4 |
| 010 | 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 |
| 011 | 5, 6, 7 |
| 012 | 14, 15, 16, 30, 31, 32, 33, 34 |
| 013 | 1 |
| 014 | 17, 18, 19, 30, 31, 2, 3, 4 |
| 015 | 20, 21, 22, 23, 24 |
| 016 | |
| 017 | |
| 018 | 3, 4, 30, 31, 25, 26, 27, 28, 29, 12, 13 |

*Spot code refer to Figure 2C

**Table 3**

Proteins detected from patient specific spots

| Symbols | Protein Name | Acc. numbers | Function |
|---|---|---|---|
| S100A8 | Protein S100-A8 | S10A8_HUMAN | apoptosis |
| PPP2R1A | Serine/threonine-protein phosphatase 2A | 2AAA_HUMAN | apoptosis, chromosome segregation |
| HSPA9 | Stress-70 protein | GRP75_BOVIN | Chaperone, cell proliferation and aging |
| PDIA6 | Protein disulfide-isomerase A6 | PDIA6_HUMAN | Chaperone, isomerase |
| HSPD1 | 60 kDa heat shock protein | CH60_HUMAN | Chaperone, protein folding |
| HSPA1A | Heat shock 70 kDa protein 1A/1B | HSP71_HUMAN | Chaperone, protein folding |
| HSPA8 | Heat shock cognate 71 kDa protein | HSP7C_HUMAN | Chaperone, protein folding |
| LMNB2 | Lamin-B2 | LMNB2_HUMAN | structural molecule activity, lipodystrophy |
| TUBA | Tubulin alpha chain | TBA_XENTR | microtubule, structural molecule activity |
| TUBB2C | Tubulin beta-2C chain | TBB2C_HUMAN | microtubule, structural molecule activity |
| TUBB5 | Tubulin beta-5 chain | TBB5_HUMAN | microtubule, structural molecule activity |
| ACT | Actin, alpha sarcomeric/cardiac | ACT2_XENLA | cytoskeleton, cell motility |
| VIM | Vimentin | VIME_HUMAN | cytoskeleton, intermediate filaments |
| DES | Desmin | DESM_HUMAN | intermediate filament, cardiomyopathy |
| MYH7 | Myosin-7 | MYH7_HUMAN | contractile protein, hypertrophic CM |
| DSP | Desmoplakin | DESP_HUMAN | desmosomal protein, cardiomyopathy |
| PSMC5 | 26S protease regulatory subunit 8 | PRS8_HUMAN | degradation of ubiquitinated proteins |
| FHL1 | Four and a half LIM domains protein 1 | FHL1_HUMAN | muscle development, hypertrophy |
| FLG2 | Filaggrin-2 | FILA2_HUMAN | cell differentiation |
| DSG1 | Desmoglein-1 | DSG1_HUMAN | mediating cell-cell adhesion |
| MFGE8 | Lactadherin | MFGM_HUMAN | cell adhesion, angiogenesis |
| JUP | Junction plakoglobin | PLAK_HUMAN | Cell adhesion, cardiomyopathy, arrhythmia |
| CS | Citrate synthase | CISY_HUMAN | Carbohydrate metabolism, Tricarboxylic acid cycle |
| ACAA2 | 3-ketoacyl-CoA thiolase | THIM_HUMAN | apoptosis and mitochondrial damage |
| CKM | Creatine kinase M-type | KCRM_HUMAN | energy transduction |
| CKMT2 | Creatine kinase S-type | KCRS_HUMAN | energy transduction |
| ACOT1 | Acyl-coenzyme A thioesterase 1 | ACOT1_HUMAN | hydrolysis of acyl-CoAs |
| SLC25A4 | ADP/ATP translocase 1 | ADT1_HUMAN | exchange of ADP and ATP |
| ACAT1 | Acetyl-CoA acetyltransferase | THIL_HUMAN | ketone body metabolism |

