分担研究者　伴信太郎

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報 告

標準模擬患者の練習状況とOSCEに対する意識：全国調査第二報

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要旨：
客観的臨床能力試験（OSCE）の導入により，標準模擬患者（SP）の需要が高まり，医学教育において重要な役割を担っている．その反面でSPの不安，不満が高まっている．SPを理解することがSPの質の向上に必要と考え，模擬患者の意識調査を実施した．目的はOSCE経験を通してSPがどのような感情を抱いているのか心理面を明らかにすることである．
1）全国532人のSPを対象に自記式調査を実施し，332人のSP（62%）から回答を得た．
2）OSCEの練習方法は「シナリオを熟読してから全体で読み合わせをし，ロールプレイをした後，演技・評価を全員で統一」が最も多かったが，時間・方法はグループ間で大きな差が見られた．
3）SPがOSCEで難しいと感じる要因は，演技では「質問に対してどこまで話したらいいかを判断すること」が73%で最も高く，評価では「受験者への基準を変えない」が66%で最も高かった．
4）OSCEに対するSPの難しさを減少させるため，指導者の確保と練習時間の充実が今後の課題と考える．
キーワード：模擬患者，意識調査，全国調査，OSCE，練習方法

Activities and attitudes of standardized patients in the objective structured clinical examination: The second report of a nationwide survey

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The role of standardized patients (SPs) has developed rapidly over the last 10 years because of medical education curriculum reform and the introduction of the objective structured clinical examination (OSCE). As the participation of SPs in medical education has increased, the anxieties and frustrations of SPs have also increased. We believe that an understanding of the attitudes of SPs would improve the quality of their activities. The purpose of this survey was to study the activities and psychological needs of Japanese SPs in the OSCE.
1) The response rate to the nationwide survey was 62% (332 of 532 SPs).
2) Role-playing and group discussion were the most common training methods, and the length of training varied from 0 to 40 hours.
3) The factors that SPs felt difficult were judging how much to respond in their performances (73%) and maintaining consistent standards in evaluating examinees (66%).
4) Our results suggest that SPs require more training and that the number of SP educators should be increased.
Key words: standardized patient, attitude survey, nationwide survey, objective structured clinical education, training method

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1. 背景・目的

1964年，Barrow氏により米国で模擬患者が紹介されて以来40年間で，医学教育は模擬患者（Simulated Patient／Standardized Patient：SP）参加型教育へと大きな変革を遂げ，その波は欧米からアジア，そして日本へも広がった[1-7]．そして，医学のみならず薬学，歯学，看護学，理学療法学教育などの分野へも広がっている[8-11]．SPの数は，医学教育改革と2005年度より本格実施された共用試験の客観的臨床能力試験（OSCE）が後押しとなり急増した[12-14]．OSCEに参加する標準模擬患者は標準化される必要があり，また学生評価は任意ではあるが実施している大学も多く，SPの責任，心理的負担も大きくなってきている．

この急速な増加にともないSPの負担感増加，質の不均一などの問題が浮上してきている．そこで，筆者らは「SPが実際にどのような内容でどれくらいの訓練を受けているのだろうか？」また，「SPはどんな不安を持っているのだろうか？」という疑問を持ち全国調査を行った．その結果，第一報では，96%のSPが満足感を持っていると答えている反面，67%のSPは負担感を持ち，「フィードバック」，「評価」，「演技」の3つのコアスキルに対し難しいと感じていることが明らかになった[7]．SPがより良い演技・評価をするためにはSP養成者による効果的な指導とSPの心理状態の安寧が必要であろう．SPが抱える問題点を明らかにし，SP養成者がSPのニーズを考慮したより良いSP養成プログラムを提供することで，SPの質の向上に貢献できるのではないかと考えた．

本論文は日本のSPを対象に実施した意識調査の結果の中で，特にOSCEに焦点を当てたものである．OSCEのための練習方法，頻度・時間などの現状とOSCE時にSPが抱く不安や問題点を明らかにすることを目的とした．

2. 対象・方法

2004年4月1日に調査時点で確認ができた59のSPグループのSPを対象に自記式アンケート調査を実施した．調査票は人口統計，活動内容，活動に伴う意識及び問題点，及び身体診察に関する意識を含む27項目（選択問題19問と自由記載8問）で構成されている．詳しい実施方法と調査票，及び基礎データの結果は模擬患者に関する研究報告書を参考にされたい[14]．データはSPSS 11.5Jを用いて統計処理した．また，自由記載項目に記載されたコメントについては2人の評価者が別々にコード化し意見を集約した．

3. 結 果

依頼文を発送した59のSPグループのうち54グループから協力可能の返答を得た．残り5グループは近々発足予定などの理由で今回の調査に含まなかった．SPの総数は532名で，最終的な調査票回収率は332名（62%）であった．

1) OSCE1回に対する練習頻度と時間

OSCEを行っていると答えた人は198人（60%）で，OSCEを行っていないと答えた人は67人（20%）いた．また，OSCEについての質問には答えているが，練習頻度と時間については無記入だったSPが67名（20%）あったが，ここでのパーセントは無記入も含むOSCE実施者を母数として計算する．図1のごとく，1回のOSCEのための練習回数は2〜4回に集中し，最多回数は12回であった．一方，時間にすると，2時間が31人（16%）で最も多く，最も多いのは40時間であった．

2) 練習方法

自由記載で「OSCEでの標準模擬患者の練習は主にどのような方法で行いますか？」と尋ねたところ，207名のSPからコメントが得られたのでその内容を質的に分析した．その結果，「SPが各自シナリオを熟読してきてから全体で読み合わせをする．そして，指導者あるいはSP等とロールプレイで練習し，演技・評価について議論し統一していく」という練習方法が最も多い方法であった．細部を見ていくと，「シナリオ熟読」「ロールプレイ／ビデオによる振り返り」「全員で議論し指導者に質問」「何度も繰り返し練習」「標準化／統一」「自己学習」の5つのプロセスがあった．一般模擬患者の場合と比べてより全体練習が多

