Multivariate factor analysis and genetic association provide evidence for clinical heterogeneity in fibromyalgia

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INTRODUCTION

❖ Fibromyalgia (FM), a common rheumatological syndrome, is characterized by a diverse set of symptoms including widespread body tenderness, spontaneous pain, fatigue, sleep disturbance, depression, and comorbidity with other somatic conditions.

❖ It is unknown whether fibromyalgia is best considered a single disorder, or a collection of conditions with overlapping symptoms but distinct pathophysiologies.

❖ We used a factor/cluster approach to explore the heterogeneity of FM clinical presentation.

❖ We then associated the derived factors and clusters with a panel of single nucleotide polymorph (SNP) markers representing over 350 genes involved in nociception, in order to identify genetic risk factors .

SUBJECTS

Complete baseline data were available for 719 FM patients enrolled in two Pfizer clinical trials.

All subjects met ACR criteria for FM, but were excluded due to 1) any serious medical condition requiring change in drug regimen; 2) major depression; 3) history of malignancy; 4) known pituitary disease; 5) any other endocrine disorder including diabetes mellitus.

Phenotypic data for these FM subjects were obtained from the following questionnaires, some of which yield several different subscales:

- FIQ – Fibromyalgia Impact Questionnaire
- HAQ – Fibromyalgia Health Assessment Questionnaire
- MAF – Multidimensional Assessment of Fatigue
- MOS-Sleep – Medical Outcome Study – Sleep Scale
- MPQ – McGill Pain Questionnaire
- SDS – Sheehan Disability Scale
- DDM – Daily Diary Measures
- SF36 – Short Form 36
- HADS – Hospital Anxiety and Depression Scale

Test	Subscale	Mean	SD	Range
Cohort Description	Age (years)	48.7	10.9	18–82
	Sex (% female)	94.4%	--	--
	Race (% White)	90.7%	--	--
FIQ	Disability in Daily Activity	64.39	13.39	23.94–99.10
HAQ	Dressing and Grooming	0.78	0.72	0–3
	Arising	1.18	0.69	0–3
	Eating	0.69	0.75	0–3
	Walking	0.92	0.76	0–3
	Hygiene	1.05	0.97	0–3
	Reach	1.22	0.76	0–3
	Grip	0.82	0.86	0–3
	Daily Activities	1.49	0.79	0–3
MAF	Severity	16.59	3.06	3–20
	Distress	7.30	2.39	1–10
	Interference	5.48	2.00	0.82–10
	Frequency	9.39	1.28	2.5–10
MOS-Sleep	Disturbance	67.27	23.63	0–100
	Adequacy	20.54	22.08	0–100
	Somnolence	50.71	24.00	0–100
	Hours of Sleep	5.37	1.56	0–12
MPQ	Affective Component	19.25	6.38	2–33
	Sensory Component	1.36	0.72	0–3
	Pain in Past Week (VAS	76.3	13.57	40–100
SDS	Social Life Disruption	5.89	2.52	0–10
	Family/Home Disruption	6.22	2.37	0–10
DDM	Mean Pain (VAS)	7.04	1.32	0–10
	Mean Sleep Quality (VAS)	6.70	1.64	0–10
SF36	General Health	57.07	11.91	25–100
	Physical Function	43.42	22.84	0–95
	Role Physical	34.92	22.54	0–100
	Bodily Pain	26.09	13.55	0–100
	Vitality	22.66	16.99	0–68.75
	Social Function	46.87	26.46	0–100
	Role Emotional	60.62	30.45	0–100
	Mental Health	57.75	21.68	0–100
HADS	Score	8.36	4.22	0–21

GENOTYPING

Pain Research Panel

- 3295 SNPs corresponding to >350 candidate genes
- targeted at genes linked to pain neurotransmission, inflammation, mood
- SNP selection weighted toward putatively functional SNPs (missense mutations, promoter regions, splice junctions)
- high linkage disequilibrium (LD) coverage of complete gene loci
- more information available online from Beckman-Coulter Genomics: *http://www.beckmangenomics.com/genomic_services/genotyping/pain_research_panel.html*

