The OPPERA Case-Control Study:
Putative Risk Factors and Mechanisms for Persistent TMD Pain

Chairpersons

Ambra Michelotti and Richard Ohrbach

OPPERA: Orofacial Pain Prospective Evaluation and Risk Assessment

The OPPERA Case-Control Study: Putative Risk Factors and Mechanisms for Persistent TMD Pain

8:00	John Kusiak	Introductory Remarks Regarding the OPPERA Program
8:05	Gary Slade	OPPERA Study Overview and Quantitative Sensory Testing
8:25	Richard Ohrbach	OPPERA Psychosocial and Clinical Profiles
8:45	William Maixner	OPPERA Study – Emerging Genetic Findings and Discoveries
9:05	Peter Svensson	Commentary
9:10	Ambra Michelotti	Discussion

The OPPERA Case-Control Study:
Putative Risk Factors and Mechanisms for Persistent TMD Pain
IADR San Diego, March 19, 2011

OPPERA Study Overview and Quantitative Sensory Testing

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Disclosure

Gary Slade is a consultant and equity stock holder in Algynomics Inc.,
a company providing research services in personalized
pain medication and diagnostics.

Scope of presentation

Part 1

- Aims of the OPPERA project
- Heuristic model
- Study design
- Overview of methods
- Sociodemographic factors associated with TMD

Part 2

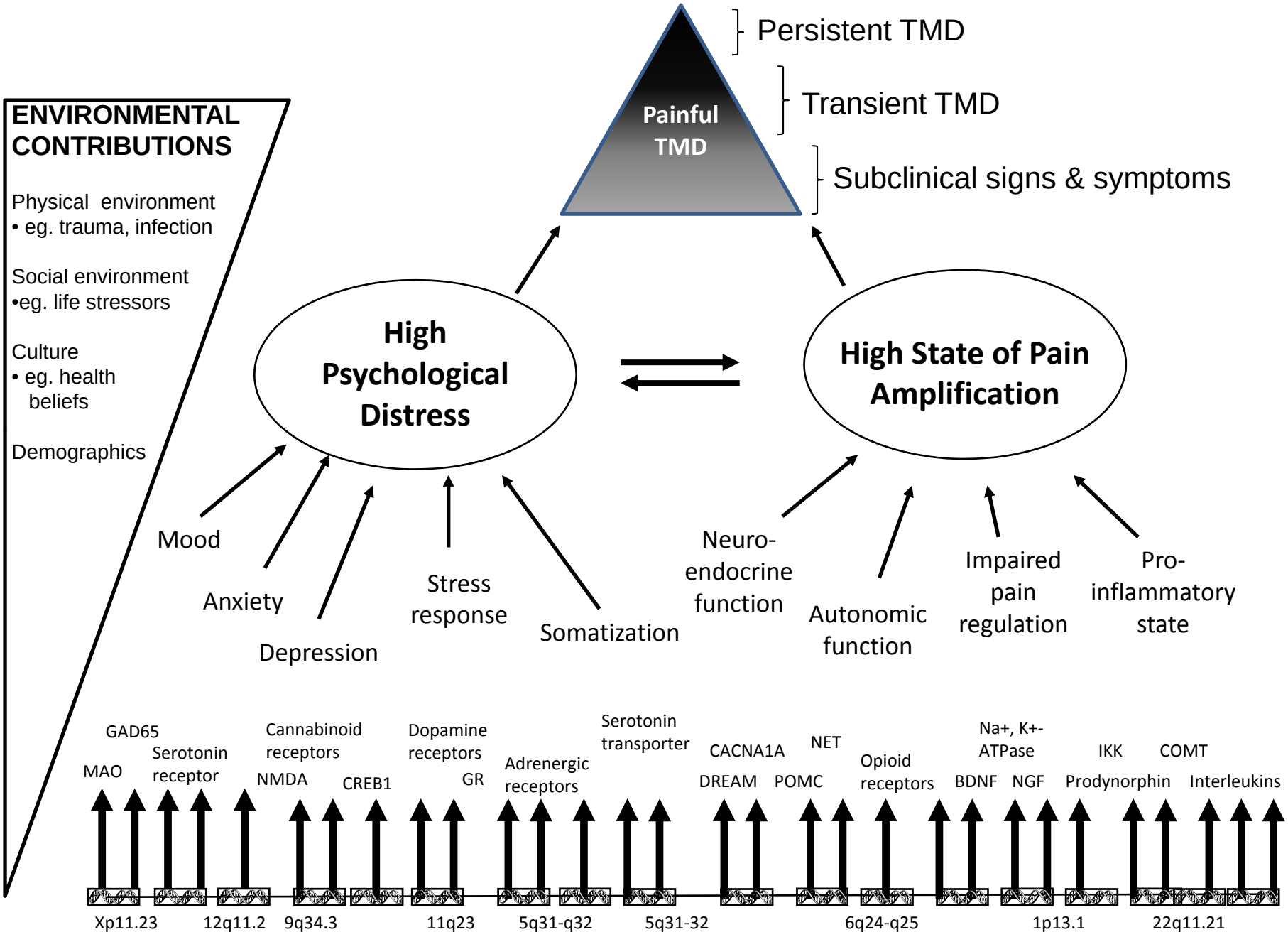
- Quantitative sensory testing (QST)
  - Univariate associations with TMD
  - Principal component analysis of 33 QST measures
  - Multivariable modeling of five QST measures

Part 1. Aims of OPPERA

To determine if

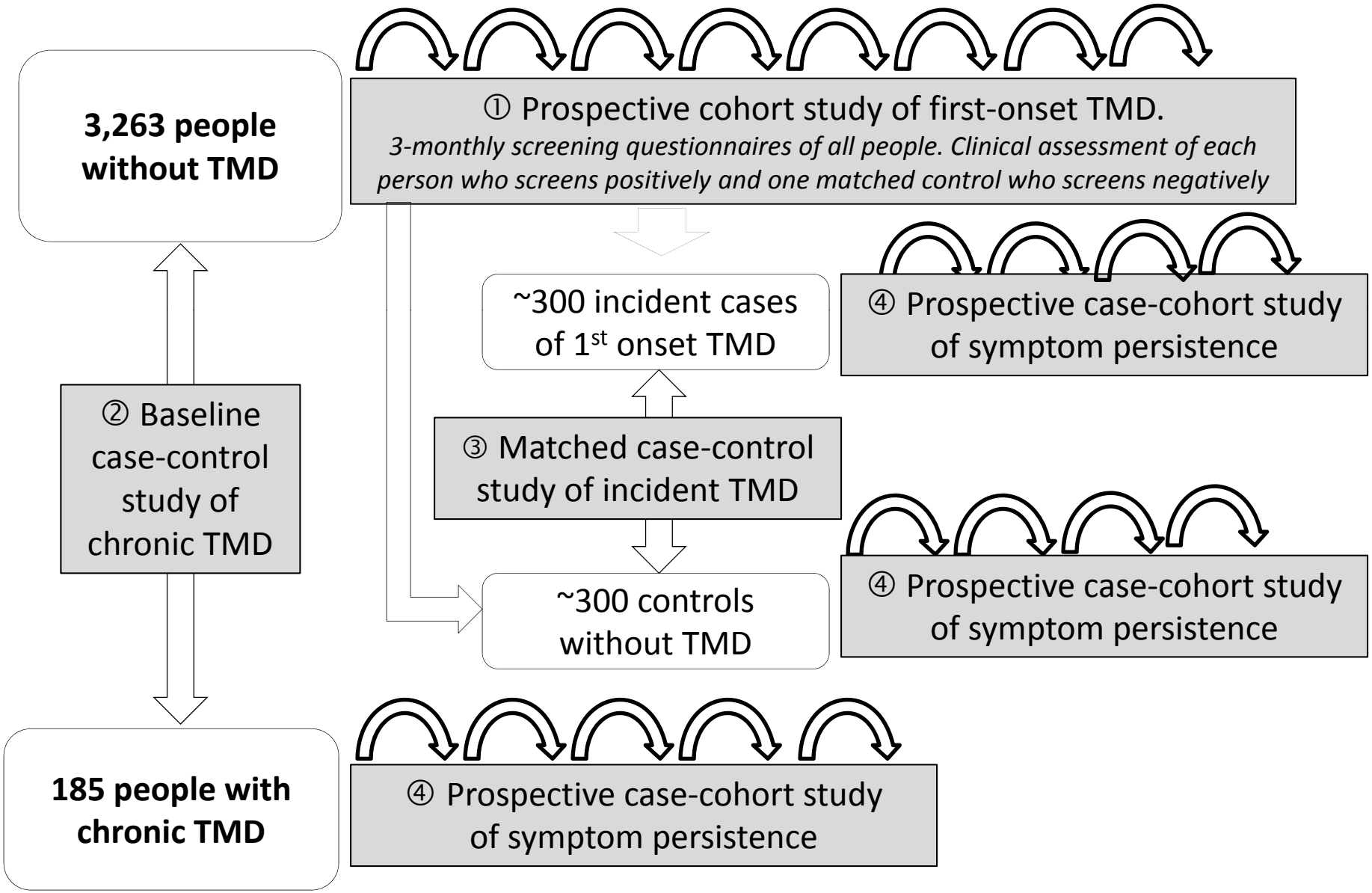
- sociodemographic characteristics,
- responses to noxious stimuli,
- psychosocial profiles, and
- genetic variants in 300 candidate genes

are associated with elevated risk of first-onset TMD and increased odds of chronic TMD.



Based on Diatchenko L et al. Idiopathic pain disorders--pathways of vulnerability. Pain 123 3:226-230, 2006

Four study designs



Baseline case-control study

- Volunteers were recruited by community-wide advertisements, emails, flyers and word-of-mouth
  - Baltimore MD, Buffalo NY, Chapel Hill NC, and Gainesville FL
  - **185 cases** with examiner-classified TMD
  - **1,633 controls** who did not have TMD when examined
- Data collection took place from May 2006 – November 2008
  - Telephone interview
  - Self-completed questionnaires
  - Clinical examination of head, neck and body[1]
  - Quantitative sensory testing
  - Autonomic function
  - Blood sample for genotyping

1. Dworkin SF and LeResche L. J. Craniomandibular Disorders, Facial and Oral Pain, 1992

Inclusion and exclusion criteria

OPPERA-wide inclusion criteria

- Aged 18-44 yrs
- Written consent to undertaken study procedures

OPPERA-wide exclusion criteria

- Nine health-related conditions (eg. kidney disease, heart disease, uncontrolled diabetes, psychiatric illness requiring hospitalization)
- Pregnant or nursing
- Traumatic facial injury or surgery during the preceding six months. [Does not include surgery only to remove teeth]
- Currently receiving orthodontic treatment

Classification of cases and controls

	Telephone interview	Clinical examination
TMD cases	• Orofacial pain ≥ 15 days in 30 days prior to interview • Orofacial pain ≥5 days/month in the five months before that	• ≥ 5 days of regional pain in past 30 days in the examiner-defined orofacial region • either ≥3 TM muscle groups or ≥1 TM joint painful to palpation or jaw movement
Controls	No orofacial pain in the month before interview and <5 days/month in the five months before that <5 headaches/month in past 3 months Do not wear night guard or occlusal splint Never diagnosed with TMD	• < 5 days of pain in past 30 days in the examiner-defined orofacial region • *Findings from muscle/joint palpation and jaw movement were not used as eligibility criteria for controls*

Kappa values for inter-examiner reliability ranged from 0.77 to 1.0

Analysis of associations with TMD

- Psychological scales/subscales and measures of pain sensitivity were computed using published algorithms
  - When up to 50% of constituent items were missing, data were imputed using expectation-maximization method

- Continuous measures were transformed to z-scores, and used as explanatory variables in binary logistic regression models to estimate standardized odds ratios for TMD

- Multivariable logistic regression models evaluated multiple explanatory variables
  - Area under ROC curve was used an indicator of the model's capacity to discriminate cases from controls

Principal component analysis

Aims were to reduce dimensionality of the data and to identify latent variables

Four steps:

1. variable selection from within major domains of the heuristic model (pain sensitivity, psychological distress, autonomic function)
2. evaluation of the correlation matrix
3. extraction of principal components and parallel analysis to select number of components
4. varimax rotation with generation of factor loadings and bootstrap estimation of 95% confidence intervals for loadings

Sample size calculations

- Primary endpoints were for the prospective cohort study
  - required sample size of 3,200 people with no TMD at baseline
  - minimum-detectable risk ratios of 2.0 for up to 10 genetic markers
  - allowing for SNPs with rare allele prevalence as low as 15%
  - specifying 99.5% confidence intervals and type II error of 0.2
  - assuming annual incidence of first onset TMD of 1.8%

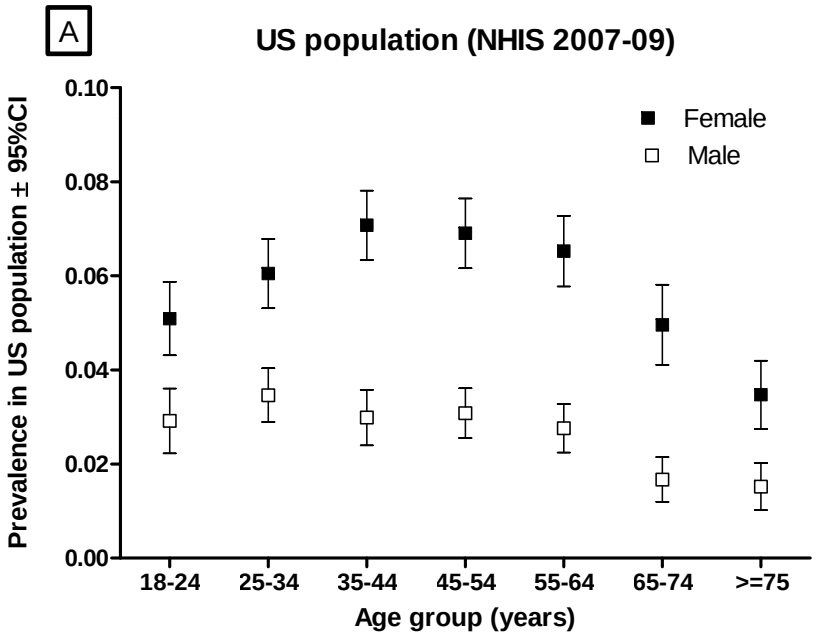
- For the baseline case-control study of 185 cases and 50% sample of controls from baseline of the prospective cohort study
  - minimum detectable odds ratios of 1.7 for binary predictor variables with exposure prevalence as low as 15%.
  - minimum-detectable standardized odds ratio of 1.25 for continuous predictor variables
  - thresholds of P=0.05 for type I error and 0.2 for type II error

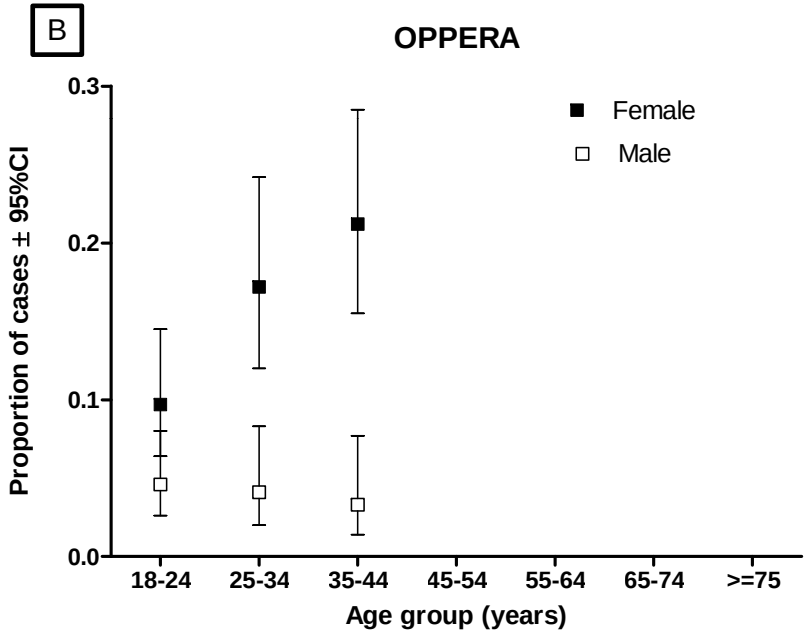
Demographic associations with TMD

	Controls		TMD cases		Adjusted* odds ratio	
Age (yrs)	No. of people	column %	No. of people	column %	OR	95%CI
35-44	344	21.1	53	28.6	2.3	1.5 ,3.6
25-34	451	27.6	60	32.4	1.8	1.2 ,2.6
18-24	838	51.3	72	38.9	ref	
Gender						
Female	925	56.6	155	83.8	4.0	2.6 ,6.0
Male	708	43.4	30	16.2	ref	
Race/ethnicity						
non-White	794	48.6	40	21.6	0.2	0.1 ,0.3
White	839	51.4	145	78.4	ref	