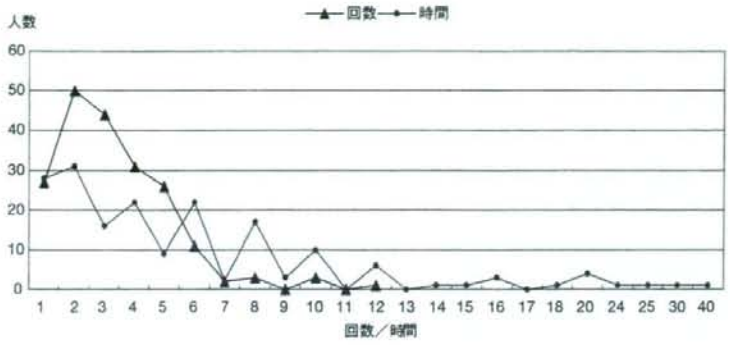

図1　OSCE1回に対する練習頻度と時間

く，内容の深い練習がされていることが伺えた．

「シナリオ熟読」に関しての意見は「なるべく全員で集まり，背景について全体像を形成していく．例えば一日のライフスタイル，家族構成，来院時の状況など」「事前に各自シナリオを読んで疑問や不明な点を全員で討議し確認し合う．重要なポイントを各自の共通事項として認識した上で練習を始める」「シナリオを繰り返し読む．曖昧な表現について議論し統一化を計る」「先生（SP養成者）のアドバイスをもらいシナリオで分からない部分を詰める」など，かなり詳細にシナリオを練り上げて標準化している様子が伺えた．

「ロールプレイ／ビデオによる振り返り」では「養成者または研修医を相手にロールプレイをし，フィードバックをした後，皆の意見を聞く」「ロールプレイをビデオに撮り，全員でビデオを見てお互いにフィードバックする」「グループ内のSP同士でロールプレイする中でメンバーが気づいた点を議論する」などの意見が挙り，SP養成者の有無にかかわらず練習方法としてロールプレイが多く用いられていた．また，毎回ではないが数回に一度順番にビデオを撮り，客観的に自己を振り返る方法も活用されていた．

次に，ロールプレイの後は「皆で議論し，養成者に質問」を行うという意見が多かった．　実際のコメントでは「SP同士でロールプレイをした後，全員で議論し養成者へ質問する」「練習後患者役がフィードバックをし，グループで気づいたことを議論する，そして最後に養成者が総括しSPの資質の向上を図る」「皆で議論し，養成者のアドバイスを受ける」など，SP全員で議論し，養成者がSPの質問に答えるという流れがあることが分かった．しかしながら，養成者のいないSPのみの練習の場合も89人（36%）あった．

「何度も繰り返し練習」に関しては「教官（SP養成者）とSP一対一で一つのシナリオに対し9回練習する」「指導者とひとりずつロールプレイをし，それをひとり3〜4回繰り返す．一回に一時間はかける」「想像できうるすべての場面を設定し練習する」「全体練習の他にも確実な状態になるまで個人的な練習と勉強を繰り返す」「シナリオ検討→打ち合わせ→場面の擦り合わせ→ロールプレイ→振り返り，これを3回繰り返したあとビデオに撮る」など，グループ内での合同学習と自己学習と両方で繰り返し練習している様子が伺えた．

最後に「標準化／統一」に関しては，「ロールプレイをし，気付いたことを述べ合い標準化していく」「評価の統一，受け答えの節度の統一をしていく」「質問に対する答え方の統一，評価にばらつきがあった場合は，理由を出し合い統一するように練習」などの意見が挙った．その他，「直前にお互いに一問一答形式で確認する」「問答集を作成」「基本的な問題集を作る」など，標準化への対応が十分にされていることが伺えた．しかし，その一方で，「練習をしていない」と答えた

SPが11名存在した．その中には「まだ決まった練習はない」「本番で学ぶ」「On the job training」「他のSPの演技を見て学ぶ」「一般模擬患者と同じ練習」などOSCEに対応するSPの練習としては不適切と思われる方法が挙っていた．

「自己学習」に関しては，「家族相手に練習する」「疾患についての論文を集めたり，医療関係の本で調べる」「イメージトレーニングをする」「ドラマ，映画を意識して見る」など，養成者やSPとの練習時間以外でも自己学習している様子が伺えた．

3) OSCEでSPを演じるときに難しいと感じること

「OSCEで標準模擬患者を演じていて難しく感じることを選んでください」との質問に対し，5項目から選択の複数回答で尋ねたところ，**図2**に示す通り，194人（73%）のSPが「質問に対してどこまで話すかを判断すること（話す程度の判断）」を最も難しいと感じていた．次に，「初めから最後の受験者まで基準を変えないで演技をすること」が178人（67%），「他のSPと演技を合わせること」が107人（40%），「受験者に対して感情移入しないようにコントロールすること」が67人（25%）であった．「特に難しいと感じない」というSPが僅かながら9人いた．

これら5項目に対しχ^2検定を用いて，性・年齢・職業・SP歴別に比較したところ，「他のSPと演技を合わせること」では60歳未満より60歳以上が有意（$p = .002$）に難しく感じているという結果になった．次に，「質問に対してどこまで話すかを判断すること」を難しいと感じていることに対して，性別において女性の方が男性より有意（$p = .007$）に難しいと感じていることが分かった．その他のコメントには，「シナリオにない質問を受けたときの対応」「集中力が持続しない」「受験者毎に気持をリセットしなくてはならないこと」「何を答えても『ありがとうございました』のOSCE特有の返答を受けたとき」などOSCE由来の難しさが目立った．

本調査当時は，OSCE時に「フィードバック」と「評価」を実施していた大学が少なくなかった．しかし，昨年度より開始した共用試験では「フィードバック」は課せられず，「評価」も任意であるため，調査時の状況を反映していない可能性がある．このため「フィードバック」に関する結果は割愛した．

4) OSCEの評価をするとき難しいと感じること

「OSCEの評価をするとき難しいと感じることを選んでください」との質問に対し，5項目から選択の複数回答で尋ねたところ，**図3**の通り，「初めから最後の受験者まで基準を変えないで評価すること（基準をかえない）」が175人（66%）で最も多く，次に「他のSPとの評価基準を合わせること」が126人（48%），「主観的評価（自分がどう感じたかで判断）と客観的評価（チェック項目をもとに出来不出来を判断）の割合の取り方」が96人（36%），「演技中に評価をするためのチェック項目にとらわれないこと」「受験者に対

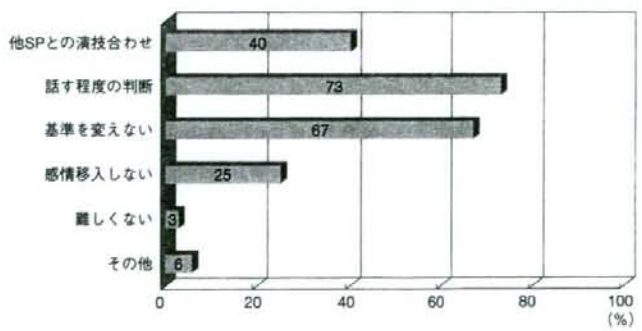

図2　OSCEでSPを演じるとき難しいと感じること

して感情移入しないようにコントロールすること」が共に2割弱であった．「特に難しいと感じない」は14人あった．

この5項目を性・年齢・職業・SP歴別にχ^2検定した結果は表1に示すとおりである．男性SPより女性SPの方が「初めから最後まで受験者の基準を変えないで評価すること」に対して有意（p = .034）に難しいと感じていた．また，「演技中に評価をするためのチェック項目にとらわれないこと」に対しては60歳以上が60歳未満より（p = .016），また非在職者（無職，専業主婦，学生）が在職者（常勤者，自営，パート）より（p = .035）有意に難しいと感じていることが分かった．また経験年数から評価の難しさを検討した結果から有意差は見られなかった．その他のコメントでは「評価に集中できない環境」，「評価が大まかすぎる」，「最初の学生の評価に引きずられる」，「迷って時間内に評価できない時，気分をリセットできない」など試験のシステムに関する意見も挙がっていた．