Quality Assessment / Control

- performed in PLINK 1.4
- samples rejected due to:
  - call rate < 95%
  - cryptic relatedness between samples
  - non-Europian ancestry
- SNP markers eliminated based on:
  - call rate < 95%
  - minor allele frequency < 2%
  - repeatability across duplicated samples > 99%
- Hardy-Weinberg disequilibrium used to detect possible calling errors
- 2760 SNPs (83.8%) passed all QC filters

PRINCIPAL COMPONENTS ANALYSIS

Principal components analysis (PCA) was employed using questionnaire data (including the HAQ, FIQ, MAF, MOS-Sleep, MPQ, and SDS) to empirically derive underlying symptom domains. PCA with varimax (orthogonal) rotation was performed using the SAS statistical package (Cary, NC). Evaluation of the eigenvalues and the scree plot revealed a four-factor solution best captured the underlying phenotypes. Factor loadings above 0.40 are represented in the accompanying table.

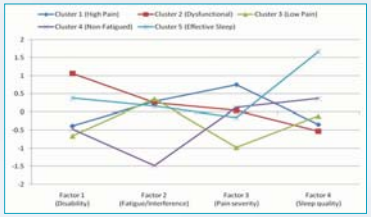
The factors were validated by determining whether they correlated with daily pain and sleep diaries and HADS and SF36 scores, for related symptom domains. Reliability was assessed by splitting the dataset in half and replicating the PCA in both halves, resulting in similar variable loadings in both halves.

Raw Variable	Factor 1 Disability	Factor 2 Fatigue/ Interference	Factor 3 Pain Severity	Factor 4 Sleep Quality
HAQ				
Dressing and Grooming	0.68			
Arising	0.67			
Eating	0.60			
Walking	0.71			
Hygiene	0.68			
Reach	0.76			
Grip	0.55			
Daily Activities	0.69			
FIQ				
Disability in Daily Activity		0.54	0.54	
MAF				
Severity		0.79		
Distress		0.71		
Interference		0.59	0.41	
Frequency		0.68		
MOS-Sleep				
Disturbance				-0.64
Adequacy				0.61
Somnolence		0.55		
Hours of Sleep				0.80
MPQ				
Affective Component			0.77	
Sensory Component			0.72	
Pain in Past Week (VAS)			0.73	
SDS				
Social Life Disruption	0.40	0.51	0.44	
Family/Home Disruption	0.44	0.53	0.43	

CLUSTERING

These four factors were then used to distinguish five clusters of FM patients with similar clinical presentations. Factor scores were subjected to an agglomerative hierarchical cluster analysis using Ward's linkage for evaluating distance between clusters.

A five-cluster solution emerged, which was labeled according to the most representative factor scores. The clusters were also validated using HADS and SF36 scores.

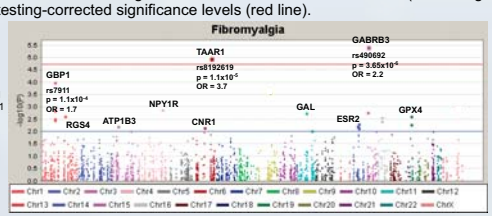


	N	Factor 1 Disability	Factor 2 Fatigue/Interference	Factor 3 Pain Severity	Factor 4 Sleep Quality
Cluster 1 (High Pain)	188	−0.38	0.29	**0.75**	−0.34
Cluster 2 (Dysfunctional)	184	**1.06**	0.25	0.04	−0.53
Cluster 3 (Low Pain)	152	−0.66	0.36	**−0.98**	−0.11
Cluster 4 (Non-Fatigued)	114	−0.47	**−1.48**	0.12	0.37
Cluster 5 (Effective Sleep)	81	0.38	0.16	−0.15	**1.67**

FIBROMYALGIA GENES

After data cleaning, genotyping was available for 370 FM subjects, and a total of 316 pain-free subjects from other scientific protocols were introduced as controls for a genetic association study. Logistic regression (adjusted for age) was utilized to compare genotype frequencies between all FM cases and controls, as well as between the separate FM clusters and cases. Several genes were associated with FM status (combining all clusters) at multiple testing-corrected significance levels (red line).