* Adjusted for study site and other demographic characteristics

Age- and gender-associations with TMD: US population and OPPERA





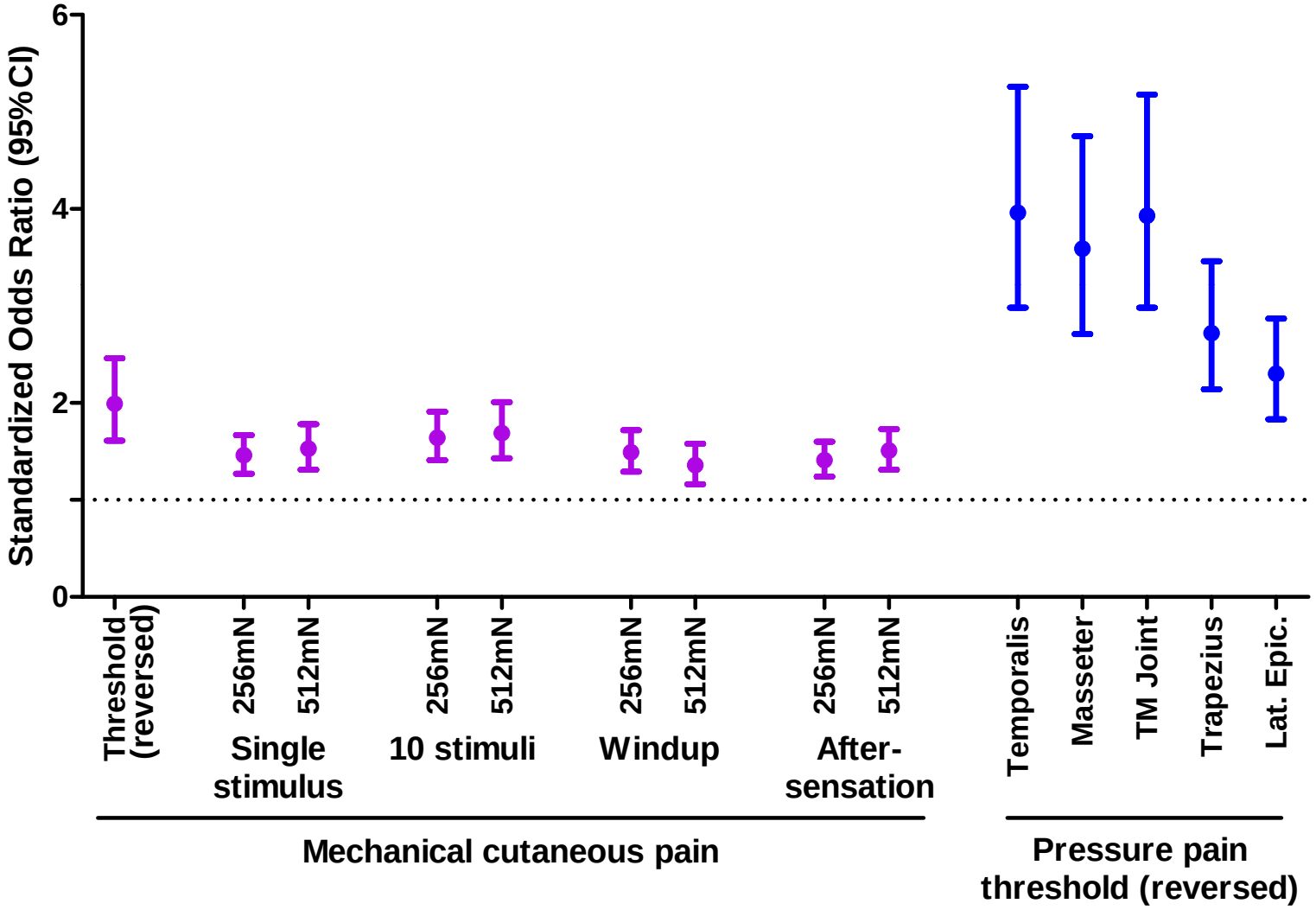
Part 2. Quantitative sensory testing

- 9 measures of sensitivity to mechanical cutaneous stimuli
  - threshold, tolerance
  - temporal summation
  - aftersensations

- Pressure pain threshold measured at 5 locations
  - masseter, temporalis, TM joint
  - trapezius, lateral epidondyle

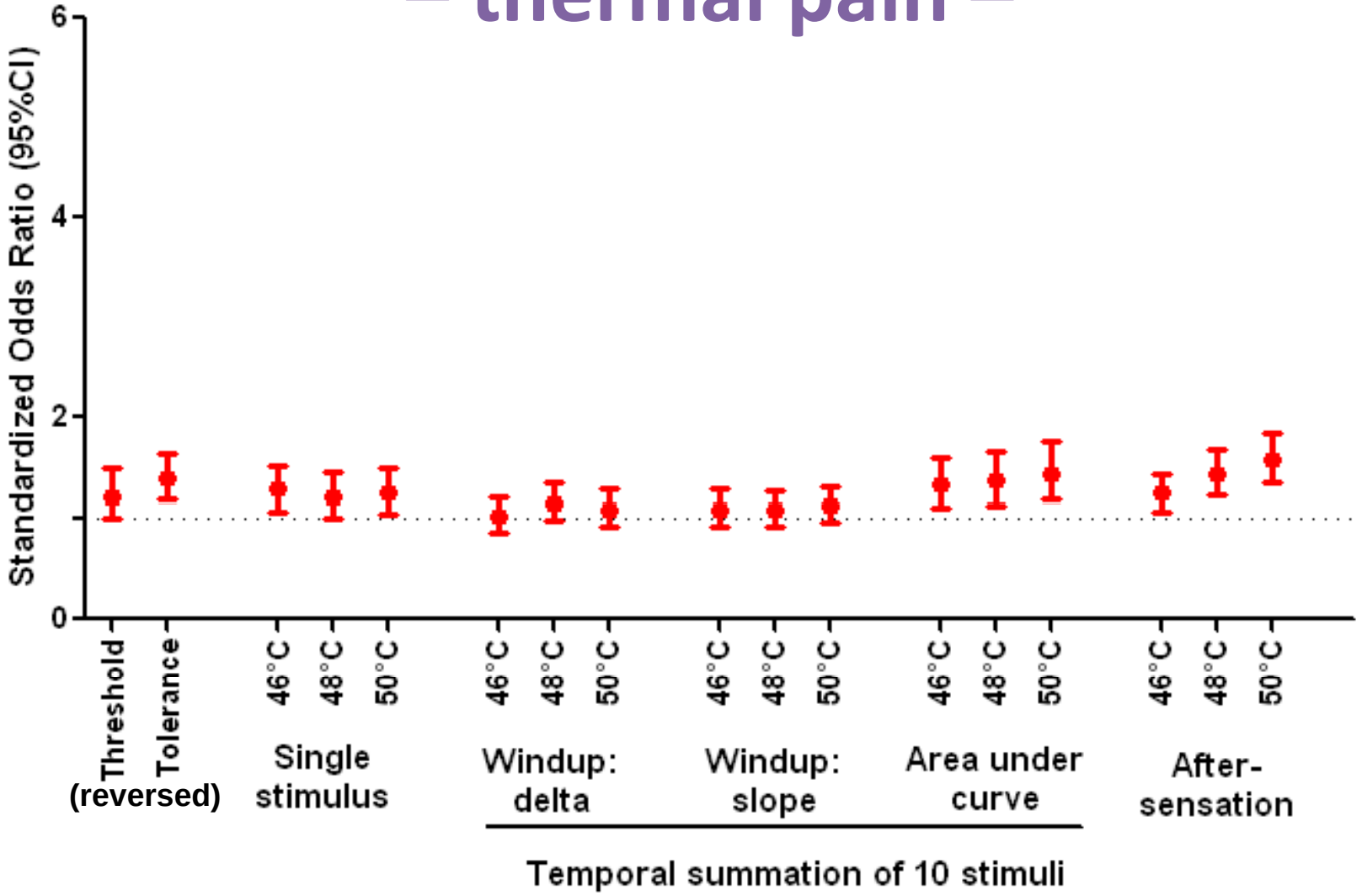
- 19 measures of sensitivity to thermal stimuli
  - threshold, tolerance
  - temporal summation
  - aftersensations

Univariate associations with TMD* – mechanical and pressure pain –



* Association between single pain measures and TMD, adjusted for study site, age, gender and race

Univariate associations with TMD* – thermal pain –



* Association between single pain measures and TMD, adjusted for study site, age, gender and race

Summary of principal component analysis

Five components accounted for 72% of variance:

1. thresholds and tolerance from multiple modalities
2. mechanical cutaneous pain ratings, aftersensations, and temporal summation
3. heat pain temporal summation ("windup")
4. overall heat pain ratings
5. heat pain aftersensations

For all components, $\alpha \geq 0.87$

Variables selected from each component

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1. thresholds and tolerance from multiple modalities
2. mechanical cutaneous pain ratings, aftersensations, and temporal summation
3. heat pain temporal summation ("windup")
4. overall heat pain ratings
5. heat pain aftersensations

For all components, $\alpha \geq 0.87$

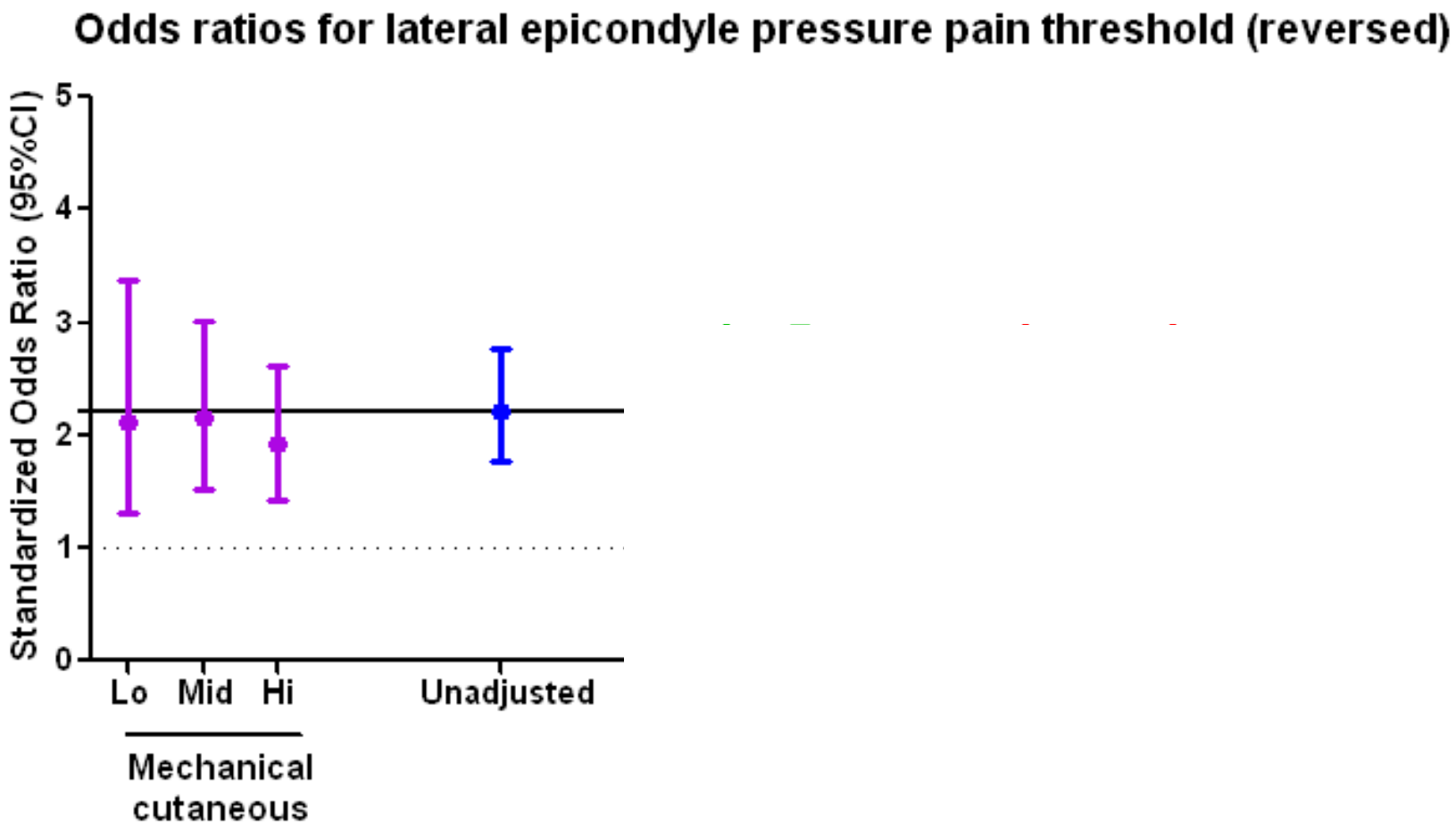
Variables selected for this analysis:

1. Pressure pain threshold at lateral epicondyle (reverse coded)
2. 0-100 rating of single cutaneous stimulus at 256 mN
3. Change from 1st rating (0-100) to greatest subsequent rating (0-100) during 48oC temporal summation
4. Area Under Curve (AUC) of 0-100 ratings during 48^{o}C temporal summation
5. 0-100 rating 30 sec after 10th thermal stimulus at 48^{o}C

Correlations among 5 QST measures

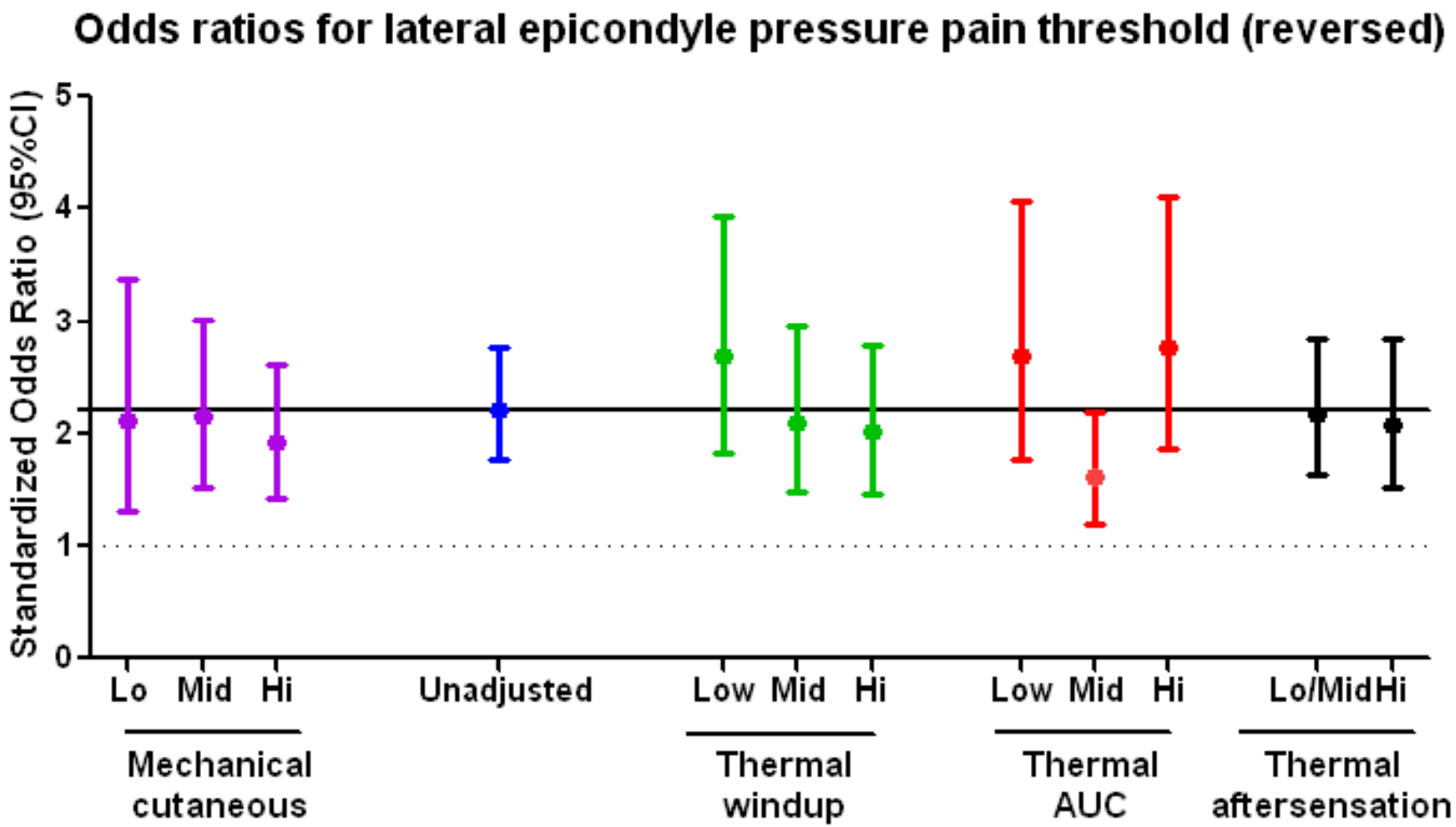
	Pearson's correlation coefficient (95% CI)			
	Pressure pain threshold	Thermal windup	Thermal area under curve	Thermal aftersensations
Mechanical cutaneous	0.18 (0.13, 0.22)	0.37 (0.33, 0.41)	-0.09 (-0.13, -0.04)	0.31 (0.27, 0.35)
Pressure pain		0.33 (0.29, 0.37)	0.02 (-0.02, 0.07)	0.16 (0.11, 0.20)
Thermal windup			0.13 (0.09, 0.18)	0.37 (0.32, 0.40)
Thermal AUC				0.01 (-0.04, 0.06)