4. 考　察

アンケート回収率は62%であったので，全国のSPの現状をおおむね反映しているものと考え

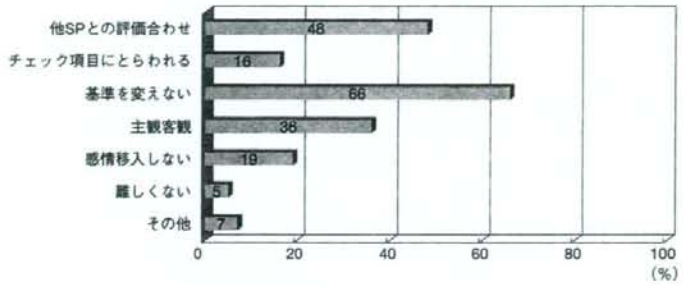

図3　OSCEの評価で難しいと感じること

表1　OSCEの評価で難しいと感じる要因の性・年齢・職業・SP歴による比較（χ^2検定）

項目	性別			年齢			職業			SP経験歴		
	男性 (n=47) なし/ある	女性 (n=207) なし/ある	p値	60未満 (n=141) なし/ある	60以上 (n=113) なし/ある	p値	非有職者 (n=168) なし/ある	有職者 (n=79) なし/ある	p値	3年未満 (n=158) なし/ある	3年以上 (n=85) なし/ある	p値
基準を変えない	30/17	99/108	**.034**	66/75	62/51	.125	85/82	34/40	.431	81/77	39/46	.253
チェック項目	42/5	169/38	.144	124/17	82/26	**.016**	135/34	71/8	**.035**	134/24	67/18	.159
他SPとの評価合せ	14/33	65/142	.489	44/97	36/77	.509	55/114	22/57	.277	48/110	28/57	.393
主観客観	32/5	125/81	.220	87/54	71/41	.443	104/65	48/30	.557	101/57	50/34	.296
感情移入しない	42/5	161/46	.051	116/25	88/25	.236	124/35	65/14	.356	127/31	68/17	.535

る．今回はSPがOSCEのためにどのように，そしてどれくらいの練習を行っているのか，また活動を通してどのような意識を抱いているのか等について考察を試みる．

標準模擬患者の練習頻度はOSCE1回に対しては2〜4回が多く，練習回数は一般模擬患者の倍になった．時間数では2時間〜40時間まで幅があった．これらの結果からOSCEのための標準模擬患者の練習量は回数・時間共にグループ間で大きな差があることが分かった．

練習方法は「SPが各自シナリオを熟読してきてから全体で読み合わせし，指導者あるいはSP等とロールプレイで練習し，演技・評価について議論し統一していく」が最も多いが，中でも，「シナリオの熟読と読み合わせ」と「演技と評価の統一」というOSCEに特化した標準化のための練習方法が組み込まれていることが特徴的といえる．しかし，練習方法は，たっぷり時間をかけ議論し，問答集まで作って練習しているグループから決まった練習方法がないグループまで，グループ毎に質のばらつきが見られた．OSCEでは演技・評価の標準化という高い技能を要求されるので，2時間練習したSPと40時間練習したSPとでは技能に差が出るのは明らかであろう．SPの個人差にも依るところが大きいが，SPが不安なくOSCEに臨むためには，筆者らの経験では最低でも8〜10時間程度の練習は必要であろうと考える．Wallaceは3時間のトレーニングが4回必要と述べている[15]試験の信頼性を保つためにはSPの質の向上・均一化は必須であり，それに答えるためにはSP養成者の確保と養成者の認識の統一，及び最低限の指導期間と指導内容のコンセンサス作りが急務と考える．

OSCEに参加する標準模擬患者としてSPを演じる難しさは「質問に対してどこまで話すかを判断すること」「初めから最後の受験者まで基準を変えないで演技をすること」「他のSPと演技を合わせること」が上位に上がり，また，OSCEの評価においてもSPは「初めから最後の受験者まで基準を変えないで評価をすること」「他のSPと評価を合わせること」が難しいことの上位に上がった．ここで興味深いのは，SPは評価で「他のSPとの標準化」より「自分の中での基準統一」の方が難しいと感じているSPが多かったことである．自分の中での統一と他のSPとの統一はいずれもOSCE特有の標準化する能力を求められることに由来する難しさである．本来コミュニケーションは言葉やしぐさなどを通して受け手に意味付けされるもので，その言葉やしぐさは「恣意性」という特徴を持つ．そのため，その時々の状況に応じてその意味が変化するもので，解釈・意味付けには個人差が伴う[16]．そこを統一するためにはSP間の解釈のすり合わせが重要である．それとともに，見落しがちなことではあるが，一人ひとりのSPの中での基準を統一する個人内統一にも注目し，3〜4回セッションを続けた後，前後の評価を比較するなどSP間・個人内の縦横の標準化のための練習を確保することも必要であろう．

本研究の限界として3点を述べる．第一に質問項目により，記入率に差があったことから，答えにくい質問があった可能性がある．第二に演技，評価に対する難しさはあくまでもSPの主観に依るものなので謙遜など文化的影響を受けている可能性があるがそれに関しては検討していない．第三に共用試験OSCEでは，評価は任意であるため，現時点への一般化はできない可能性がある．今後の課題として，継続的な調査を行うことと，日本だけでなく他の国々でのSPの実態を調査しSP養成の参考にしたいと考えている．

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Identification of Marker Genes for Differential Diagnosis of Chronic Fatigue Syndrome

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Chronic fatigue syndrome (CFS) is a clinically defined condition characterized by long-lasting disabling fatigue. Because of the unknown mechanism underlying this syndrome, there still is no specific biomarker for objective assessment of the pathological fatigue. We have compared gene expression profiles in peripheral blood between 11 drug-free patients with CFS and age- and sex-matched healthy subjects using a custom microarray carrying complementary DNA probes for 1,467 stress-responsive genes. We identified 12 genes whose mRNA levels were changed significantly in CFS patients. Of these 12 genes, quantitative real-time PCR validated the changes in 9 genes encoding granzyme in activated T or natural killer cells (*GZMA*), energy regulators (*ATP5J2, COX5B*, and *DBI*), proteasome subunits (*PSMA3* and *PSMA4*), putative protein kinase *c* inhibitor (*HINT*), GTPase (*ARHC*), and signal transducers and activators of transcription 5A (*STAT5A*). Next, we performed the same microarray analysis on 3 additional CFS patients and 20 other patients with the chief complaint of long-lasting fatigue related to other disorders (non-CFS patients) and found that the relative mRNA expression of 9 genes classified 79% (11/14) of CFS and 85% (17/20) of the non-CFS patients. Finally, real-time PCR measurements of the levels of the 9 involved mRNAs were done in another group of 18 CFS and 12 non-CFS patients. The expression pattern correctly classified 94% (17/18) of CFS and 92% (11/12) of non-CFS patients. Our results suggest that the defined gene cluster (9 genes) may be useful for detecting pathological responses in CFS patients and for differential diagnosis of this syndrome.