1. **GABRB3** = GABA receptor B3 subunit
2. **TAAR1** = trace amine-associated receptor 1
3. **GBP1** = G protein-binding protein 1
4. **NPY1R** = neuropeptide Y receptor 1
5. **GAL** = galanin
6. **GPX4** = glutathione peroxidase 4
7. **RGS4** = regulator of G protein signaling 4
8. **ESR2** = estrogen receptor 2
9. **ATP1B3** = Na+/K+ transporting ATPase beta 3
10. **CNR1** = cannabinoid receptor 1



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FACTOR/CLUSTER ASSOCIATIONS

The PCA components were associated with Pain Research Panel SNPs as quantitative traits in a linear regression, incorporating age as a covariate.

Factor 1 Disability			Factor 2 Fatigue/Interference			Factor 3 Pain Severity			Factor 4 Sleep Quality		
GENE	SNP	P	GENE	SNP	P	GENE	SNP	P	GENE	SNP	P
PRKCE	rs3754565	0.00045	PRKG1	rs1409351	5.60E−05	P2RX7	rs208288	0.0013	EPHB2	rs4654824	0.00067
RAP1A	rs10776733	0.00053	ETV1	rs10215655	0.00028	GRIA4	rs1445604	0.0024	CHUK	rs11597086	0.0013
FPRL1	rs10853843	0.0016	CCKAR	rs2725301	0.00034	GRIA1	rs3813470	0.0025	CALCA	rs1553005	0.0014
NTSR1	rs3746780	0.0026	TAAR2	rs3813353	0.00079	SLC1A3	rs10491374	0.0030	GRK5	rs4751716	0.0022
CACNA1A	rs2074879	0.0026	D8H	rs3025388	0.0010	CX3CL1	rs170364	0.0038	ACCN1	rs28935	0.0027
DRD3	rs10934256	0.0028	GLRA2	rs6630811	0.0015	GABRB3	rs4906902	0.0040	FACL2	rs10027540	0.0029
ACCN1	rs8069909	0.0036	CDK5	rs756785	0.0021	5−HT1B	rs130058	0.0053	DRD3	rs2630349	0.0031
NRG1	rs7007436	0.0036	MME	rs989692	0.0032	CX3CR1	rs2853707	0.0055	KLKB1	rs4253252	0.0035
GRIA1	rs4385264	0.0038	ESR1	rs9340820	0.0039	MAP2K1	rs4776783	0.012	CNR1	rs12720071	0.0039
ADRBK2	rs1344079	0.0039	PENK	rs2576573	0.0049	CPN1	rs3829161	0.013	SCN8A	rs1905248	0.0040

The clusters were contrasted against the healthy controls to detect SNPs that associate with the development of clinically-relevant subtypes of FM.