Stratified associations: pressure pain



* Odds ratios for TMD, adjusted for study site, age, gender and race

Stratified associations: pressure pain



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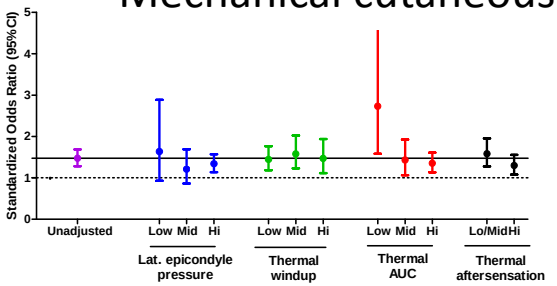
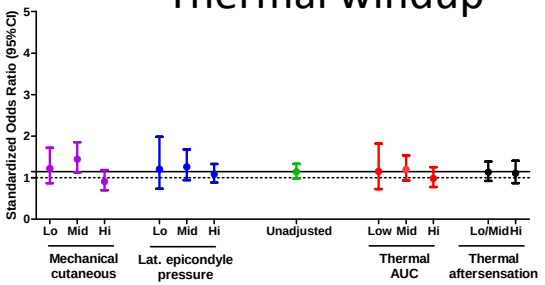
Summary of stratified associations

- In general, standardized odds ratios for one QST measure did not vary across strata of low, mid and high tertiles of the other QST measures
- This suggests independence of effects, that is:
  - no confounding
  - no interaction

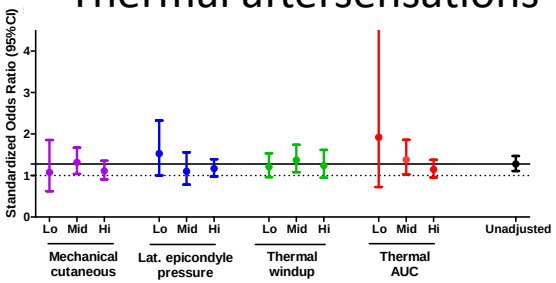
Mechanical cutaneous



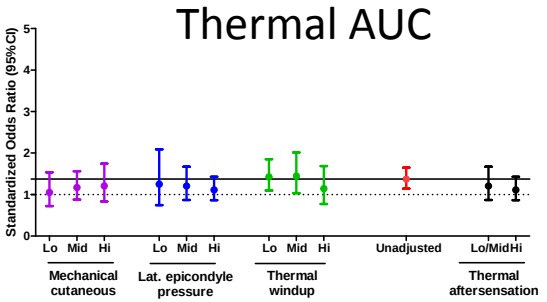
Thermal windup



Thermal aftersensations



Thermal AUC



Multivariable models for odds of TMD - stepwise selection of five variables -

Explanatory variable	Odds ratios from multivariable logistic regression model Explanatory variables coded as z-scores
Pressure pain threshold	2.0
Mechanical cutaneous	1.4
Thermal windup	1.2
Thermal area under curve	ns
Thermal aftersensation	ns
Model discrimination (AUC)	*0.81*

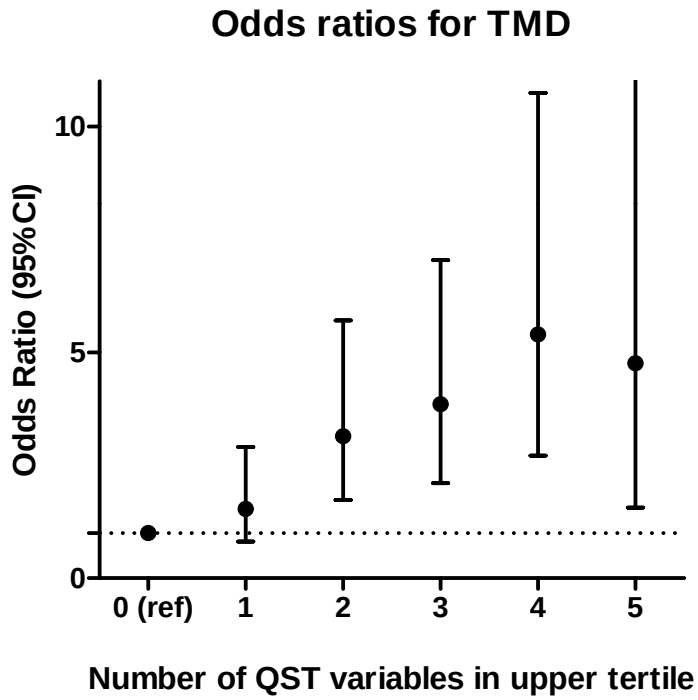
* Models additional adjust for study site, age, gender and race

Multivariable models for odds of TMD - stepwise selection of five variables -

	Odds ratios from multivariable logistic regression model	
Explanatory variable	Explanatory variables coded as z-scores	Explanatory variables dichotomized
Pressure pain threshold	2.0	2.3
Mechanical cutaneous	1.4	1.9
Thermal windup	1.2	ns
Thermal area under curve	ns	ns
Thermal aftersensation	ns	1.6
Model discrimination (AUC)	*0.81*	*0.80*

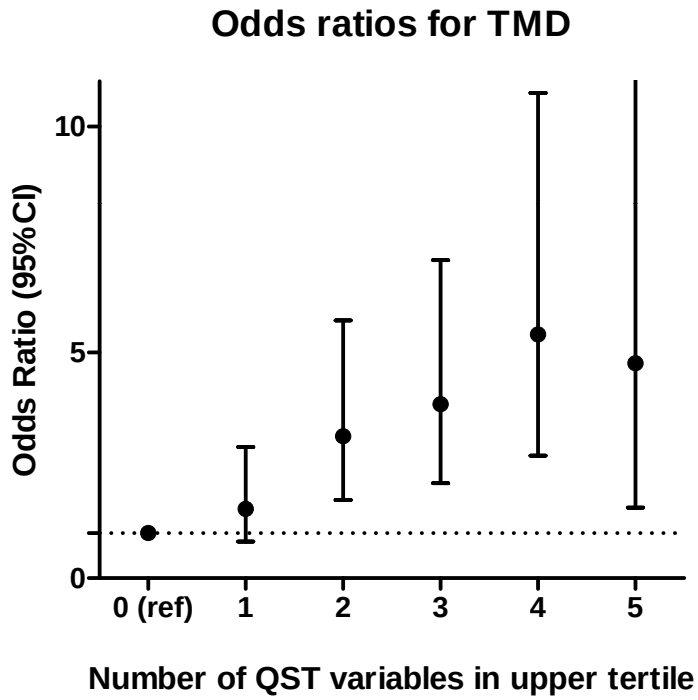
* Models additional adjust for study site, age, gender and race

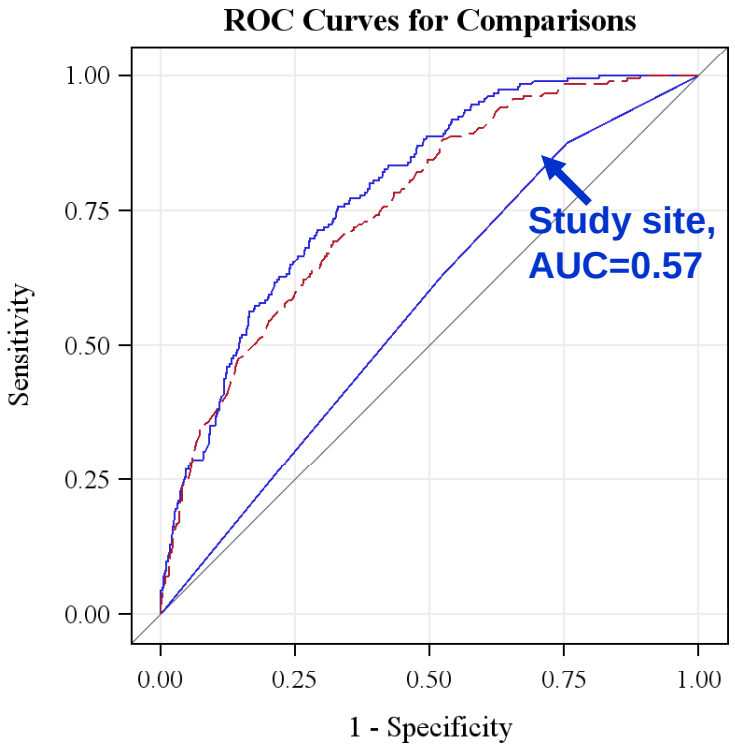
Multivariable models for odds of TMD - count of QST values in upper tertile -



* Odds ratios for TMD, adjusted for study site, age, gender and race

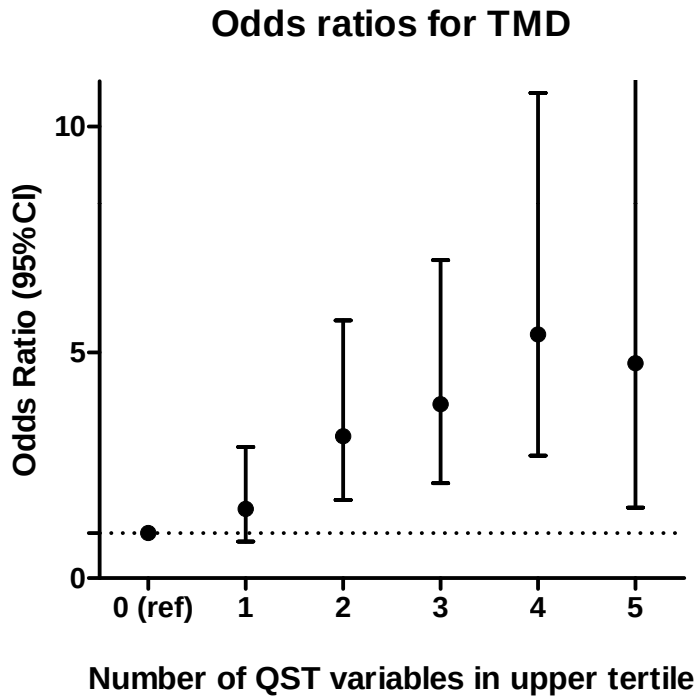
Multivariable models for odds of TMD - count of QST values in upper tertile -

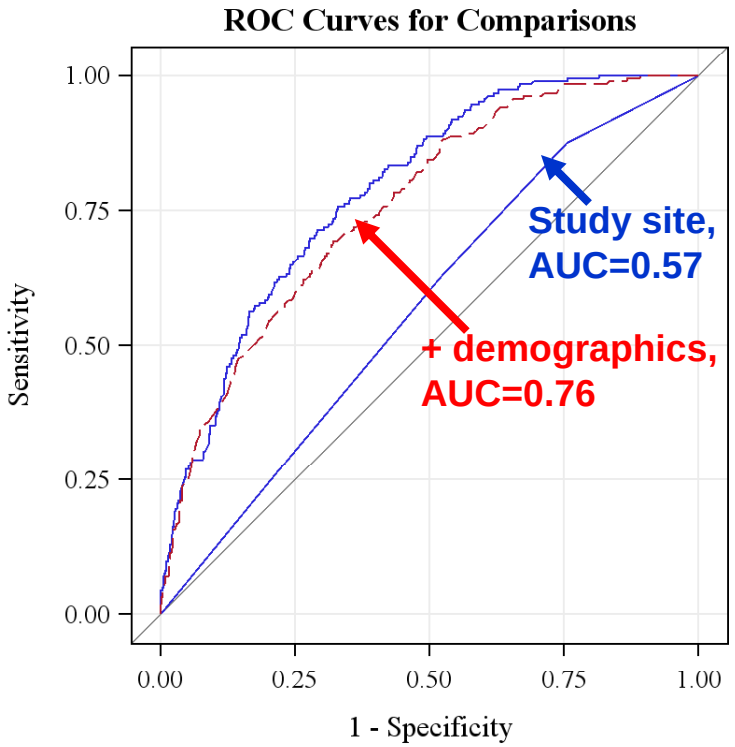




* Odds ratios for TMD, adjusted for study site, age, gender and race

Multivariable models for odds of TMD - count of QST values in upper tertile -





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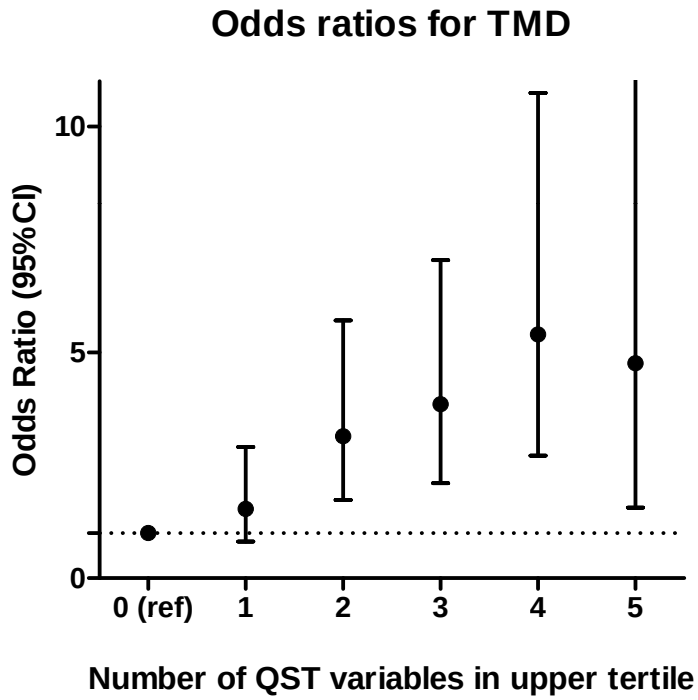
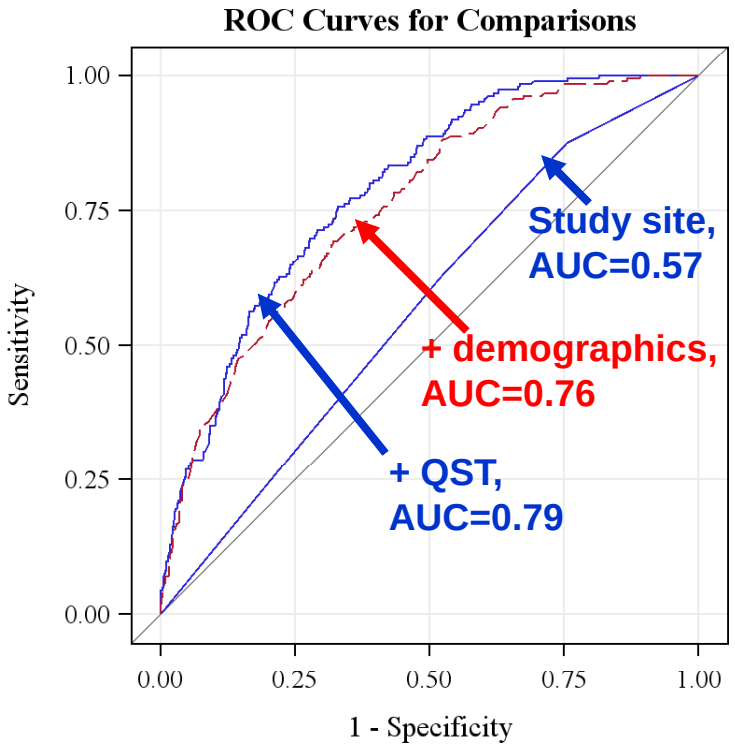
Multivariable models for odds of TMD - count of QST values in upper tertile -





* Odds ratios for TMD, adjusted for study site, age, gender and race

Conclusions

In the baseline OPPERA case-control study:

- Greater age, female gender and white race were associated with increased odds of TMD
- Moderately strong univariate associations with TMD were found for pressure pain thresholds at multiple body sites and rating of mechanical cutaneous pain at the finger
  - Weaker associations with TMD were found for other mechanical cutaneous measures and for thermal measures at the arm
- Exploratory factor analysis of 33 QST measures identified five principal components
  - thresholds to various stimuli
  - mechanical cutaneous pain
  - thermal ratings, windup, and aftersensations

continued

Conclusions

- Stratified analysis of five QST variables, one from each component, suggested they were independently associated with TMD

- Conventional, stepwise multivariable logistic regression modeling identified three variables that contributed to odds of TMD
  - they added only modestly to sociodemographic characteristics in discriminating cases from controls

- A person-level summary variable, created by counting the number of variables with values in the upper tertile of the distribution, provided similar ability to discriminate cases from controls
  - it showed increasing odds of TMD associated with each additional high score, up to four high scores

Acknowledgments

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Psychosocial and Clinical Profiles

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Supported by NIH/NIDCR Grant U01DE17018

Aims of OPPERA

To determine if:

- sociodemographic characteristics,
- responses to noxious stimuli,
- psychosocial profiles, and
- genetic variants in 300 candidate genes

are associated with elevated risk of first-onset TMD and increased odds of chronic TMD.