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INTRODUCTION

Chronic fatigue syndrome (CFS) is a clinically defined condition characterized by long-lasting disabling fatigue, resulting in severe impairment in daily functioning and associated symptoms such as memory and concentration difficulties, muscle aches, sleep disturbances, and headache (1). CFS poses a diagnostic challenge because of the unknown mechanism underlying this syndrome and the difficulty in making an objective assessment of pathological fatigue.

Several groups have been searching for reliable biomarkers for diagnosing CFS and have shown altered gene expression profiles in peripheral blood leukocyte populations, which can distinguish the majority of CFS cases (2–5). Recently, a data-intensive analysis has been conducted successfully by the Wichita CFS project (6). In the 2-day in-hospital study, gene expression levels of 20,000 genes in isolated peripheral blood mononuclear cells were analyzed to identify biologically and clinically meaningful signatures of gene expression relevant to classification, diagnosis, and treatment of CFS (6).

Peripheral leukocytes express receptors for stress mediators, such as hormones, neurotransmitters, growth factors, and cytokines. Also, leukocytes produce a number of mediators, including cytokines, some of which can activate the hypothalamus-pituitary-adrenal (HPA) axis (7). Leukocytes may be potential targets for mediators eliciting pathological responses associated with stress-related disorders. CFS has been hypothesized to involve an abnormal response to various stressful experiences such as infection, overwork, or psychological stresses resulting in immunologic dysfunction, dysregulation of the HPA axis, and dysautonomia (8–11). At the same time, psychological and sociocultural factors, when present in patients with CFS, also influence the severity of the illness and treatment outcome (8–10). In

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fact, CFS is accompanied frequently by psychiatric disorders such as mood disorders, and the clinical manifestations of these two conditions partly overlap. Therefore, it is important that physicians are able to make the differential diagnosis between CFS and mood disorders, particularly major depression. However, at present, we have no reliable laboratory tool linking or separating these two disease states (10).

We developed a custom cDNA microarray specifically designed to measure mRNA levels of 1,467 stress-responsive genes in blood (12). Using this microarray, a whole-blood RNA collection system, and real-time PCR, we have identified a cluster of nine genes in blood as marker genes useful for differential diagnosis of CFS.

MATERIALS AND METHODS

Subjects

The present study was approved by the institutional review boards of the Nagoya University School of Medicine. After the experimental procedures were fully explained, written informed consent was obtained from all patients. All procedures were in accordance with the institutional guidelines and the Helsinki Declaration. Patients were recruited from a series of patients referred to the Department of General Medicine, Nagoya University Hospital, Nagoya, Japan. Initially, 11 patients with CFS (four males and seven females; aged 33.4 ± 9.4 years) were selected according to the Centers for Disease Control and Prevention criteria for CFS (1). Next, for a discriminating analysis of CFS versus non-CFS patients, 3 patients with CFS and 20 patients who presented with the chief complaint of general fatigue related to other disorders (non-CFS patients) were enrolled additionally in microarray analysis. Finally, 18 CFS and 12 non-CFS patients also were enrolled in quantitative real-time PCR assay for checking the validity of differential diagnosis. We obtained clinical information concerning current disability, duration of illness, number and nature of accompanying symptoms (Table 1), the clinical data on blood chemistry, and complete blood cell counts (CBC) (Supplementary Table 1) by standard laboratory tests. To confirm the diagnosis, all CFS and non-CFS patients underwent a psychiatric evaluation by a psychiatrist accustomed to confirming CFS patients' diagnoses. Age- and sex-matched healthy volunteers were recruited randomly to each experiment as controls. The controls were free of medication and underwent comprehensive medical examination for past and current health problems. Three months prior to enrollment in this study, all patients were removed from medications being taken.

Measures

RNA preparation, amplification, and hybridization. Venous blood (5 or 10 mL) was taken from patients and healthy volunteers under fasting conditions before lunch. Whole blood was poured directly into the PAXgene Blood RNA tube (Becton Dickinson, Franklin Lakes, NJ, USA). Total RNA was extracted from the whole blood mixture using a PAXgene Blood RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Contaminating DNA was removed using an RNase-free DNase kit included in the PAXgene Blood RNA kit (Qiagen). The quality of the purified RNA and its applicability for microarray analysis was assessed by the Agilent 2100 Bioanalyzer using an RNA 6000 Nano Labchip kit (Agilent Technologies, Palo Alto, CA, USA). Quality of RNA was considered to be acceptable when the RIN value was > 8.0. All RNA samples fulfilled this criterion. The labeling of RNA was done by an indirect aminoallyl labeling methodology. Five μg of total RNA was first reverse transcribed with oligo dT primer conjugating T7 sequence. The yield of first strand cDNA complementary to poly(A) RNA was amplified by using a MEGAscript T7 *in vitro* RNA transcription kit (Applied Biosystems, Foster City, CA, USA). Amplified RNA (6 μg) was reverse transcribed by using random hexamer and aminoallyl-dUTP. The synthesized cDNA was labeled by reaction with a dye (NHS-ester Cy5 or Cy3; Amersham Biosciences, Piscataway, NJ, USA). Cy5-cDNAs prepared from each patient were mixed with the equivalent amount of Cy3-cDNAs from the respective healthy subject, and the mixture was applied to the cDNA microarray. Hybridization was performed at 62° C for 12 h. After washing, fluorescence intensity at each spot was assayed using a scanner (ScanArray 5000; GSI-Lumonics, Billerica, MA, USA).

Microarray analysis. The construction of our microarray already has been described (12). To minimize non-specific hybridization reactions, mainly with hemoglobin RNAs, we selected 1,467 genes whose mRNAs were confirmed to be detectable in whole blood RNA samples by reverse transcriptase-PCR. The genes carried on our cDNA microarray are categorized into stress hormones, neurotransmitters, cytokines, growth factors, receptors, signal transduction molecules, transcription factors, heat shock proteins, growth- or apoptosis-associated factors, metabolic enzymes, and others (see Supplementary Table 2).