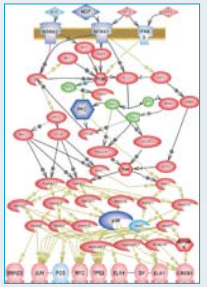
Cluster 1 (n=67) High Pain			Cluster 2 (n=76) Dysfunctional			Cluster 3 (n=66) Adaptive Copers			Cluster 4 (n=49) Non−Fatigued			Cluster 5 (n=24) Effective Sleepers		
GENE	P	OR	GENE	P	OR	GENE	P	OR	GENE	P	OR	GENE	P	OR
GABRB3	3.8E−05	0.20	TAAR1	0.00092	4.2	ATP6V1B2	0.0014	0.44	CALM2	3.1E−05	3.9	TAAR1	7.0E−07	14.1
IL−8	0.0011	1.9	APP	0.0010	2.0	NTSR1	0.0018	0.51	GALR1	0.00091	6.0	KCNJ9	3.8E−05	4.3
GRIN2A	0.0014	1.9	IFRD1	0.0014	1.9	GBP1	0.0027	1.9	DDC	0.0038	2.0	ADORA3	0.0013	2.7
VIL2	0.0018	2.0	ACCN2	0.0016	0.48	PRKCE	0.0030	1.8	SCN10A	0.0041	2.0	AGTR1	0.0027	2.8
PRKCE	0.0023	0.53	GPX4	0.0022	0.54	EPHB3	0.0036	2.4	CAMK4	0.0061	2.7	CALCA	0.0035	2.5
TAAR1	0.0029	3.6	GAL	0.0024	0.15	APP	0.0041	0.51	RGS2	0.0067	2.0	RUNX1	0.0036	2.9
NALP12	0.0033	0.39	GRIA4	0.0028	0.54	KCNJ6	0.0042	2.0	CHRNA5	0.0076	1.9	GBP2	0.0038	2.5
NTRK2	0.0035	2.0	GBP2	0.0030	1.8	CX3CR1	0.0048	1.8	ITGAM	0.0080	1.9	SCN10A	0.0052	2.5
EPHB2	0.0036	0.51	GBP1	0.0030	1.8	EPHB4	0.0058	1.7	KCNJ5	0.0084	0.53	FACL2	0.0056	2.5
TRPM8	0.0038	0.50	DRD3	0.0036	1.8	DDX24	0.0061	0.56	PRKACB	0.0092	2.7	ADRA1D	0.0075	2.8

PATHWAY ANALYSIS

While the contribution of a specific genetic marker may be weak, the interaction between several genetic markers of genes within convergent molecular pathways is likely crucial for disease development.

To explore pain-related pathways that underlie FM, we employed *Pathway Studio* (Ariadne Genomics, Rockville MD), which utilizes the ResNet database containing more than 1.25 million events of regulation and interaction among cellular processes.

Fibromyalgia Pathways	P
EDG3/5 -> AP-1/ELK-SRF signaling	0.033
IGF1R -> MEF/MYOD/MYOG signaling	0.035
NGFR -> NF-kB signaling	0.042
AGER -> NF-kB signaling	0.045
DDR1 -> NF-kB signaling	0.046
EctodysplasinR -> NF-kB signaling	0.046
FibronectinR -> NF-kB signaling	0.046
PTAFR -> NF-kB signaling	0.046
TLR -> AP-1 signaling	0.049
ErythropoietinR -> NF-kB signaling	0.049



Cluster Pathways	P
Cluster 1 (High Pain)	
NTRK -> FOXO/MYCN signaling	0.007
NGFR -> MEF signaling	0.015
TLR3 -> NF-kB signaling	0.043
NTRK -> AP-1/CREB/ELK-SRF/MYC /SMAD3/TP53 signaling	0.048
Cluster 2 (Dysfunctional)	
none	--
Cluster 3 (Low Pain)	
CCR1 -> STAT signaling	0.042
Cluster 4 (Low Pain)	
T-cell receptor -> CREBBP signaling pathway	0.018
TGFBR -> ATF/GADD/MAX/TP53 signaling	0.029
TGFBR -> MEF/MYOD/MYOG signaling	0.029
CCR5 -> TP53 signaling	0.044
Cluster 5 (Effective Sleepers)	
EGFR/ERBB -> STAT signaling	0.007
Skeletal Myogenesis Control	0.013
EGFR/ERBB2 -> CTNNB signaling	0.016
Gonadotrope Cell Activation	0.028
AngiotensinR -> STAT signaling	0.031
EGFR -> CTNND signaling	0.038
EGFR -> ZNF259 signaling	0.038

CONCLUSIONS

- Clusters of FM patients were identified based on symptom patterns of pain, fatigue, disability, and sleep
- Some genetic variants broadly associated with FM status (including GABRB3, TAAR1, and GPB1) were observed within distinct symptom clusters, although others were seen across cluster divisions
- The clustering analysis, as well as all genetic associations and pathway results, need to be confirmed in independent replication studies
- Phenotypic subgroups may reflect the influences of specific cellular pathways, providing rationale for individualized treatment of patients with FM