Scope of presentation

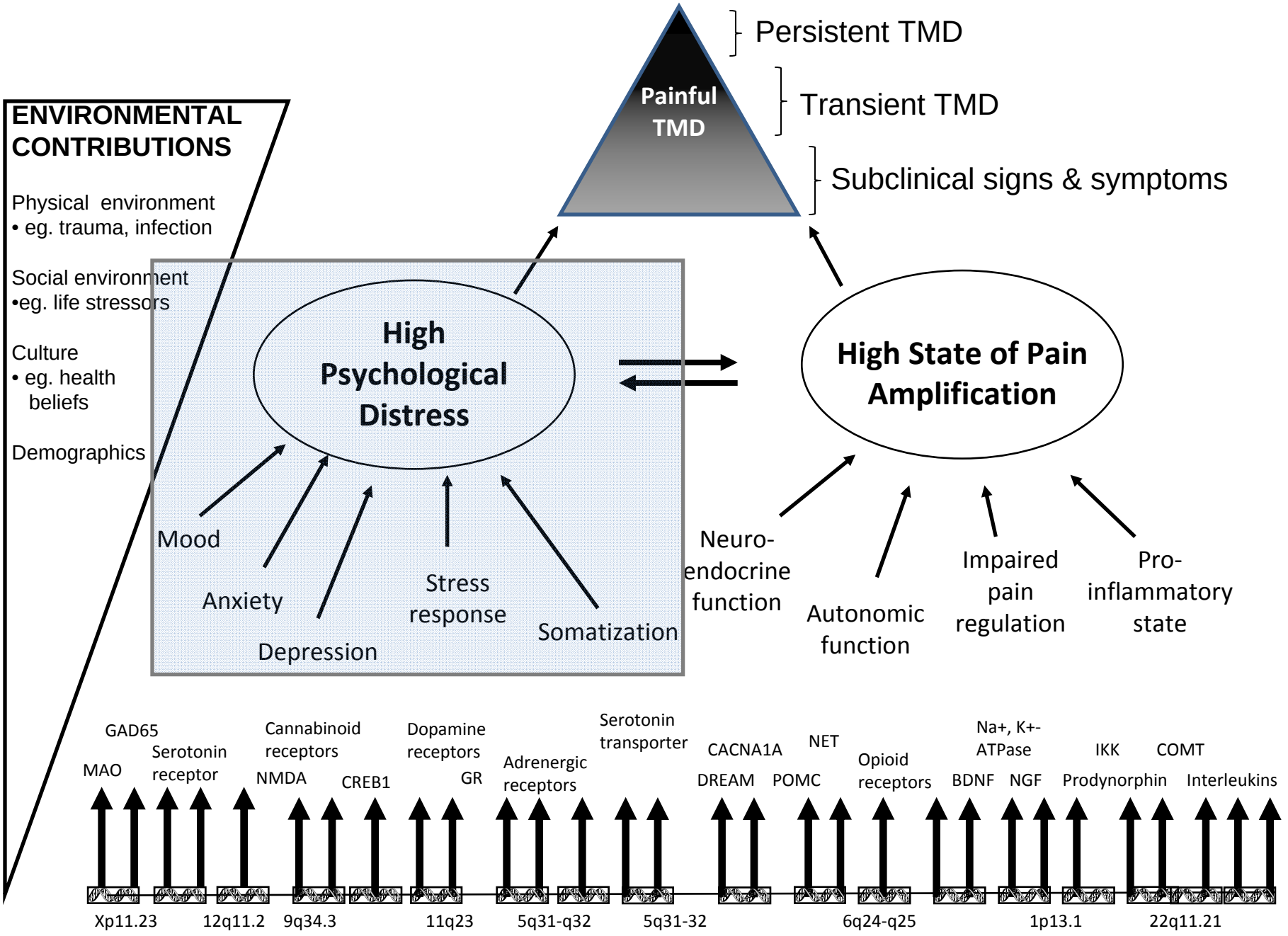
With a cohort of non-TMD controls and chronic TMD cases, assess associations using the following variables:

Part 1

- Psychological constructs and measures
  - Univariate associations with TMD
  - Principal component analysis of 21 psychological measures
  - Multivariable modeling of 4 psychological measures

Part 2

- Clinical constructs and measures
  - Univariate associations with TMD
  - Multivariable modeling of six clinical measures

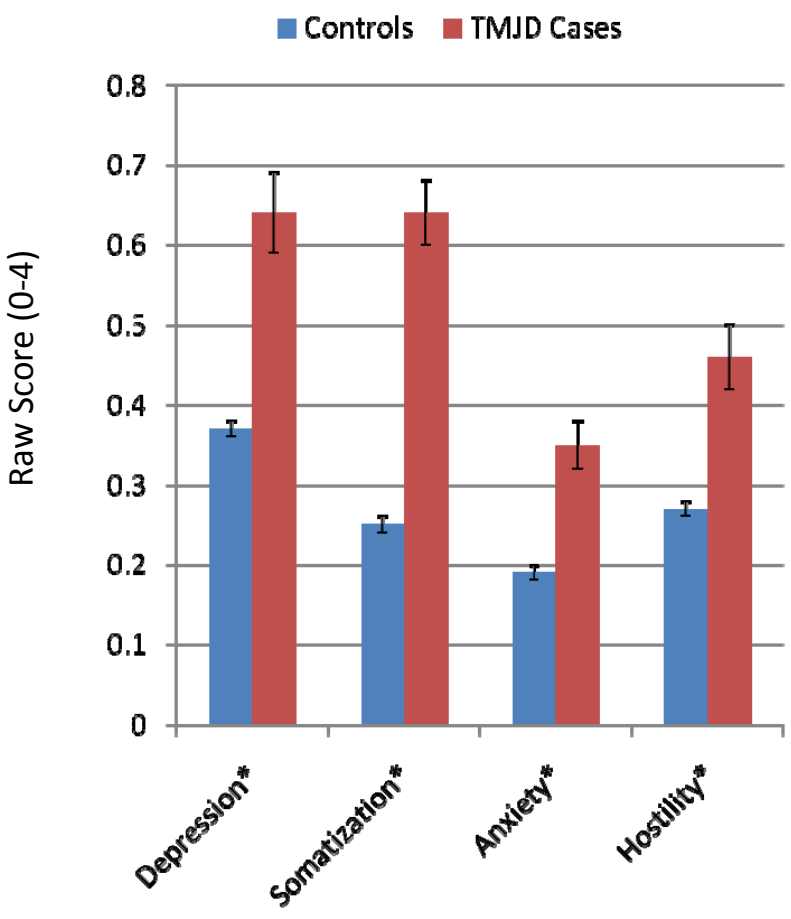

Persistent TMD
Painful TMD
Transient TMD
Subclinical signs & symptoms
ENVIRONMENTAL CONTRIBUTIONS
Physical environment
• eg. trauma, infection
Social environment
•eg. life stressors
Culture
• eg. health beliefs
Demographics
High Psychological Distress
High State of Pain Amplification
Mood
Anxiety
Depression
Stress response
Somatization
Neuro-endocrine function
Autonomic function
Impaired pain regulation
Pro-inflammatory state
GAD65
MAO
Serotonin receptor
NMDA
Cannabinoid receptors
CREB1
Dopamine receptors
GR
Adrenergic receptors
Serotonin transporter
CACNA1A
DREAM
NET
POMC
Opioid receptors
Na+, K+-ATPase
BDNF
NGF
IKK
Prodynorphin
COMT
Interleukins
Xp11.23
12q11.2
9q34.3
11q23
5q31-q32
5q31-32
6q24-q25
1p13.1
22q11.21

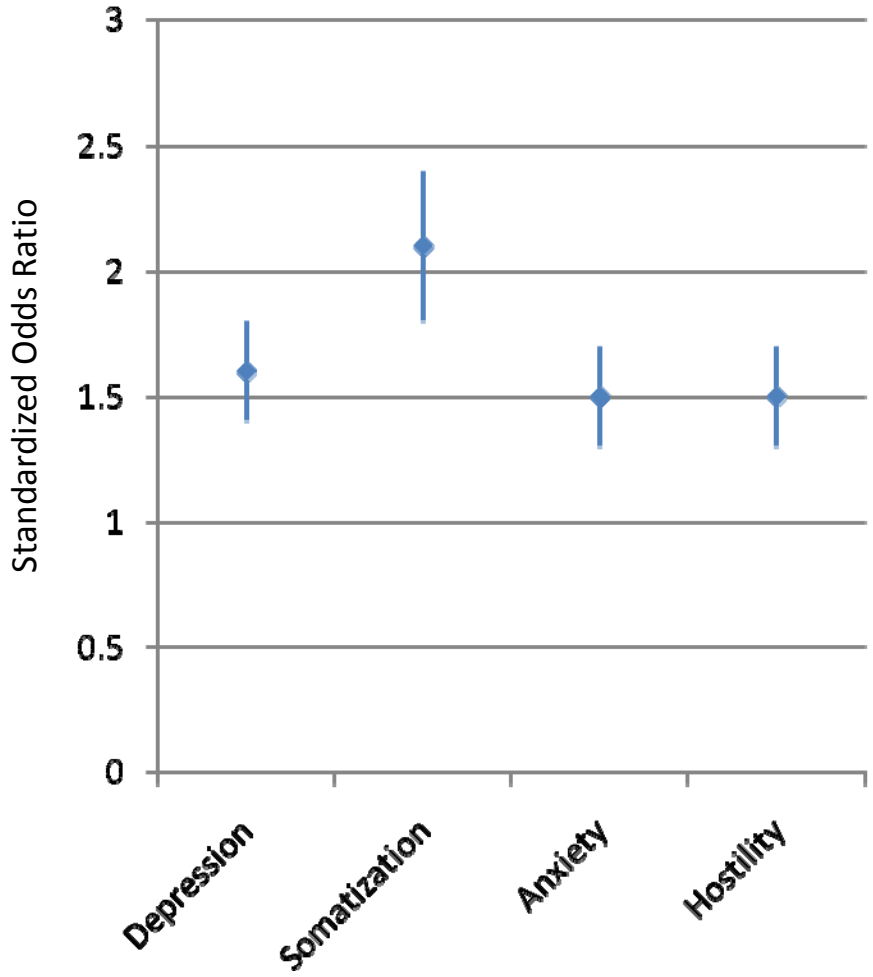
Psychological Questionnaires

- Pre-Clinic
  - Coping Strategies Questionnaire-Revised (CSQ-R)
  - Eysenck Personality Questionnaire-Revised (EPQ)
  - Kohn Reactivity Scale (KOHN)
  - Life Experiences Survey (LES)
  - Lifetime Stressor List & PSTD Checklist for Civilians (LSL/PCL-C)
  - Perceived Stress Scale (PSS)
  - Trait Anxiety Inventory
  - Pennebaker Inventory for Limbic Languidness (PILL)
  - Pain Catastrophizing Scale (PCS)
  - Symptom Checklist 90-Revised (SCL-90R)

- In-Clinic
  - Profile of Mood States-Bipolar (POMS-Bi)
  - State Anxiety Inventory
  - In-Vivo Coping

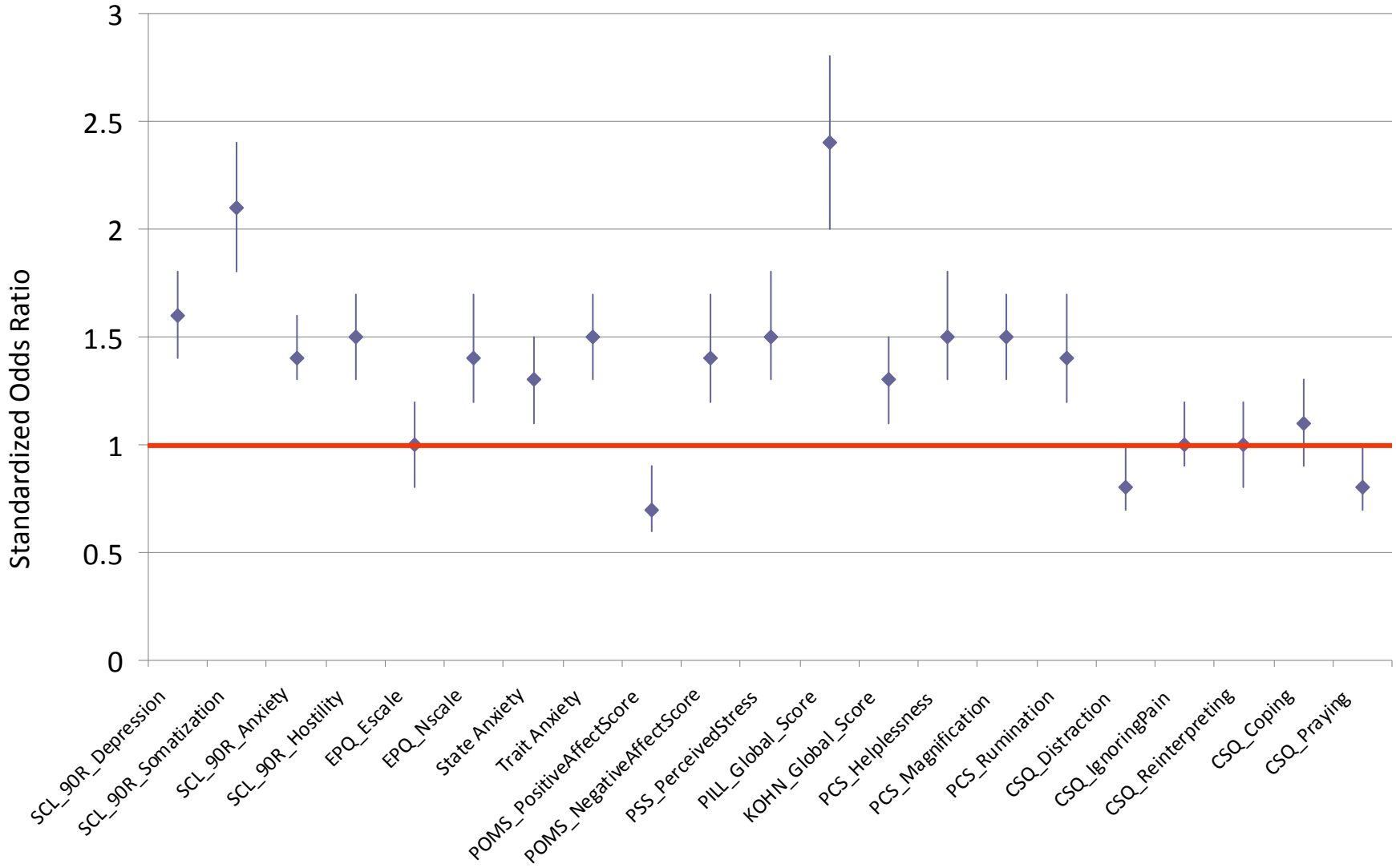
SCL-90R





* p < .0001

Standardized Odds Ratios – Psychosocial Measures



SOR adjusted for study site, age, gender, and race.
Effect estimates use imputation for missing data, total n=1808.