Signal intensities of Cy5 and Cy3 were quantified and analyzed by subtracting backgrounds, using the QuantArray software (GSI-Lumonics). The global normalization was performed by scaling the Cy3 signal intensities to the median Cy5:Cy3 ratio. The normalized values for duplicate cDNA probes were averaged. Then, we selected 1,072 genes having fluorescence intensities higher than a cut-off value of 300 in either Cy5 or Cy3 conditions among all samples. The relative expression values (Cy5:Cy3) for 1,072 genes were subjected to hierarchical clustering using the GeneSpring 7.3 software (Agilent). Average linkage and cosine θ were used for clustering algorithm and the calculations of distance metric, respectively. After the Cy5:Cy3 ratios of 1,072 genes were transformed to logarithm base 2, statistical significance between CFS patients and age- and sex-matched controls was examined by the paired *t* test statistic using the

Table 1. Clinical features of patients

Patient Number	Age	Gender	Duration of illness (years)	Clinical diagnosis	Symptoms according to the revised CDC criteria for CFS								
					Unexplained fatigue	Impairment in memory	Sore throat	Tender nodes	Muscle pain	Multijoint pain	Headaches	Un-refreshing sleep	Post-exertional malaise
1	41	F	12	CFS	+	+	–	–	–	+	+	+	+
2	25	M	2	CFS + Adjustment disorder	+	+	–	–	–	–	+	+	+
3	35	M	3	CFS	+	–	–	+	–	+	+	+	+
4	30	F	5	CFS	+	+	+	+	+	+	+	+	+
5	27	F	1	CFS	+	+	–	–	+	+	+	+	+
6	25	F	7	CFS + Mood disorder	+	+	+	–	–	+	+	+	+
7	26	F	3	CFS	+	+	–	–	+	+	+	+	+
8	46	F	13	CFS	+	+	–	–	–	+	+	+	+
9	29	M	2	CFS + Mood disorder	+	+	–	+	–	–	+	+	+
10	30	M	1	CFS	+	+	–	–	+	–	+	–	+
11	53	F	5	CFS	+	+	–	+	–	–	+	+	+
				Additionally enrolled patients for microarray analysis									
12	28	F	2	CFS + Adjustment disorder	+	+	–	+	–	–	+	+	–
13	24	F	8	CFS	+	+	+	+	–	–	–	+	+
14	25	M	5	CFS + Mood disorder	+	+	+	–	+	+	–	+	+
15	30	F	3	Mood disorder	+	+	+	–	+	+	+	+	+
16	24	F	3	Somatoform disorder	+	–	+	+	–	+	–	+	–
17	45	F	12	Personality disorder	+	+	–	–	–	–	–	–	+
18	38	F	3	Mood disorder	+	–	–	–	–	+	+	+	–
19	39	F	1	Adjustment disorder	+	+	+	–	+	+	+	+	–
20	25	F	0.5	Eosinophillia	+	–	–	+	+	+	+	+	–
21	42	M	13	Diabetes mellitus type II + hyper-CK-emia	+	–	–	+	–	–	–	+	–
22	34	M	0.5	Mood disorder	+	+	–	–	+	–	–	+	+
23	15	M	1	Sleep disorder	+	+	–	+	–	–	–	+	+
24	42	F	8	Mood disorder	+	+	–	+	–	+	+	+	–
25	35	F	5	Anxiety disorder	+	+	–	–	–	–	+	+	–
26	60	F	5	Mood disorder	+	–	+	–	–	+	+	+	–
27	23	F	1	Mood disorder	+	+	+	–	–	+	+	+	–
28	41	F	2	Mood disorder	+	–	–	+	+	–	–	+	–
29	30	M	4	Adjustment disorder	+	+	+	–	+	–	+	+	+
30	17	F	1	Adjustment disorder	+	–	+	–	+	+	+	–	–
31	46	M	7	Unexplained fatigue	+	+	+	–	+	+	+	–	+
32	31	F	4	Unexplained fatigue	+	+	+	+	+	–	+	+	+
33	23	F	0.5	Unexplained fatigue	+	–	+	+	–	–	–	+	+
34	33	M	3	Adjustment disorder	+	+	–	–	–	–	+	+	+

Continued

Table 1. *Continued*

					Additionally enrolled patients for quantitative real-time PCR								
35	45	F	0.5	CFS	+	+	+	+	+	+	+	+	+
36	13	F	0.75	CFS + Adjustment disorder	+	+	+	–	+	+	+	+	+
37	28	F	7	CFS + Adjustment disorder	+	+	+	–	+	–	–	+	+
38	31	F	2.5	CFS	+	–	+	+	+	+	+	+	+
39	24	F	4	CFS	+	–	+	+	+	+	+	+	+
40	25	M	2	CFS + Adjustment disorder	+	+	+	–	+	–	+	+	+
41	25	F	3	CFS + Adjustment disorder	+	+	+	–	+	–	+	+	+
42	35	M	10	CFS	+	+	+	–	+	+	+	+	+
43	27	F	10	CFS	+	+	–	–	+	+	+	+	+
44	29	M	3	CFS	+	+	+	–	–	–	+	+	+
45	41	F	15	CFS	+	+	+	–	–	+	+	+	+
46	45	M	7	CFS	+	+	+	+	+	+	+	+	+
47	46	F	0.8	CFS + Secondary depression	+	+	+	–	+	–	+	–	–
48	35	M	1.1	CFS + Major depression	+	+	–	+	–	+	–	+	+
49	53	M	6	CFS	+	+	+	–	–	+	–	+	+
50	26	M	4	CFS + Secondary depression	+	+	+	–	+	+	+	+	+
51	38	M	6	CFS	+	+	+	–	–	–	+	+	+
52	26	F	1	CFS	+	+	+	+	+	+	+	+	+
53	37	F	1	Major depression	+	+	–	–	+	+	–	+	+
54	48	F	1.5	Adjustment disorder	+	+	+	–	+	+	–	+	+
55	31	F	5	Adjustment disorder	+	–	–	–	–	+	+	–	+
56	28	M	5	Major depression	+	+	+	–	+	+	+	+	+
57	26	M	2	Unexplained fatigue	+	+	–	–	–	–	–	+	+
58	72	F	5	Unexplained fatigue	+	+	–	–	+	+	+	+	+
59	41	F	4	Adjustment disorder	+	+	+	+	–	+	+	+	+
60	38	F	9	Adjustment disorder	+	–	–	–	+	–	+	–	+
61	33	F	7	Adjustment disorder	+	+	–	–	–	–	+	+	+
62	32	M	2.5	Hypo-cerebrospinal fluid	+	–	–	–	+	–	–	+	+
63	28	F	4	Mood disorder	+	+	–	–	–	+	+	+	+
64	37	M	1	Major depression + Sleep apnea	+	+	–	–	+	–	–	+	+

CK, creatine kinase.