| Symbols | Protein Name | Acc. numbers | Function |
|---|---|---|---|
| HADH | Hydroxyacyl-coenzyme A dehydrogenase | HCDH_HUMAN | Fatty acid metabolism |
| FH | Fumarate hydratase | FUMH_HUMAN | fumarate metabolic process |
| MAOB | Amine oxidase | AOFB_HUMAN | metabolism of neuroactive and vasoactive amines |
| MT-CO2 | Cytochrome c oxidase subunit 2 | COX2_HUMAN | electron transport |
| UQCRC1 | Cytochrome b-c1 complex subunit 1 | QCR1_HUMAN | electron transport, ATP |
| UQCRC2 | Cytochrome b-c1 complex subunit 2 | QCR2_HUMAN | electron transport, ATP |
| SAMM50 | Sorting and assembly machinery component 50 homolog | SAM50_HUMAN | mitochondrial function |
| NDUFS1 | NADH-ubiquinone oxidoreductase | NDUS1_HUMAN | NADH oxidation |
| PDHX | Pyruvate dehydrogenase protein X component | ODPX_HUMAN | pyruvate metabolic, acyltransferase activity |
| TUFM | Elongation factor Tu | EFTU_HUMAN | Elongation factor |
| ATP5A1 | ATP synthase subunit alpha | ATPA_HUMAN | oxidative phosphorylation |
| ATP5B | ATP synthase subunit beta | ATPB_HUMAN | oxidative phosphorylation |
| ATP5C1 | ATP synthase subunit gamma | ATPG_HUMAN | oxidative phosphorylation |
| DECR1 | 2,4-dienoyl-CoA reductase | DECR_HUMAN | Oxidoreductase |
| ALDH2 | Aldehyde dehydrogenase | ALDH2_HUMAN | Oxidoreductase |
| DLD | Dihydrolipoyl dehydrogenase | DLDH_HUMAN | Oxidoreductase |
| DLAT | Dihydrolipoyllysine-residue acetyltransferase- pyruvate dehydrogenase complex | ODP2_HUMAN | Oxidoreductase |
| DLST | Dihydrolipoyllysine-residue succinyltransferase-2-oxoglutarate dehydrogenase complex | ODO2_HUMAN | Oxidoreductase |
| ECSIT | Evolutionarily conserved signaling intermediate in Toll pathway | ECSIT_HUMAN | Oxidoreductase |
| HSDL2 | Hydroxysteroid dehydrogenase-like protein 2 | HSDL2_HUMAN | Oxidoreductase |
| IDH2 | Isocitrate dehydrogenase | IDHP_HUMAN | Oxidoreductase |
| LDHA | L-lactate dehydrogenase A chain | LDHA_HUMAN | Oxidoreductase |
| MDH2 | Malate dehydrogenase | MDHM_HUMAN | Oxidoreductase |
| ACADM | Medium-chain specific acyl-CoA dehydrogenase | ACADM_HUMAN | Oxidoreductase |
| LDHD | Probable D-lactate dehydrogenase | LDHD_HUMAN | Oxidoreductase |
| AIFM1 | Apoptosis-inducing factor 1 | AIFM1_HUMAN | oxidoreductase, apoptosis |
| VDAC1 | Voltage-dependent anion-selective channel protein 1 | VDAC1_HUMAN | Oxidoreductase, apoptosis |
| ACADVL | Very long-chain specific acyl-CoA dehydrogenase | ACADV_HUMAN | Oxidoreductase, cardiomyopathy |
| PRKAR1A | cAMP-dependent protein kinase type I-alpha regulatory subunit | KAP0_HUMAN | Post-translational modification |
| PRKAR2A | cAMP-dependent protein kinase type II-alpha regulatory subunit | KAP2_HUMAN | Post-translational modification |
| LDB3 | LIM domain-binding protein 3 | LDB3_HUMAN | PKC-mediated signaling |