Component Loadings for PCA Model in Controls (n=1633)

	Component 1	Component 2	Component 3	Component 4
STAIY1 State-Trait Anxiety Inventory	**0.78**	0.13	-0.11	0.00
STAIY2 State-Trait Anxiety Inventory	**0.79**	0.14	0.03	0.01
Overall Positive Affect Score	**-0.85**	0.17	0.11	0.06
Overall Negative Affect Score	**0.49**	0.39	-0.05	0.05
PSS Perceived Stress Scale	**0.69**	0.16	0.05	0.00
EPQ-R Extraversion Scale	**-0.61**	0.21	0.13	-0.05
EPQ-R Neuroticism Scale	**0.55**	0.18	0.17	-0.01
SCL 90R Depression Scale	0.29	**0.69**	0.05	0.02
SCL 90R Somatization Full Scale	-0.13	**0.84**	0.11	0.01
SCL 90R Anxiety Scale	0.10	**0.81**	0.04	0.01
SCL 90R Hostility Scale	0.09	**0.74**	0.00	0.00
PILL Global Score	-0.12	**0.67**	0.13	0.03
CSQ_Praying Scale	0.02	-0.20	**0.55**	0.31
Global Kohn Score	0.26	-0.17	**0.46**	-0.25
PCS Rumination	-0.10	0.08	**0.88**	-0.07
PCS Magnification	-0.05	0.15	**0.79**	0.01
PCS Helplessness	-0.01	0.12	**0.83**	-0.05
CSQ_Distraction Scale	0.05	-0.19	0.32	**0.67**
CSQ_Ignoring Pain Scale	-0.04	0.13	-0.33	**0.79**
CSQ_Distancing Scale	0.13	-0.02	0.02	**0.72**
CSQ_Coping Scale	-0.12	0.07	-0.01	**0.79**
Cumulative Variance	**0.18**	**0.35**	**0.49**	**0.60**
Cronbach's Alpha	**0.87**	**0.85**	**0.54**	**0.74**

Component Loadings for PCA Model in Controls (n=1633)

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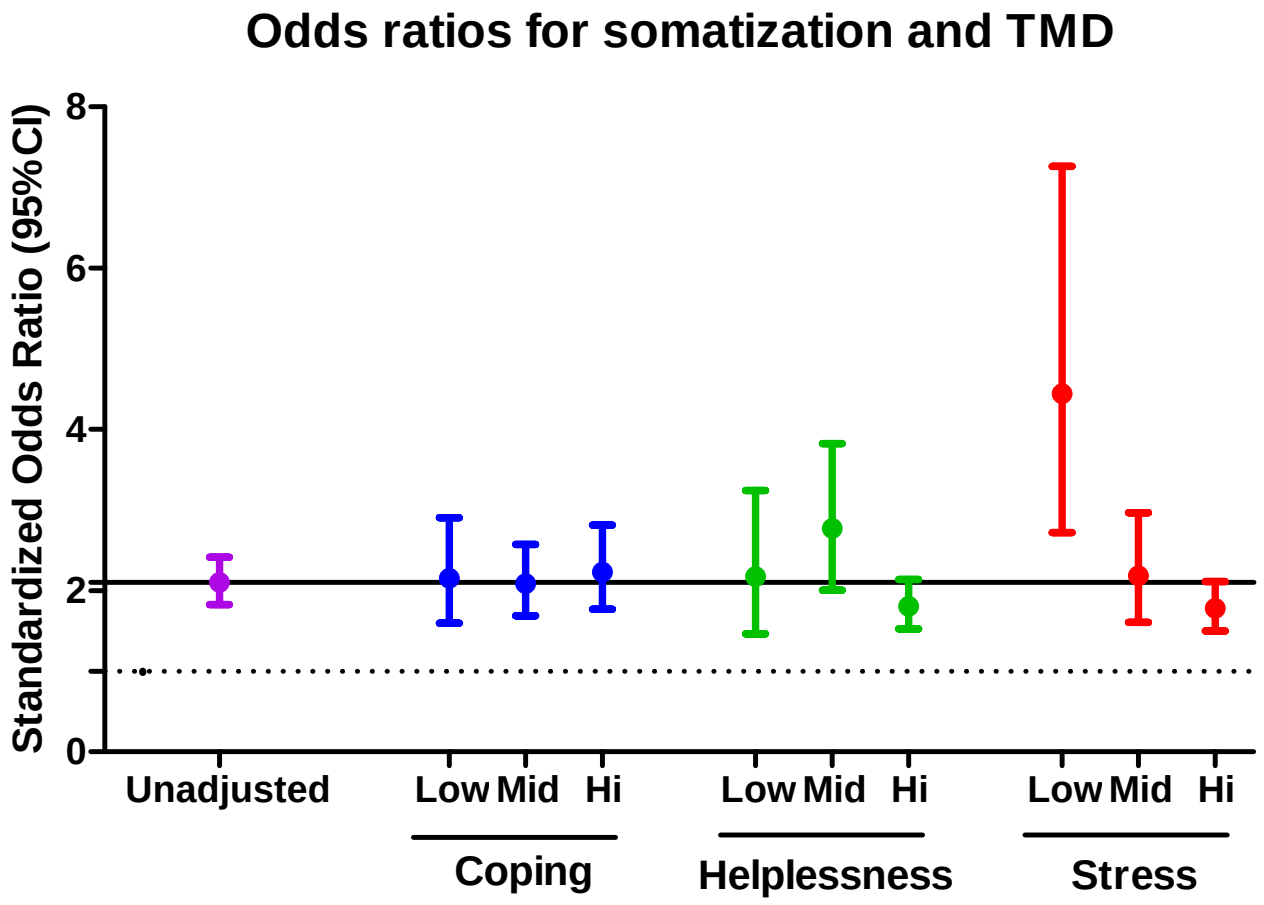
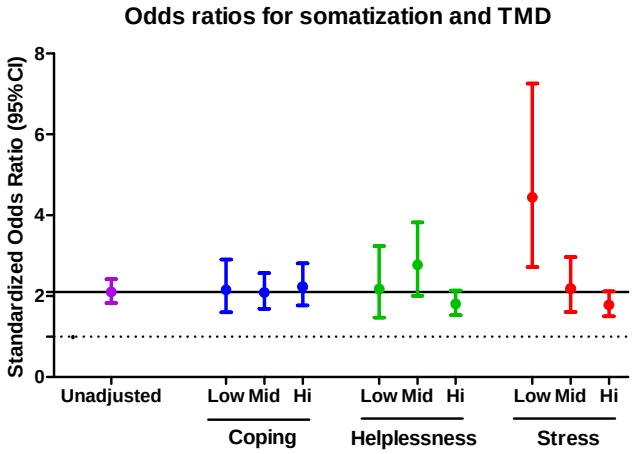
PILL Global Score	-0.12	**0.67**	0.13	0.03
CSQ_Praying Scale	0.02	-0.20	**0.55**	0.31
Global Kohn Score	0.26	-0.17	**0.46**	-0.25
PCS Rumination	-0.10	0.08	**0.88**	-0.07
PCS Magnification	-0.05	0.15	**0.79**	0.01
PCS Helplessness	-0.01	0.12	**0.83**	-0.05
CSQ_Distraction Scale	0.05	-0.19	0.32	**0.67**
CSQ_Ignoring Pain Scale	-0.04	0.13	-0.33	**0.79**
CSQ_Distancing Scale	0.13	-0.02	0.02	**0.72**
CSQ_Coping Scale	-0.12	0.07	-0.01	**0.79**
Cumulative Variance	**0.18**	**0.35**	**0.49**	**0.60**
Cronbach's Alpha	**0.87**	**0.85**	**0.54**	**0.74**

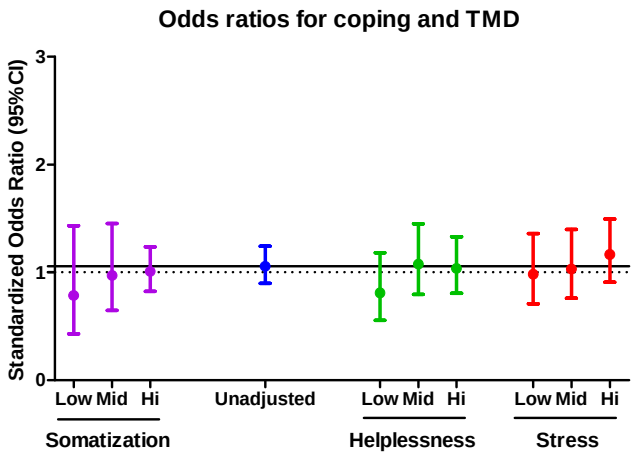
	PSS	Somat	Helplessness
Somat	0.35		
Helplessness	0.40	0.39	
Coping	-0.1	0.07	0.04

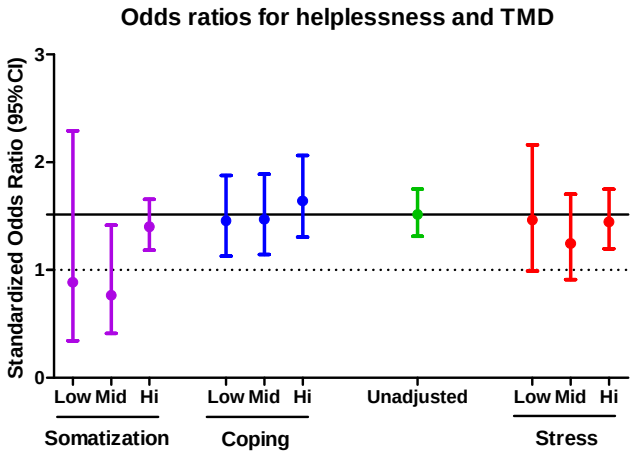
Somatization

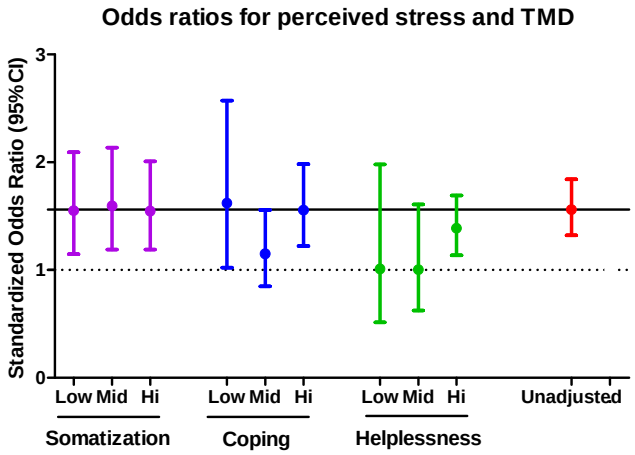


Stratified associations: four psychology measures

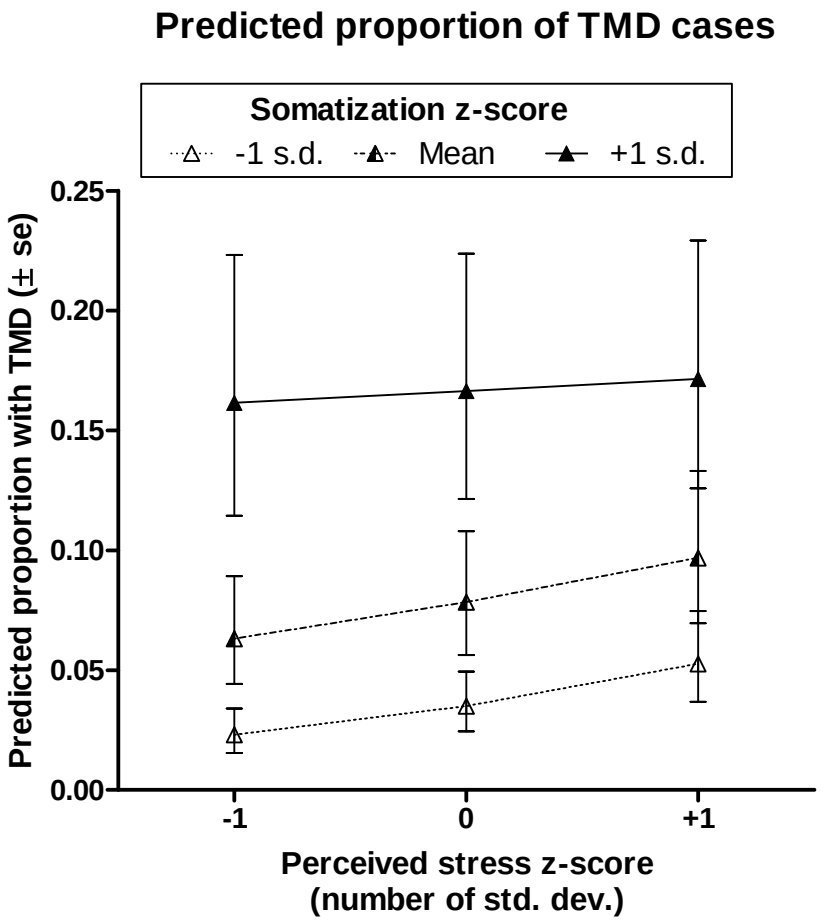








Predicted proportion of TMD cases



Multivariable models for odds of TMD - stepwise selection of four variables -

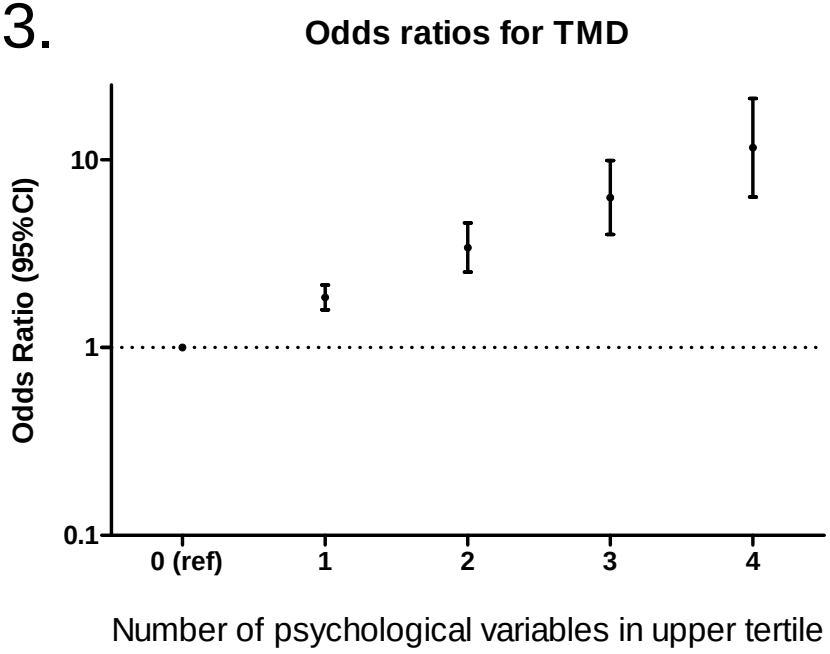
Explanatory variable	Odds ratios from multivariable logistic regression model: Explanatory variables coded as z-scores	Explanatory variables dichotomized
Stress (PSS)	1.1	1.5
Somatization (SCL90)	2.0	4.7
Helplessness (PCS)	1.1	1.5
Coping (CSQ)	ns	ns
Model discrimination (AUC)	*##*	*##*

* Models additional adjust for study site, age, gender and race

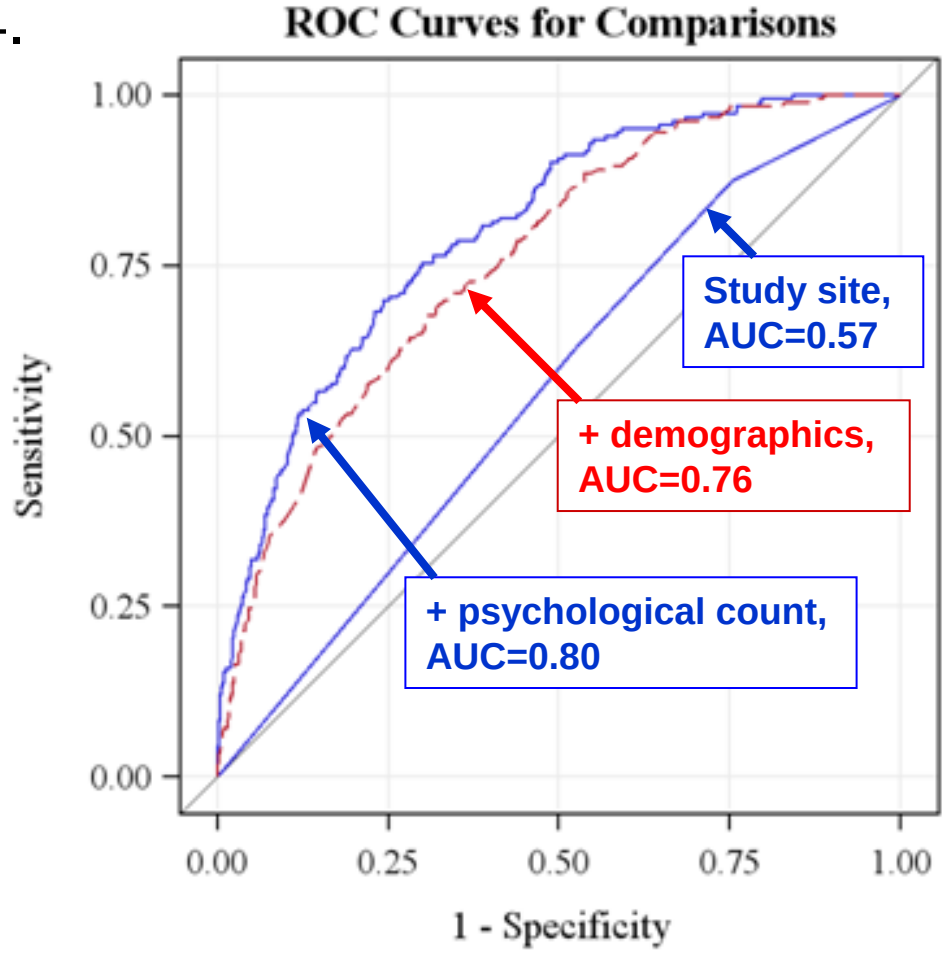
1. **Selected variables:**
   - **Somatization (SCL90R)**
   - **Perceived Stress (PSS-10)**
   - **Coping (CSQ)**
   - **Helplessness (PCS)**

2. Create count variable: increment if value is in upper tertile for each scale.

3.



4.