Cyber-T stats program written in the R stats language (see http://cybert.microarray.ics.uci.edu/help/index.html) (13). Statistical significance was defined as a Bonferroni-corrected *P* value of < 0.05, after the problem of a multiple test was addressed.

Quantitative real-time PCR. cDNA was prepared from total RNA (0.5 μg) using oligo dT primer according to the instructions of SuperScript II reverse transcriptase kit (Invitrogen, Carlsbad, CA, USA). The mRNA levels of ten target genes based on GenBank accession numbers (Table 2) were analyzed by quantitative real-time PCR using predesigned, gene-specific TaqMan probes and primer sets (search the batch ID for each gene in Table 2 at https://products.appliedbiosystems.com/ab/en/US/adirect/ab?cmd=ABGEBatchSearch) and the ABI-PRISM 7500 sequence detection system (Applied Biosystems, Foster City, CA, USA). Appropriate predesigned TaqMan probe and primer sets for detecting specific mRNA types of *COX7C* and *HSPA2* were not available because of their gene structure (Applied Biosystems). Each PCR reaction was performed according to the protocol of TaqMan Universal PCR Mastermix (Applied Biosystems), and data were analyzed using SDS 2.2 software (Applied Biosystems). A no template control and a no RT step control also were run for every reaction to see that the amplification was not off genomic DNA. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an endogenous quantity control, and quantity values were normalized to *GAPDH* mRNA expression. After the relative ratio of each mRNA between CFS patients and control subjects was calculated, the unpaired *t* test was performed to compare the relative ratio for each gene in the microarray ($n = 11$) versus quantitative real time PCR results ($n = 11$). Finally, we also examined relative mRNA ratios of the target genes in newly enrolled 18 CFS and 12 non-CFS patients using age- and sex-matched healthy subjects as controls by quantitative real-time PCR.

Table 2. List of TaqMan probes and primer sets

Gene symbol	GenBank accession number	Batch ID	Slope in standard curve (Ct/Quantity)
GZMA	M18737	Hs00196206_m1	-3.58
ATP5J2	AF047436	Hs00934710_m1	-3.30
DBI	BC006466	Hs01554584_m1	-3.69
HINT	U51004	Hs00602163_m1	-3.19
KIAA0194	D83778	Hs00412357_m1	-3.77
COX5B	NM_001862	Hs00426948_m1	-3.24
ARHC	L25081	Hs00237129_m1	-3.70
PSMA4	BC005361	Hs00160566_m1	-3.88
PSMA3	BC005265	Hs00541061_m1	-3.09
STAT5A	L41142	Hs00559643_m1	-3.29
GAPDH	M33197	Hs99999905_m1	-3.29

All supplementary materials are available online at molmed.org.

RESULTS

Clinical Features of CFS Patients

The initially enrolled 11 patients with CFS were comprised of four males and seven females whose median age was 33.4 ± 9.4 years. The clinical features of the 11 patients are shown in Table 1. All patients were complaining of debilitating fatigue lasting for longer than one year, and the median duration of fatigue was 4.9 years. Five patients (patients 3, 6, 7, 9, and 10) reported some linkage between infectious episodes and onset of their symptoms, but none of them were positive for serum antibody against *Coxiella burnetti* (Q fever) that could trigger CFS-like symptoms (14), human herpes viruses type 6 and 7, Epstein-Barr virus, or cytomegalovirus (data not shown). Biochemical data also demonstrated the absence of current active infection (data not shown). CBC of one patient (patient 6) was not measured at the time of sampling for microarray analysis, since the medical record from another hospital described no abnormalities in CBC and leukocyte populations. CBC data and white blood cell differential counts of the other patients were normal (Supplementary Table 1). All 11 patients showed normal BMI values (20.3 ± 2.2, mean ± SD) (see Supplementary Table 1). Three CFS patients (patients 2, 6, and 9) had past histories of major depression or adjustment disorder. We consulted the psychiatrist and confirmed that these disorders were not active during the experimental period.

Gene Expression Profile in Patients with CFS

Hierarchical cluster analysis of 1,072 genes whose fluorescence intensities were higher than a cut-off value of 300 in either Cy5 or Cy3 conditions among all samples suggested the presence of genes that commonly changed among all patients, compared with age- and sex-matched controls (data not shown). The statistic analysis of 1,072 genes (paired *t* test; Bonferroni [experiment-wide false positive rate] adjusted *P* value = 0.05) identified 12 genes whose mRNA levels were changed significantly in CFS patients compared with the healthy controls (Figure 1). Several upregulated genes were categorized into regulators of energy metabolism; a mitochondrial ATP subunit (*ATP5J2*; f subunit of the F0 complex), nuclear-encoded subunits of the mitochondrial cytochrome *c* (*COX7C* and *COX5B*), and an intracellular acyl-coenzyme A transporter (*DBI*). The significantly upregulated genes also contained a cytotoxic T lymphocyte- and natural killer cell-specific serine protease, granzyme A (*GZMA*), a member of the ras homolog gene family (*ARHC*), and proteasome subunits (*PSMA3* and *PSMA4*). The mRNA levels of two other genes encoding a protein with unknown function (*KIAA0194*), and a putative pro-

Figure 1. Identification of 12 CFS-associated genes in patients with CFS. The expressions of 1,072 genes were compared between 11 patients with CFS and age- and sex-matched healthy controls, and 12 common genes were identified that were significantly changed in CFS patients according to the paired *t* test using the cyber-T stats program (Bonferroni wide false positive rate = 0.05). Heatmap values represent the relative ratios of gene expressions between CFS and age- and sex-matched healthy controls. Hierarchical clustering was performed by the results of average linkage and distance metric of cosine θ. Gene symbols, *P* values, GenBank accession numbers, and names of the 12 genes are listed. *P* values are based on paired *t* tests. Patient's numbers indicated at the bottom of column are corresponding to the numbers in Table 1.

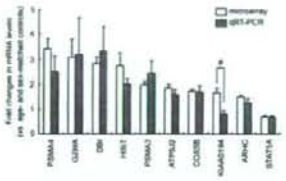

Figure 2. Relative expression of 10 marker genes in patients with CFS by microarray and real-time PCR. RNA prepared from 11 patients with CFS and age- and sex-matched healthy controls was subjected to TaqMan real-time PCR as described in the method section. Of the 12 genes, 10 mRNA levels were measured and then normalized to *GAPDH* mRNA expression. After the relative ratios of 10 mRNAs between CFS patients and control subjects were calculated, they were compared between microarray (empty bars) and real time PCR results (solid bars). Values are mean fold changes ± SD ($n = 11$). $^{\#}P < 0.05$ by the paired *t* test.

tein kinase C inhibitor of the HINT family (*HINT*) also were elevated significantly. In contrast, CFS patients showed decreased mRNA levels of heat shock 70-KDa protein 2 (*HSPA2*) and a member of the signal transducer and activator of transcription (STAT) family of transcription factors (*STAT5A*).