| Symbols | Protein Name | Acc. numbers | Function |
|---|---|---|---|
| PGK1 | Phosphoglycerate kinase 1 | PGK1_HUMAN | Kinase, transferase |
| PKM2 | Pyruvate kinase isozymes M1/M2 | KPYM_HUMAN | pyruvate, apoptosis, calcium signaling |
| PTRF | Polymerase I and transcript release factor | PTRF_HUMAN | caveolae formation, cell membrane repair |
| APOD | Apolipoprotein D | APOD_HUMAN | ligands transport |
| PACSIN3 | Protein kinase C and casein kinase substrate in neurons protein 3 | PACN3_HUMAN | vesicle formation and transport |
| SRL | Sarcalumenin | SRCA_HUMAN | regulation of calcium transport |
| FGB | Fibrinogen beta chain | FIBB_HUMAN | platelet aggregation |
| FGG | Fibrinogen gamma chain | FIBG_HUMAN | platelet aggregation |
| ANXA2 | Annexin A2 | ANXA2_HUMAN | stress response, exocytosis |
| ANXA6 | Annexin A6 | ANXA6_HUMAN | regulate intracellular Ca2+ release |
| ANXA11 | Annexin A11 | ANX11_HUMAN | cytokinesis |
| ANXA7 | Annexin A7 | ANXA7_HUMAN | membrane fusion, exocytosis |

**Table 4**

Canonical pathways involved in proteins targeted by autoantibodies

| Ingenuity Canonical Pathways | Molecules (Symbols) |
|---|---|
| Mitochondrial Dysfunction | ATP5C1, NDUFS1, ATP5B, UQCRC2, MT-CO2, UQCRC1, AIFM1 |
| Oxidative Phosphorylation | ATP5C1, NDUFS1, ATP5B, UQCRC2, MT-CO2, UQCRC1, ATP5F1 |
| Pyruvate Metabolism | PKM2, LDHD, DLAT, PDHX, ACAT1 |
| Valine, Leucine and Isoleucine Degradation | ACADVL, ACAT1, ACAA2, HADH |
| Citrate Cycle | CS, DLST, IDH2 |
| Purine Metabolism | PKM2, ATP5C1, ATP5B, MYH7, HSPD1, ATP5F1 |
| Fatty Acid Metabolism | ACADVL, ACAT1, ACAA2, HADH |
| Lysine Degradation | DLST, ACAT1, HADH |
| Extrinsic Prothrombin Activation Pathway | FGB, FGG |
| Fatty Acid Elongation in Mitochondria | ACAA2, HADH |
| Glycolysis/Gluconeogenesis | PKM2, DLAT, PDHX |
| Intrinsic Prothrombin Activation Pathway | FGB, FGG |
| Alanine and Aspartate Metabolism | DLAT, PDHX |
| Acute Phase Response Signaling | ECSIT, FGB, FGG |
| Butanoate Metabolism | ACAT1, HADH |
| Propanoate Metabolism | ACADVL, ACAT1 |
| IL-1 Signaling | ECSIT, PRKAR2A |
| Synthesis and Degradation of Ketone Bodies | ACAT1 |
| Cardiomyocyte Differentiation via BMP Receptors | MYH7 |
| AMPK Signaling | CKM, PRKAR2A |
| Tryptophan Metabolism | ACAT1, HADH |
| Tight Junction Signaling | PRKAR2A, MYH7 |
| Calcium Signaling | PRKAR2A, MYH7 |
| ILK Signaling | MYH7, DSP |
| Huntington's Disease Signaling | ATP5B, HSPA9 |
| Protein Ubiquitination Pathway | HSPA9, HSPD1 |
| Glucocorticoid Receptor Signaling | HSPA9, FGG |
| Caveolar-mediated Signaling | PTRF |
| Nitric Oxide Signaling in the Cardiovascular System | PRKAR2A |

| Ingenuity Canonical Pathways | Molecules (Symbols) |
|---|---|
| Arginine and Proline Metabolism | CKM |
| Melanocyte Development and Pigmentation Signaling | PRKAR2A |
| α-Adrenergic Signaling | PRKAR2A |
| Cardiac β-adrenergic Signaling | PRKAR2A |
| Insulin Receptor Signaling | PRKAR2A |
| Role of NFAT in Cardiac Hypertrophy | PRKAR2A |
| cAMP-mediated signaling | PRKAR2A |
| Cardiac Hypertrophy Signaling | PRKAR2A |