5. Clinically sensible index of only 4 psychological variables achieved 0.68 Sensitivity and 0.75 Specificity in predicting cases and controls

Conclusions

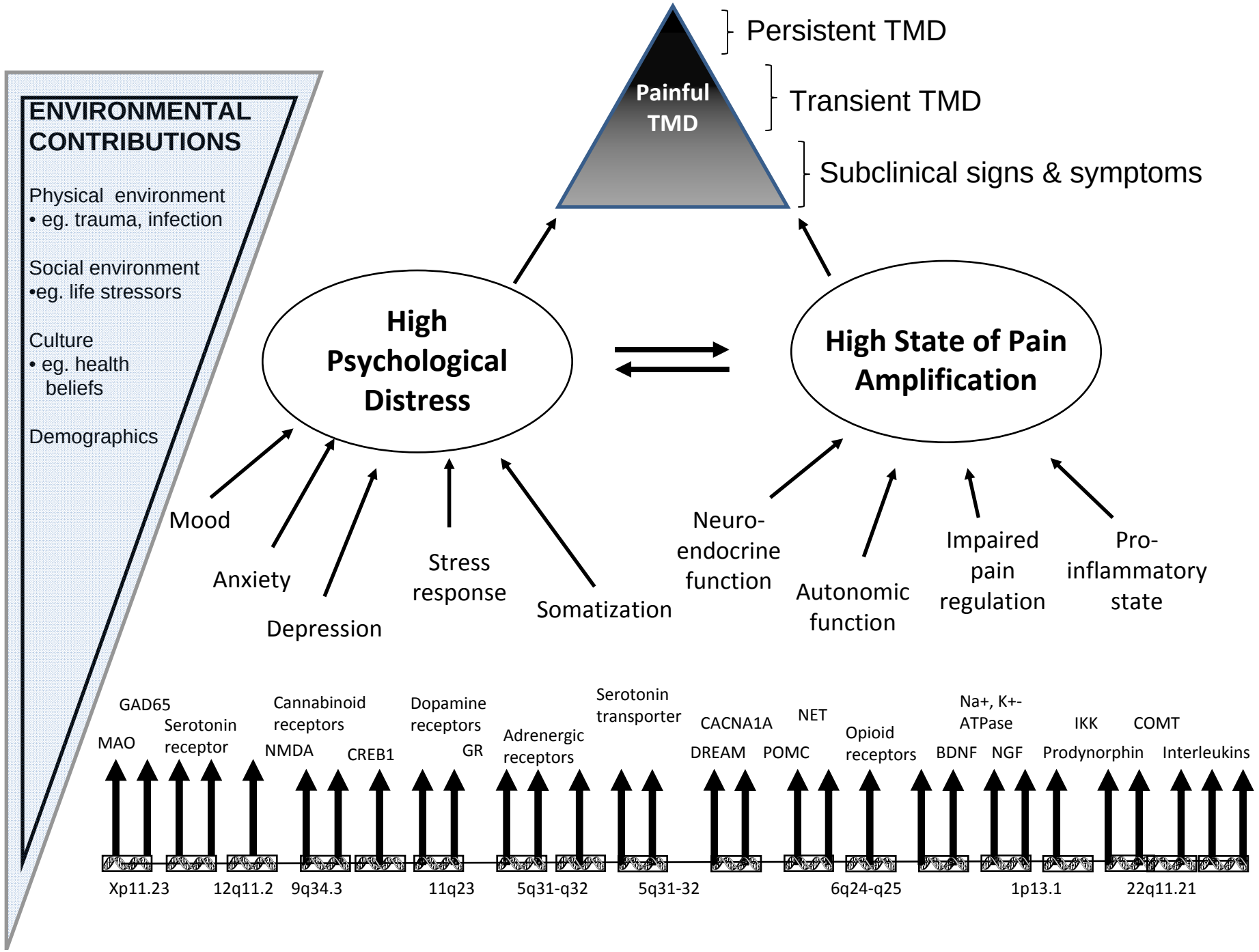
In the baseline OPPERA case-control study:

- Multiple psychosocial variables were associated with increased odds of chronic TMD (but most are modest in magnitude)
- Exploratory factor analysis of 21 measures suggests 4 major psychosocial factors:
    - Overall psychological function
    - Affective Distress/Stress
    - Passive Coping
    - Active Coping
- Stratified analysis of four psychological variables, one from each factor, suggested they were independently associated with TMD

continued

Conclusions

- Conventional, stepwise multivariable logistic regression modeling
  - identified three variables that contributed to the odds of chronic TMD, but
  - they added only modestly to sociodemographic characteristics in discriminating cases from controls
- A person-level summary variable, created by counting the number of variables with values in the upper tertile of the distribution,
  - provided similar ability to discriminate chronic cases from controls
  - showed increasing odds of TMD associated with each additional high score, up to four high scores

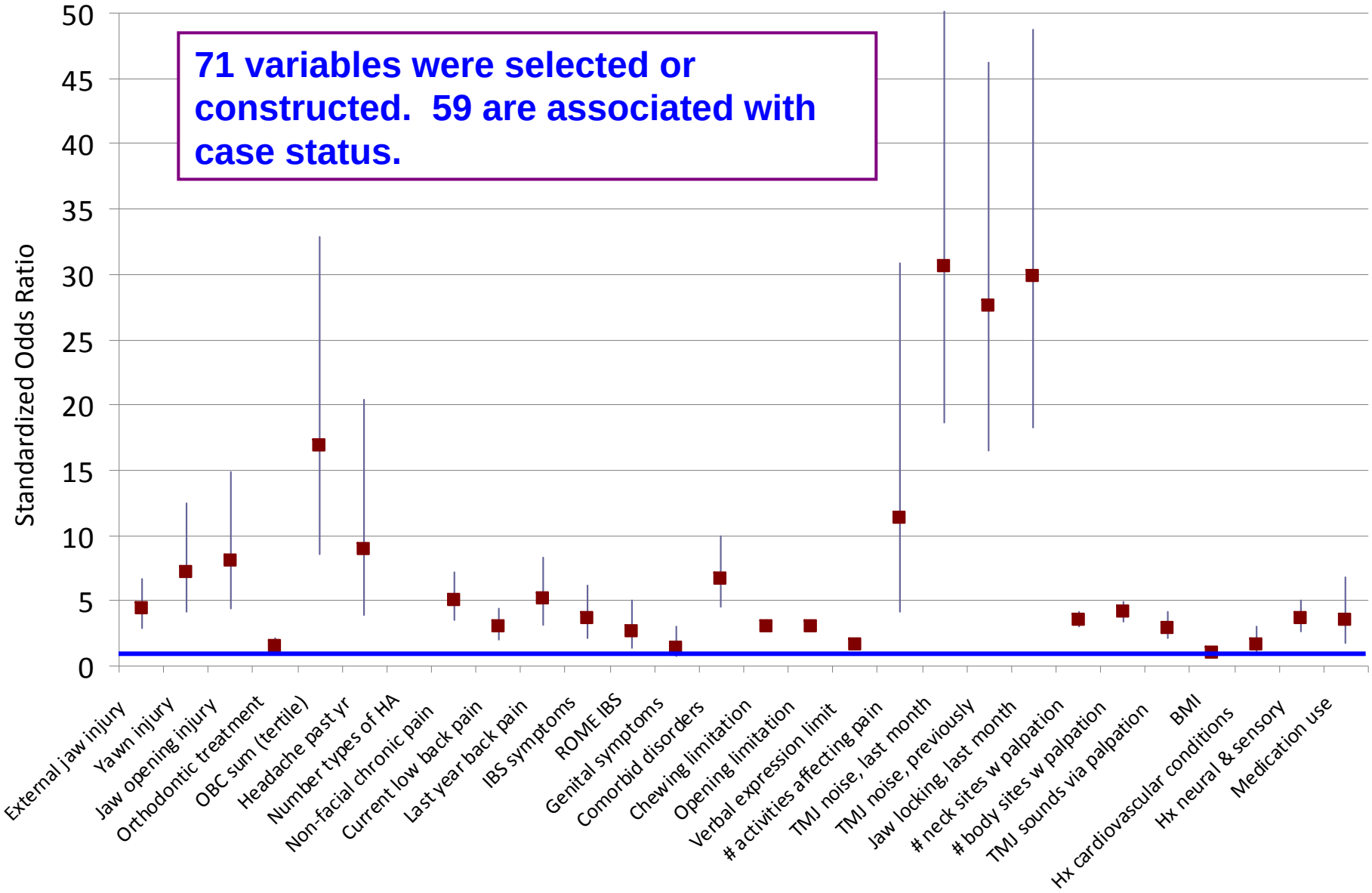

ENVIRONMENTAL CONTRIBUTIONS
Physical environment
• eg. trauma, infection
Social environment
•eg. life stressors
Culture
• eg. health beliefs
Demographics
Painful TMD
Persistent TMD
Transient TMD
Subclinical signs & symptoms
High Psychological Distress
High State of Pain Amplification
Mood
Anxiety
Depression
Stress response
Somatization
Neuro-endocrine function
Autonomic function
Impaired pain regulation
Pro-inflammatory state
GAD65
MAO
Serotonin receptor
NMDA
Cannabinoid receptors
CREB1
Dopamine receptors
GR
Adrenergic receptors
Serotonin transporter
CACNA1A
DREAM
NET
POMC
Opioid receptors
Na+, K+-ATPase
BDNF
NGF
IKK
Prodynorphin
COMT
Interleukins
Xp11.23
12q11.2
9q34.3
11q23
5q31-q32
5q31-32
6q24-q25
1p13.1
22q11.21

Overview of Clinical Data

- Condition-specific physical variables
  - Jaw trauma
  - Sleep bruxism
  - Overuse behaviors
  - Orthodontic treatment
  - Limitation in jaw function
  - Associated musculoskeletal dysfun
  - Interference in TMJ function
  - Non-pain symptoms
- Clinical examination variables
  - Mobility
  - Movement pain
  - TMJ noise
  - Masticatory palpation pain
  - Non-masticatory palpation pain
- Health-related variables
  - Headache
  - Low back pain
  - Irritable bowel syndrome and abdominal problems
  - Gynecologic
  - Functional symptoms and body pain
  - Anthropometric
  - Medical history

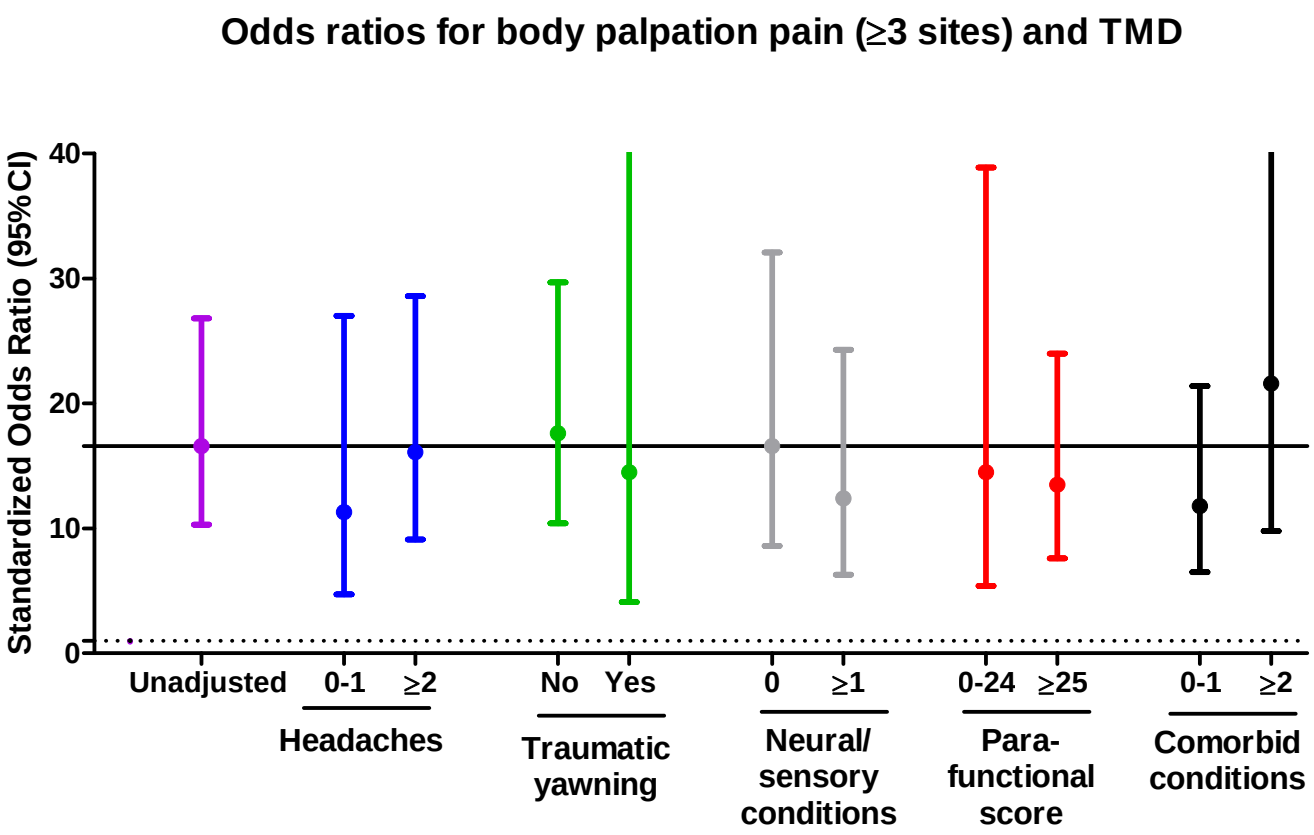
610 clinical items distributed across 65 content areas.

Standardized Odds Ratios - Clinical Measures

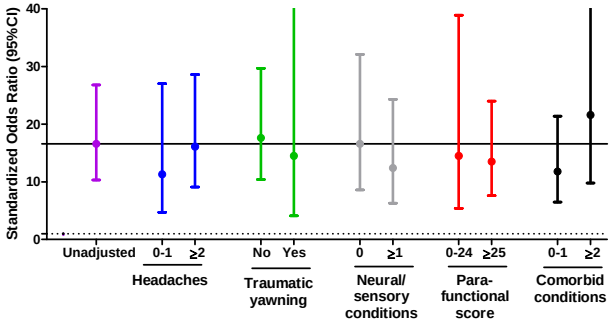


SOR adjusted for study site, age, gender, and race.

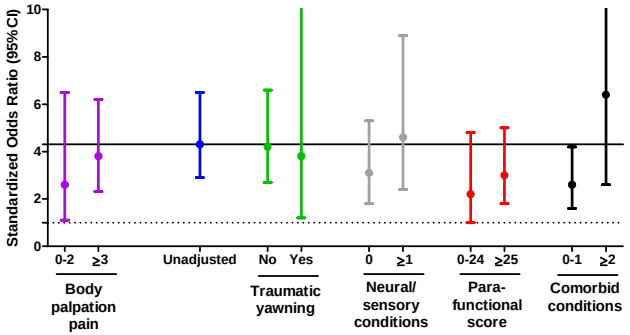
Body Palpation Score



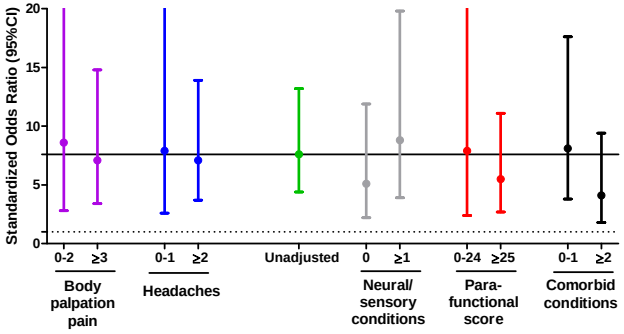
Odds ratios for body palpation pain (≥3 sites) and TMD



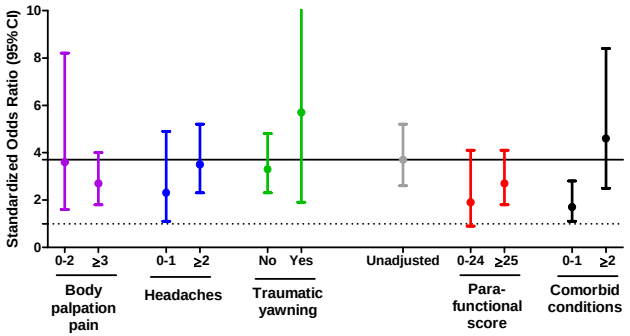
Odds ratios for headache (≥2 types) and TMD



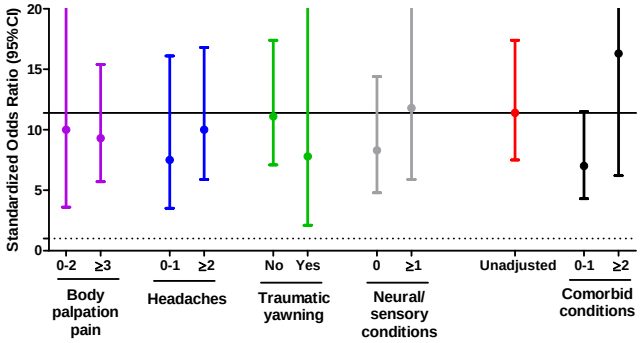
Odds ratios for traumatic yawning and TMD