Quantitative Real-Time PCR

Although we applied the indirect labeling method to reduce the extent of cyanine dye bias in our microarray assay, there may be some concerns regarding the possibility of the genes exhibiting some dye-bias in dual-labeled spotted cDNA microarrays. Therefore, we performed TaqMan real-time PCR to validate the microarray data. The mRNA levels of two genes (*COX7C* and *HSPA2*) were not measured, since appropriate TaqMan probes for these genes were not available. As for the rest of genes, we confirmed that each PCR reaction had a similar efficiency of reaction when we checked the slope in a standard curve for each PCR reaction using the same total RNA from whole blood as standard (Table 2). Among the 10 mRNA levels measured, real-time PCR did not demonstrate any significant change in the *KIAA0194* mRNA level, while the other nine mRNA levels in CFS patients were confirmed to be changed significantly (Figure 2).

Microarray Analysis of CFS and Non-CFS Patients with Prolonged Fatigue

To test whether gene expression profiling could be useful for differential diagnosis of CFS, 3 CFS patients and 20 patients who presented with the chief complaint of general fatigue related to other disorders (non-CFS patients) were enrolled in this study additionally. Relative gene expression levels of the CFS and non-CFS patients also were measured by the dual-labeled cDNA microarray using age- and sex-matched healthy subjects as controls. RNA samples from the newly added patients (3 CFS and 20 non-CFS) and age- and sex-matched healthy controls were labeled Cy5 and Cy3, respectively. All 20 non-CFS patients complained of abnormal fatigue lasting for more than 6 months, while their clinical features did not completely meet the CDC criteria for CFS (Table 1). First, we compared gene expression profiles of 1,072 genes in 14 CFS patients, including 3 additionally enrolled patients, and 20 non-CFS patients. Hierarchical cluster analysis of the relative mRNA levels of 1,072 genes showed that gene expression patterns could be classified roughly into CFS and non-CFS patterns, but it was difficult to draw a margin between the two patterns (data not shown).

Next, we tested whether the changes in nine genes, whose expressions were confirmed to be changed significantly between 11 CFS patients and healthy subjects by both microarray (see Figure 1) and quantitative real-time PCR (see Figure 2), could exclude non-CFS patients. As shown in Figure 3, the hierarchical clustering of the expression of nine genes classified 34 patients into two groups (A and B) or 3 groups (A, B1, and B2). Group A branches contained 13 CFS patients and 3 non-CFS patients (mood disorder, 30-year-old female; somatoform disorder, 24-year-old female; personality

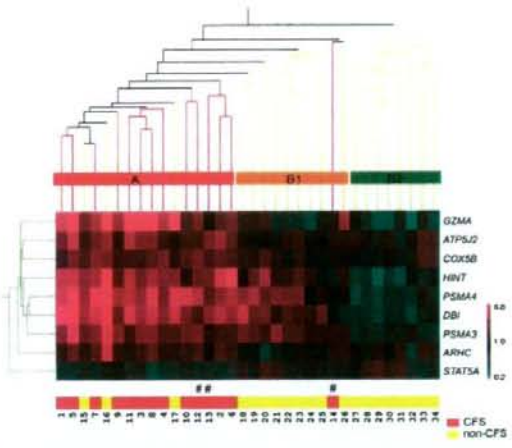

Figure 3. Expression of nine marker genes in CFS and non-CFS cases. RNA was prepared from whole blood of 3 additionally enrolled patients with CFS and 20 patients with the chief complaint of general fatigue related to other disorders, and subjected to the microarray analysis. The expression patterns of nine genes in these patients were compared with those of the initially enrolled 11 patients with CFS by hierarchical cluster analysis. CFS and non-CFS cases are indicated as red and yellow bars, respectively. Patient's numbers indicated at the bottom of columns are corresponding to the numbers in Table 1. #Three newly enrolled patients with CFS.

disorder, 45-year-old female). Among 10 branches of group B1, only 1 CFS patient was included. All branches of group B2 were composed of non-CFS patients. Thus, the cluster analysis of relative mRNA levels of nine genes measured by the microarray suggested that the nine marker genes might be useful for differential diagnosis of CFS.

Use of Nine Marker Genes for Differential Diagnosis of CFS

Finally, we tested whether the nine marker genes could be useful for differential diagnosis of CFS. To correctly assess this issue, we omitted the 11 patients in whom we had identified the nine genes. A total of 18 newly enrolled CFS patients and 12 non-CFS patients (Table 1) were subjected to quantitative real time RT-PCR analysis using *GAPDH* mRNA as an endogenous quantity control. Age- and sex-matched healthy subjects (total 30 subjects) also were used as controls of individual patients. As shown in Figure 4A, the expression levels of six genes (*PSMA4, PSMA3, HINT1, DBI, GZMA,* and *ATHC*) out of nine genes in 18 CFS patients were significantly different from that in 12 non-CFS patients. The hierarchical clustering of the expression of nine genes classified 30 patients into two groups (*a* and *b*) (Figure 4B). Group *a* branches contained 17 CFS patients and 1 non-CFS patient (major depression, 37 F). Among 12 branches of group *b*, 1 case of CFS was included. Thus, the expression pattern of nine genes could distinguish the majority of our CFS patients from non-CFS patients.

DISCUSSION

The microarray or differential display approach has been used to examine the CFS-specific gene expression in peripheral blood mononuclear cells (2–5). Two sources are commonly used for preparation of RNA, whole blood, or its leukocyte populations (15). Because of advantages and disadvantages associated with both systems (16–20), at present there is no consensus regarding the optimal technique for isolation of RNA from peripheral blood. A whole-blood RNA collection system is appealing, particularly in clinical settings, since the RNA isolation method is easy to use and reduces operator time and sample volume. In addition, this system reduces the risk of exposure of laboratory personnel to biohazards relative to the risk involved in isolation of leukocyte populations.

Using RNA from whole blood, we show here that both microarray analysis and real-time PCR identify nine genes whose mRNA expression are significantly different in 11 patients with CFS, compared with age- and sex-matched healthy controls. Although the individual genes identified as CFS-related genes did not overlap with those identified in other studies (2–5), most them could be categorized into distinct clusters, including host defense, energy metabolism, or small G protein-dependent signal transduction (2–5). The significance of our study can be considered from three different perspectives.