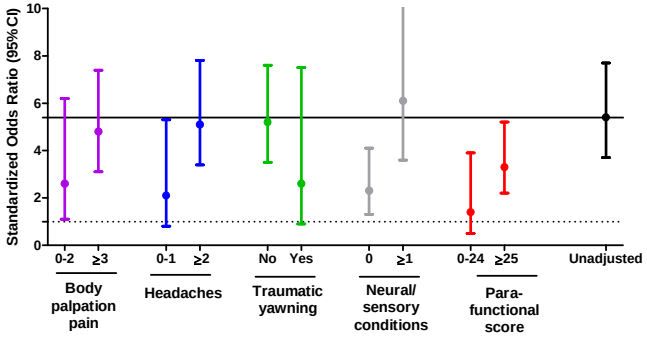
Odds ratios for neural/sensory conditions and TMD



Odds ratios for parafunction score (≥ 25) and TMD



Odds ratios for comorbid conditions (≥ 2) and TMD



Multivariable models for odds of TMD - stepwise selection of four variables -

Explanatory variable (categorical)	Odds ratios from multivariable logistic regression model
Body palpation (3 or more, vs 0-2)	11.8
Yawn as trauma (yes, vs no)	5.7
Number headache types (2 or more, vs 0-1)	2.0
Hx neural sensory conditions (yes, vs no)	1.6
Comorbid disorders (2 or more, vs 0-1)	1.7
Parafunction (25-62, vs 0-24)	6.0
Model discrimination (AUC)	*###*

* Models additional adjust for study site, age, gender and race

1. **Selected variables:**

   # headache types (CPSQ)

   Jaw injury, yawning (CPSQ)

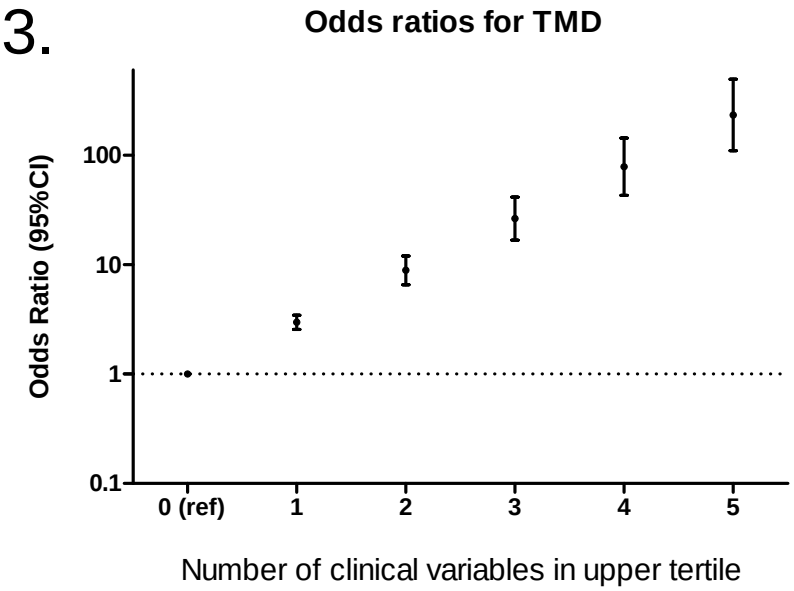
   Comorbid disorder count (CPSQ)

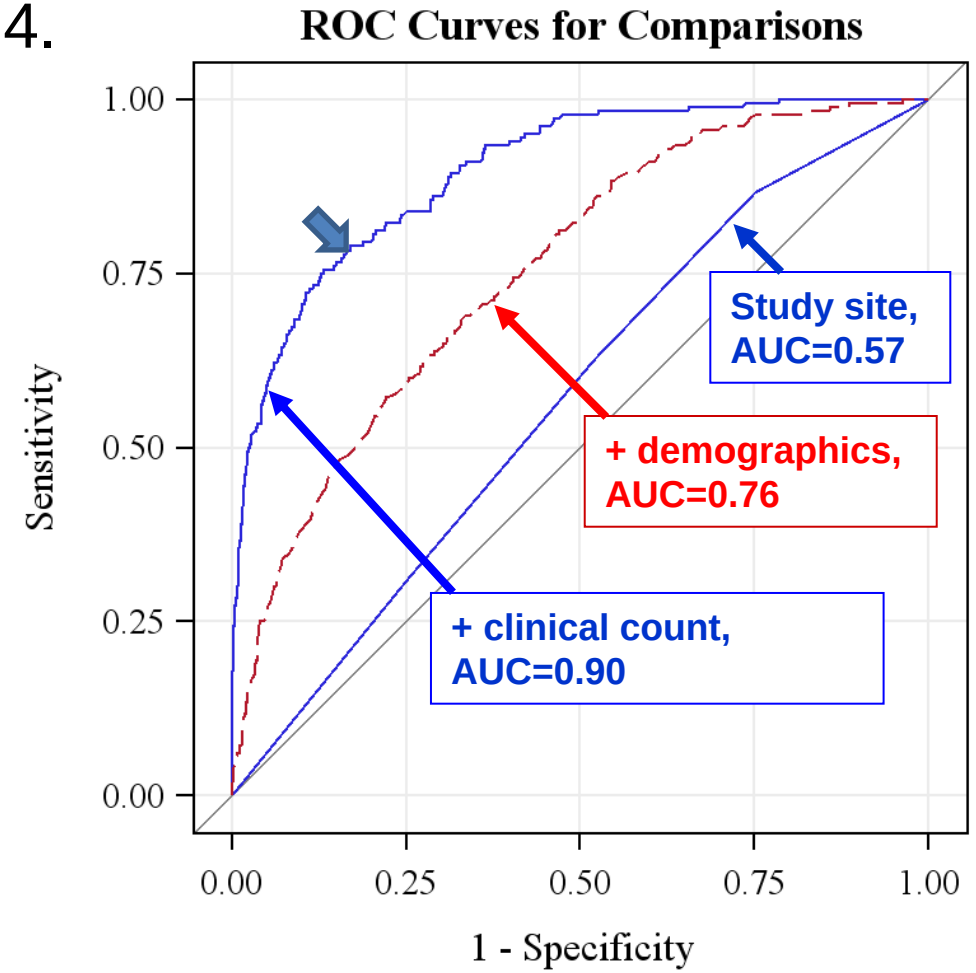
   Parafunction score (OBC)

   # body sites positive to palpation

   Hx neural/sensory conditions

2. Create count variable: increment if value is in upper tertile for each scale.

3. 

4. 

5. Clinically sensible index of 6 diverse clinical variables achieved 0.76 Sensitivity and 0.85 Specificity in predicting cases and controls.

Conclusions

1. TMD cases are sicker.
2. History of injury is more common among cases.
3. History of orthodontic treatment is more common among cases
4. History of headache, and more types of headache, are more common among cases.
5. History of back pain, and more episodes of back pain, are more common among cases.
6. History of IBS is more common among cases.
7. Comorbid conditions are more common among cases.
8. Cases report more TMJ noises.
9. Examiners detect more sounds in cases than controls.
10. Closed lock and open lock history are common among cases.
11. Pain from palpation is symmetrical
12. Distribution of palpation findings among muscles sites are as expected.
13. Cases report more oral behaviors.

continued

Conclusions

In the baseline OPPERA case-control study:

- Multiple clinical variables were associated with increased odds of chronic TMD -- and most have meaningful magnitude
- Stratified analysis of six clinical variables, selected from diverse domains, suggested they were independently associated with chronic TMD
- Conventional, stepwise multivariable logistic regression modeling identified six variables that contributed to the odds of chronic TMD
- A person-level summary variable, created by counting the number of variables with values in the upper tertile of the distribution, provided similar ability to discriminate chronic cases from controls & showed increasing odds of TMD associated with each additional high score, up to six high scores

Acknowledgments

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- Personnel involved in this part of the project:

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Gary Slade (Stats/EpiCore)
Flora Mulkey (Stats/EpiCore)
Yoly Gonzalez (UB)
Sharon Gordon (UMB)
Pei Feng Lim (UNC)
Margaret Ribeiro-Dasilva (UF)
Chuck Greene (EAP member)
Karon Cook (EAP member)

The OPPERA Case-Control Study:
Putative Risk Factors and Mechanisms for Persistent TMD Pain
IADR San Diego, March 19, 2011

OPPERA Study – Emerging Genetic Findings and Discoveries

William Maixner
University of North Carolina – Chapel Hill

Supported by NIH/NIDCR Grant U01DE17018

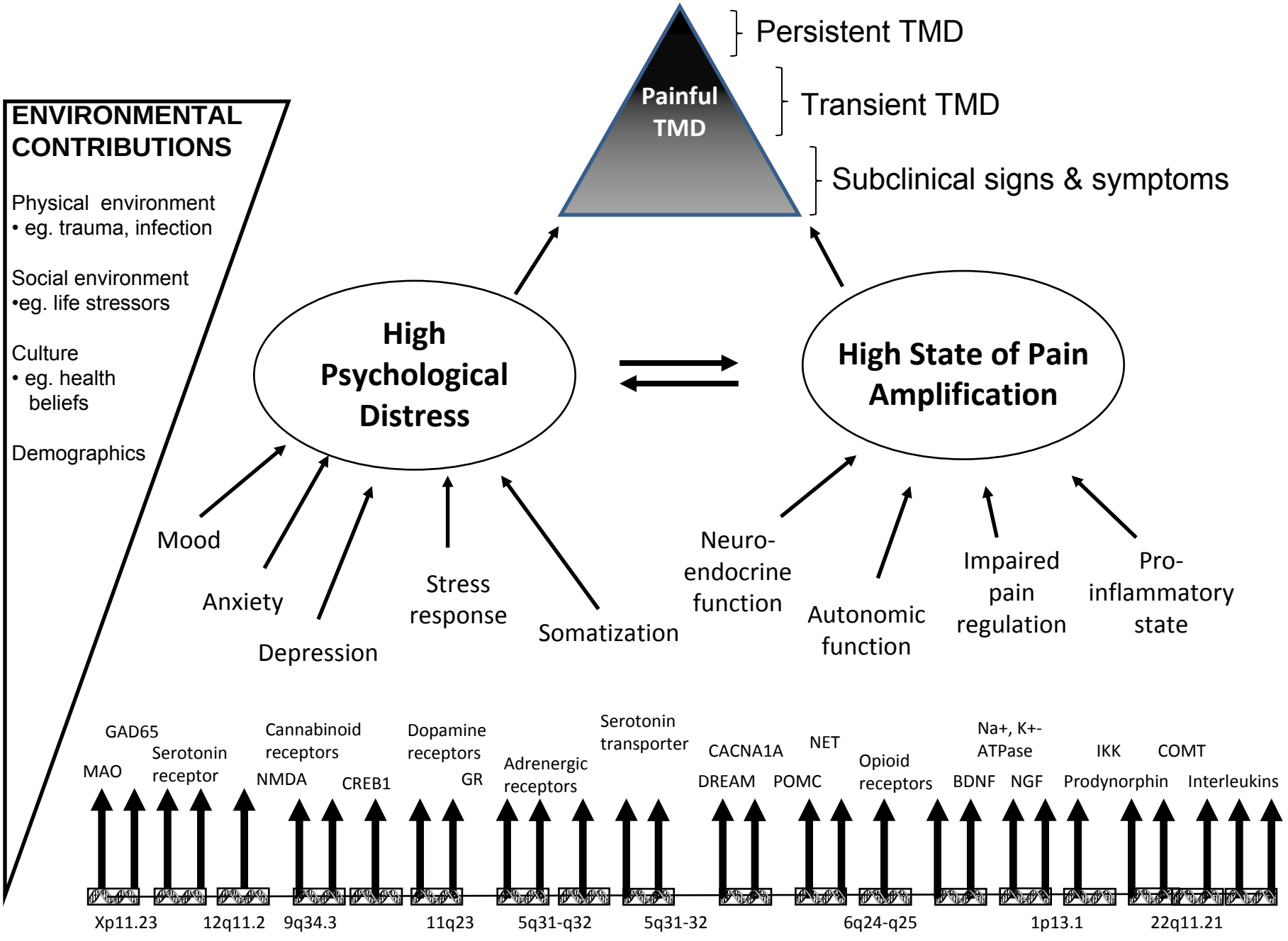
Disclosure

William Maixner is a founder, officer and equity stock holder in Algynomics Inc., a company providing research services in personalized pain medication and diagnostics.

Operational Aims from OPPERA's Baseline Case-Control Study

- To identify a set of SNPs, genes and cellular pathways that distinguish cases from controls

- To create a set of SNPs, genes and cellular pathways that capture the main etiologic constructs for TMD


ENVIRONMENTAL CONTRIBUTIONS
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• eg. trauma, infection
Social environment
•eg. life stressors
Culture
• eg. health beliefs
Demographics
Painful TMD
Persistent TMD
Transient TMD
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High Psychological Distress
High State of Pain Amplification
Mood
Anxiety
Depression
Stress response
Somatization
Neuro-endocrine function
Autonomic function
Impaired pain regulation
Pro-inflammatory state
GAD65
MAO
Serotonin receptor
NMDA
Cannabinoid receptors
CREB1
Dopamine receptors
GR
Adrenergic receptors
Serotonin transporter
CACNA1A
DREAM
NET
POMC
Opioid receptors
Na+, K+-ATPase
BDNF
NGF
IKK
Prodynorphin
COMT
Interleukins
Xp11.23
12q11.2
9q34.3
11q23
5q31-q32
5q31-32
6q24-q25
1p13.1
22q11.21

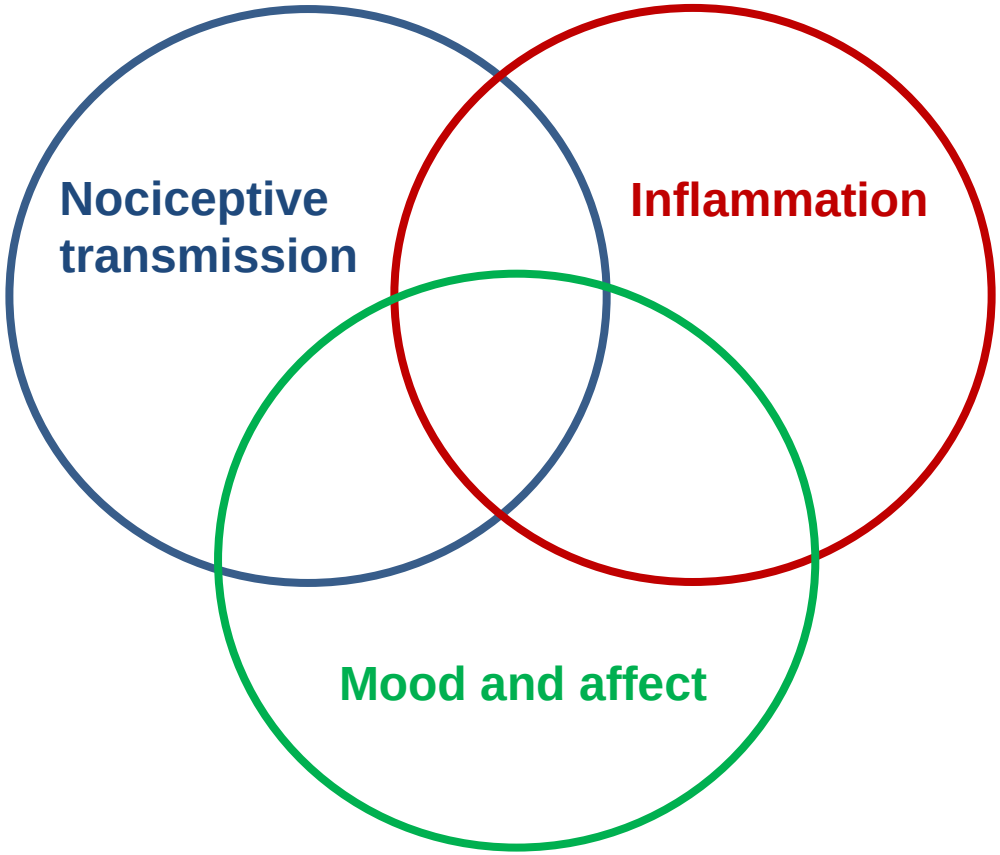
Association Methods

Candidate Gene	Genome-Wide Association
Hypothesis driven (“confirmatory”)	Hypothesis neutral (“exploratory”)
Relatively inexpensive per sample	Expensive per sample (but price decreasing)
Relatively expensive per SNP	Very inexpensive per SNP
Good power in moderately sized studies	Requires very large sample sizes
Results are easily interpreted	Results may require much work to interpret
Limited to few genes at a time	“Genome-wide” coverage

Pain Research Panel

Assessment of 3295 SNPs from 350 genes implicated in key pathways that regulate the perception of pain