First, the identified genes are informative in considering the pathophysiology of CFS. The upregulated *GZMA* encodes a T cell- and natural killer cell-specific serine protease that functions as a common component necessary for lysis of target cells by these cytotoxic cells. The proteasome subunits *PSMA3* and *PSMA4* also were upregulated. The proteasome is the central proteolytic system that also plays an important role in the major histocompatibility complex-class I antigen processing. Previous studies identified genes involved in T cell activation (2–5). Our findings also suggest that patients with CFS may have altered immunity, such as that involved in anti-viral defense. As reported in other studies (3,5), we also have identified genes encoding molecules catalyzing oxidative phosphorylation in mitochondria (*COX5B* and

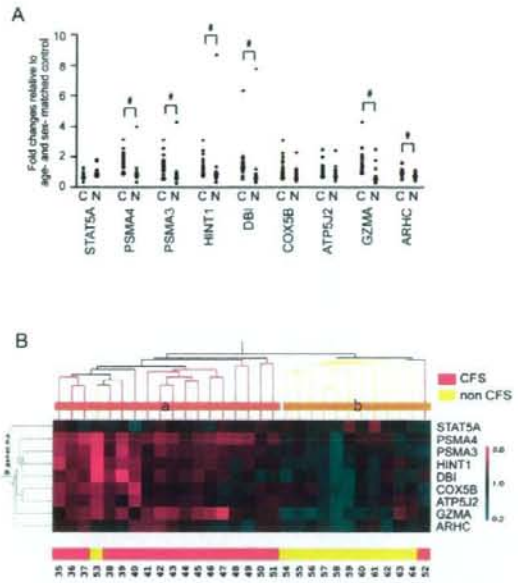

Figure 4. Differentiation of CFS and non-CFS cases by expression levels of nine marker genes. (A) The expressions of nine marker genes were validated by real-time PCR, and their mean values were compared in 18 newly enrolled CFS patients (indicated as "C") with 12 non-CFS patients (indicated as "N"). Values are mean fold changes ± SD. $^{\#}P < 0.05$ by the unpaired *t* test. (B) The expression levels of nine genes from 18 CFS and 12 non-CFS patients were differentiated using hierarchical cluster analysis by the results of average linkage and distance metric of cosine θ. CFS and non-CFS patients are indicated as red and yellow bars, respectively. Patient's numbers indicated at the bottom of columns are corresponding to the numbers in Table 1.

ATP5J2). *COX5B* and *ATP5J2* encode a cytochrome *c* oxidase subunit and a subunit of the mitochondrial proton channel, respectively. Although we were unable to measure the mRNA level of another cytochrome *c* oxidase subunit (*COX7C*) by real time PCR, these nuclear-encoded subunits (*COX5B* and *COX7C*) function in the regulation and assembly of the cytochrome *c* oxidase complex and mitochondrial ATPase. In addition, our CFS patients had significantly increased *DBI* mRNA levels. The diazepam binding inhibitor (DBI) is known as a GABA receptor modulator or acyl-coenzyme A (acyl-CoA) binding protein (ACBP). ACBP binds thiol esters of long fatty acids and coenzyme A in a one-to-one binding mode with high specificity and affinity. This molecule is suggested to act as an intracellular acyl-CoA transporter and to form a pool of ACBP-acyl-CoA complex that is an important intermediate in lipid synthesis and fatty acid degradation that participates in regulating intermediary metabolism and gene expression. The increased mRNA expression of *DBI*, *COX5B*, and *ATP5J2* strongly suggests abnormalities in energy metabolism in our CFS patients.

We also found that the *STAT5A* mRNA level was decreased significantly in CFS patients. The protein encoded by *STAT5A* is a member of the STAT family of transcription factors. STAT-5 mediates the signal transduction triggered by various cell ligands, such as IL2, IL4, colony-stimulating factor 1, and growth hormones. Adult growth hormone deficiency (AGHD) is a CFS-like disorder characterized by fatigue, tiredness, and myalgia; replacement therapy with human growth hormone improves these symptoms (21). Growth hormone activates STAT1, 3, 5A, and 5B in different cell systems (22). Webb *et al.* reported that STAT-5 isoform, but not STAT-1 or STAT3, were increased markedly in skeletal muscles in patients with AGHD and suggested that the STAT5 signal transduction pathway in skeletal muscle might be abnormal in AGHD (21). The decreased expression of *STAT5A* mRNA in peripheral blood cells from CFS patients suggests that the abnormality in STAT5 signaling might be associated with symptoms of CFS.

In the Wichita study directed by CDC (6), fatigue-associated gene expression patterns in isolated blood mononuclear cells were identified by several groups sharing the same data sets. Most of the groups in that study did not divide subjects into CFS and non-CFS cases by CDC classification but focused instead on fatigue itself and accompanying symptoms for elucidation of fatigue-associated genes. It was confirmed that 9 of 16 genes reported by Kaushik *et al.* as differentially expressed genes in CFS (5) also were included among fatigue-associated genes measured by quantitative trait analysis (QTA) in the Wichita study (23). Our study also revealed that two genes, *STAT5A* and *COX5B*, were categorized in the same pathways as *STAT5B* and *COX7A2*, which were identified as fatigue-associated genes according to QTA. *STAT5A* and *COX5B* belong to the Jak-STAT signaling pathway and oxidative phosphorylation pathway, respectively. Furthermore, Fang *et al.* in the

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《原著論文》

模擬患者の協力を得た身体診察実習の今後の方向性

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Perceptions of simulated/standardized Patients (SPs) and SP trainers to have SPs involved in physical examination in the future.

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A 27-item questionnaire of simulated/standardized patients (SPs) and a 39-item questionnaire of SP trainers were completed to evaluate the present status of SPs' and SP trainers' activities and attitudes toward physical examinations. Thirty-three SP trainers (61%) and 332 SPs (62%) responded. Of the respondents, 54 SPs had experiences with physical examination trainings. About 76% of SPs without physical examinations experiences and SP trainers and 98% of SPs with physical examinations experiences perceived that medical students learn clinical skills and communication skills during physical examinations more effectively if SPs participated in their trainings. As for examined body areas, about 80% of SPs expressed highly favorable attitudes toward examination of head & neck, arms and legs, however, only about 25 % of SPs expressed favorable attitudes toward examination of chest, back and abdomen with disrobing. SPs' males or over 50 years old are more accepting of chest, back and abdomen examinations. These results indicate that SPs would be willing to participate in physical examination training for medical students, with varying levels of willingness depending on gender, age and the body areas in question. SPs recognized the value of participating in physical examination. Information is important in encouraging the SPs to take part. Experience brings acceptance therefore it is suggested that SPs have a phased program, beginning with head, arms and legs, then progressing to abdomen and chest.

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