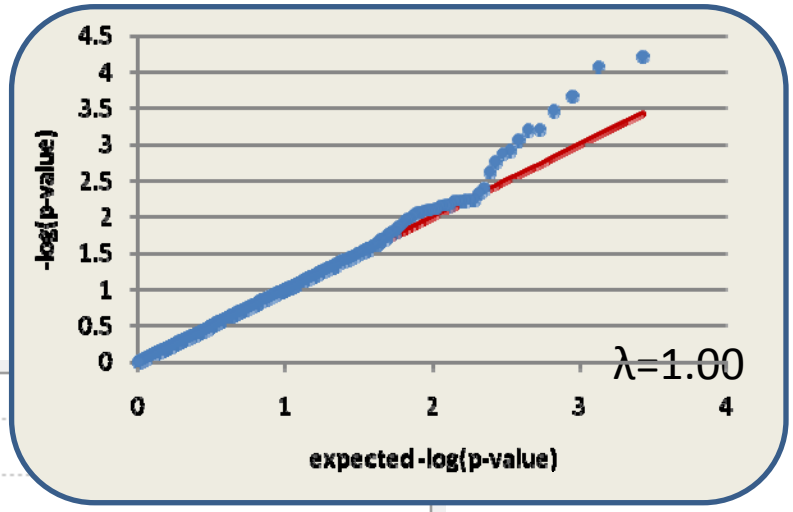
- Affymetrix ParAllele microarray platform using Molecular Inversion Probe (MIP) technology
- 3 domains relevant to hypothesized risk pathways
- Genes coding for proteins that mediate or modify the therapeutic effects of pharmacological agents used to treat pain
- SNP choice selective for putatively functional loci
- LD coverage of entire gene at $r^2 > 0.8$
- 160 ancestry-informative markers

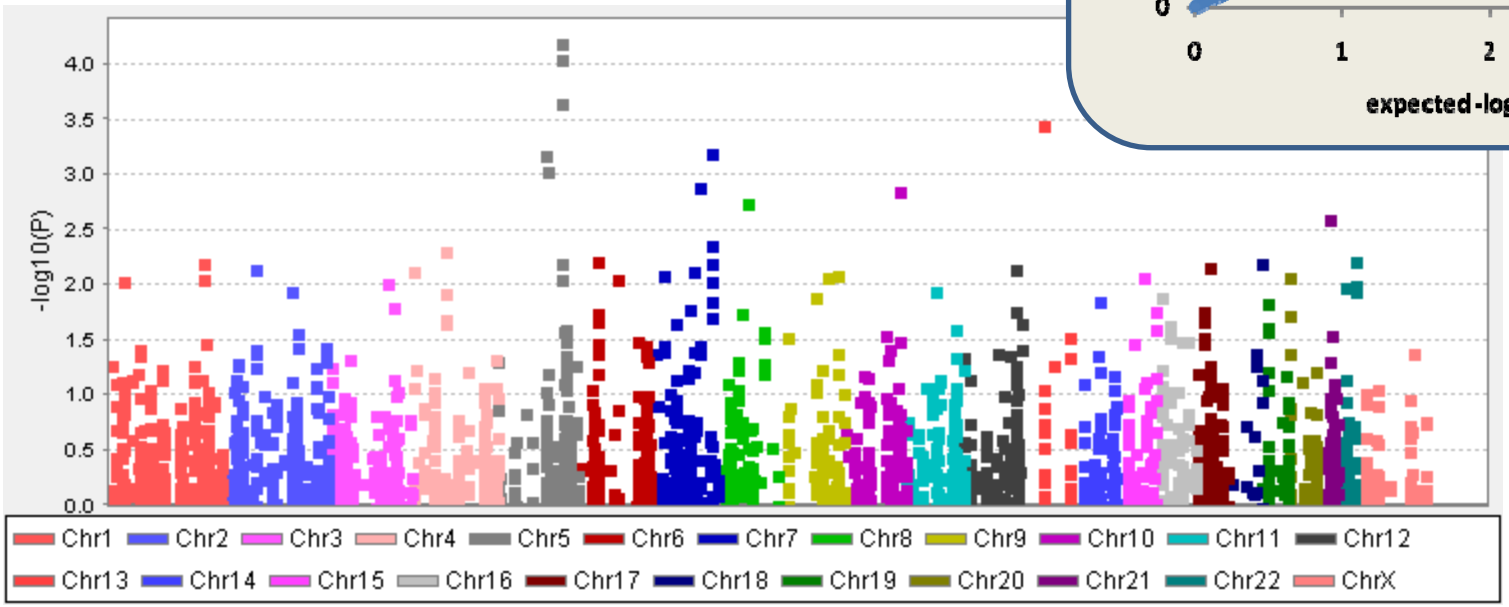


Association Tests

348 combined TMD cases vs 1612 controls

CHR	SNP	GENE	Call Rate	MAF (W)	MAF (B)	OR	P
5	rs2963155	NR3C1	99.90	0.195	0.24	0.63	6.15E-05
5	rs9324918	NR3C1	99.95	0.133	0.18	0.56	8.41E-05
5	rs33389	NR3C1	99.95	0.133	0.15	0.57	0.00022
13	rs9316233	HTR2A	99.95	0.207	0.42	0.64	0.00034
7	rs7800170	CHRM2	99.95	0.531	0.66	0.72	0.00062





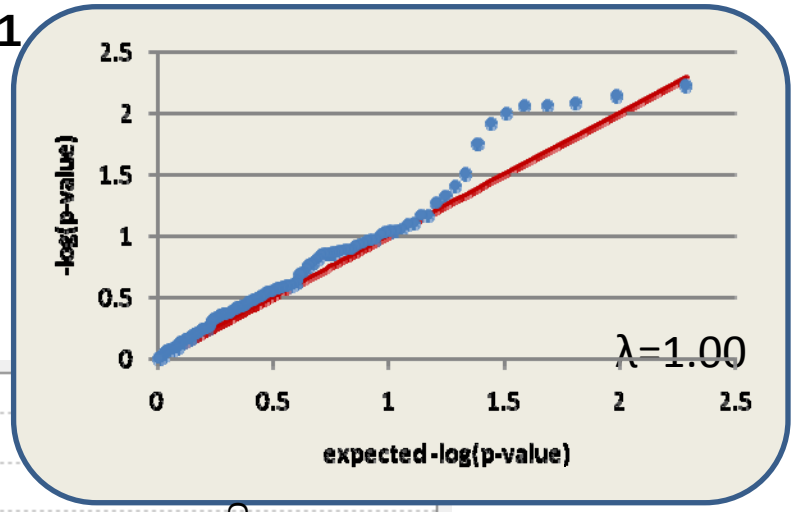
Logistic Regression model:

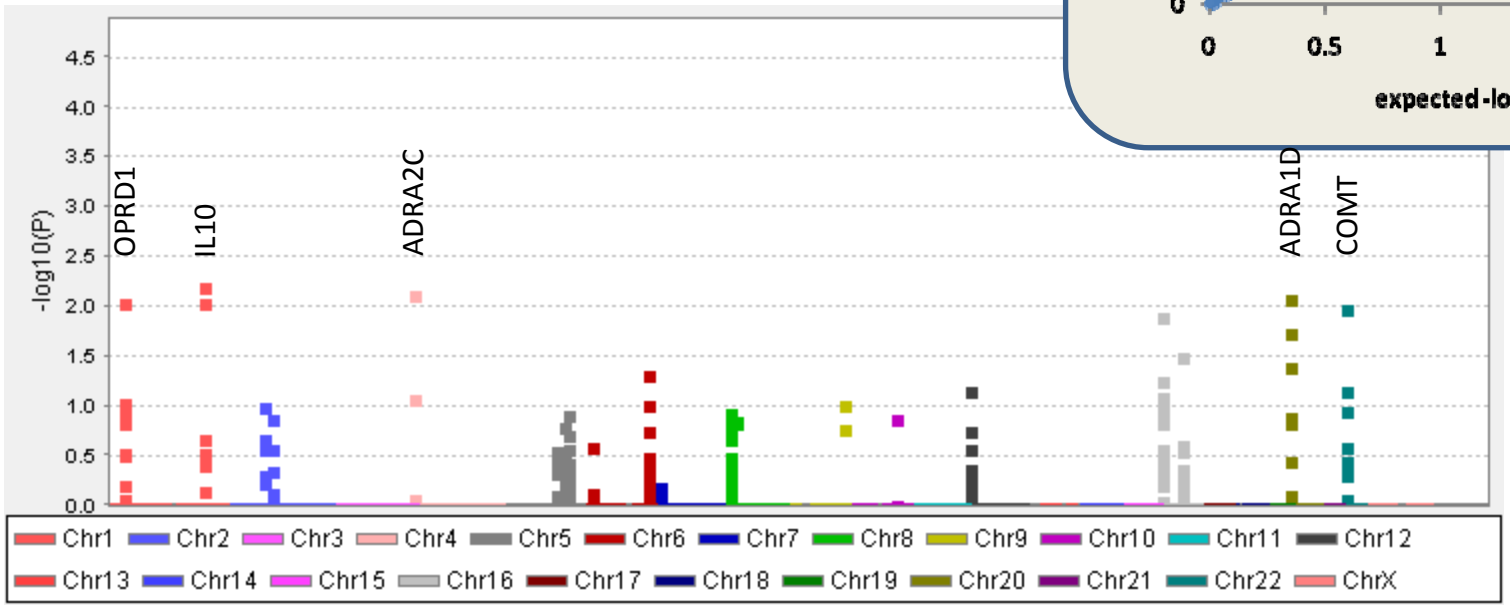
$y = \beta\emptyset + \beta1(\text{allele dosage}) + \beta2(\text{sex}) + \beta3\text{-}6(5\text{ sites}) + \beta7\text{-}8(2\text{ race eigenvectors}) + e$

Association Tests

348 combined TMD cases vs 1612 controls: Tier 1

CHR	SNP	GENE	Call Rate	MAF (W)	MAF (B)	OR	P
1	rs3024496	IL10	100.0%	0.52	0.36	0.76	0.0059
4	rs7696139	ADRA2C	99.64%	0.22	0.60	0.74	0.0072
20	rs1556832	ADRA1D	100.0%	0.53	0.23	1.29	0.0082
1	rs1800896	IL10	99.95%	0.53	0.27	0.77	0.0086
1	rs2236857	OPRD1	99.85%	0.27	0.33	1.32	0.0087





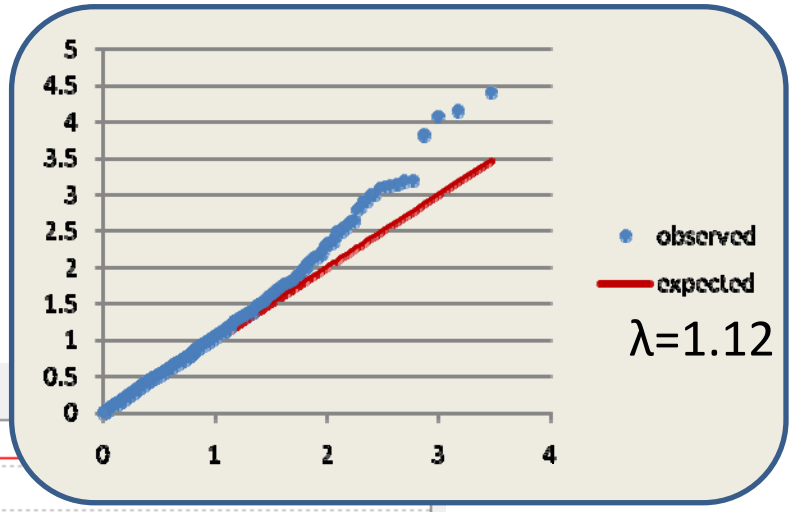
Logistic Regression model:

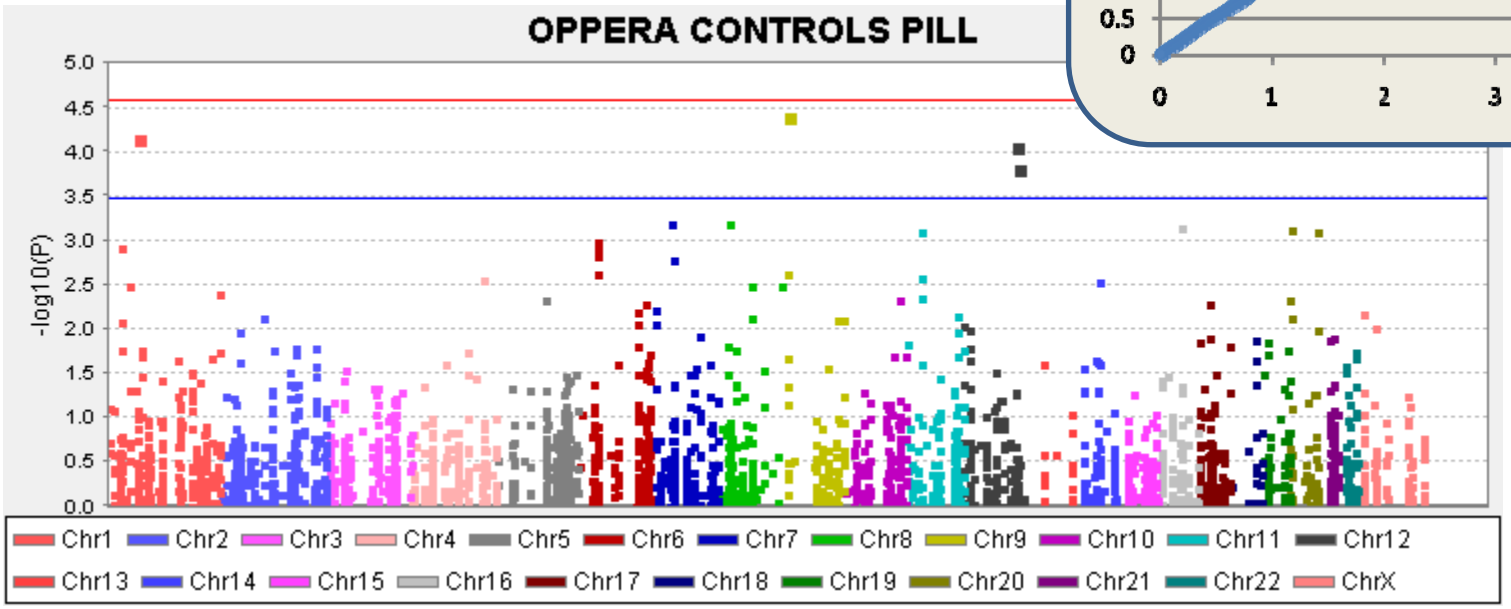
$y = \beta\emptyset + \beta1(\text{allele dosage}) + \beta2(\text{sex}) + \beta3\text{-}6(5\text{ sites}) + \beta7\text{-}8(2\text{ race eigenvectors}) + e$

Association Tests

1434 controls with PILL (somatization) score

CHR	SNP	GENE	Call Rate	MAF (W)	MAF (B)	BETA	P
9	rs3765550	MPDZ	100.0%	0.44	0.18	3.47	3.94E-05
1	rs2498982	INADL	100.0%	0.44	0.28	3.19	7.00E-05
12	rs9658478	NOS1	99.88%	0.00	0.07	10.64	8.33E-05
12	rs208288	P2RX7	100.0%	0.08	0.25	4.57	0.00015
8	rs1563945	PNOC	100.0%	0.15	0.32	-3.44	0.00063





Linear Regression model:

$y = \beta\emptyset + \beta1(\text{allele dosage}) + \beta2\text{-}4(4 \text{ sites}) + \beta5\text{-}6(2 \text{ race eigenvectors}) + e$

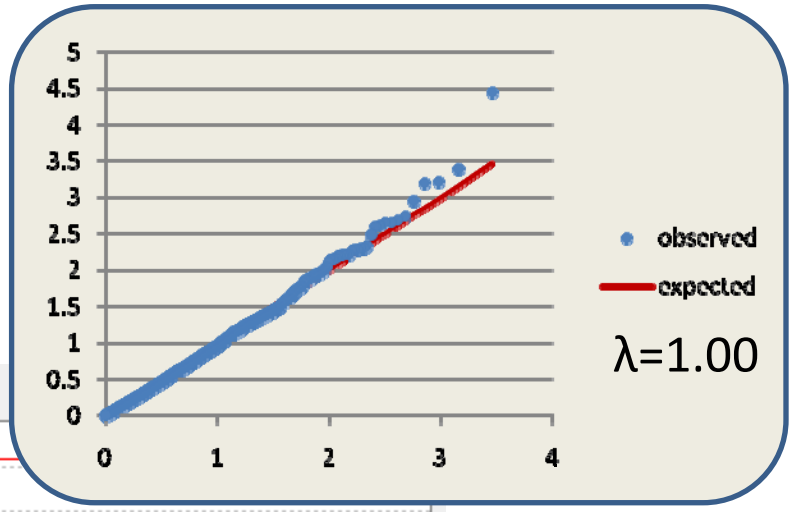
Putative Genetic Polymorphims Associated with TMD Case Status

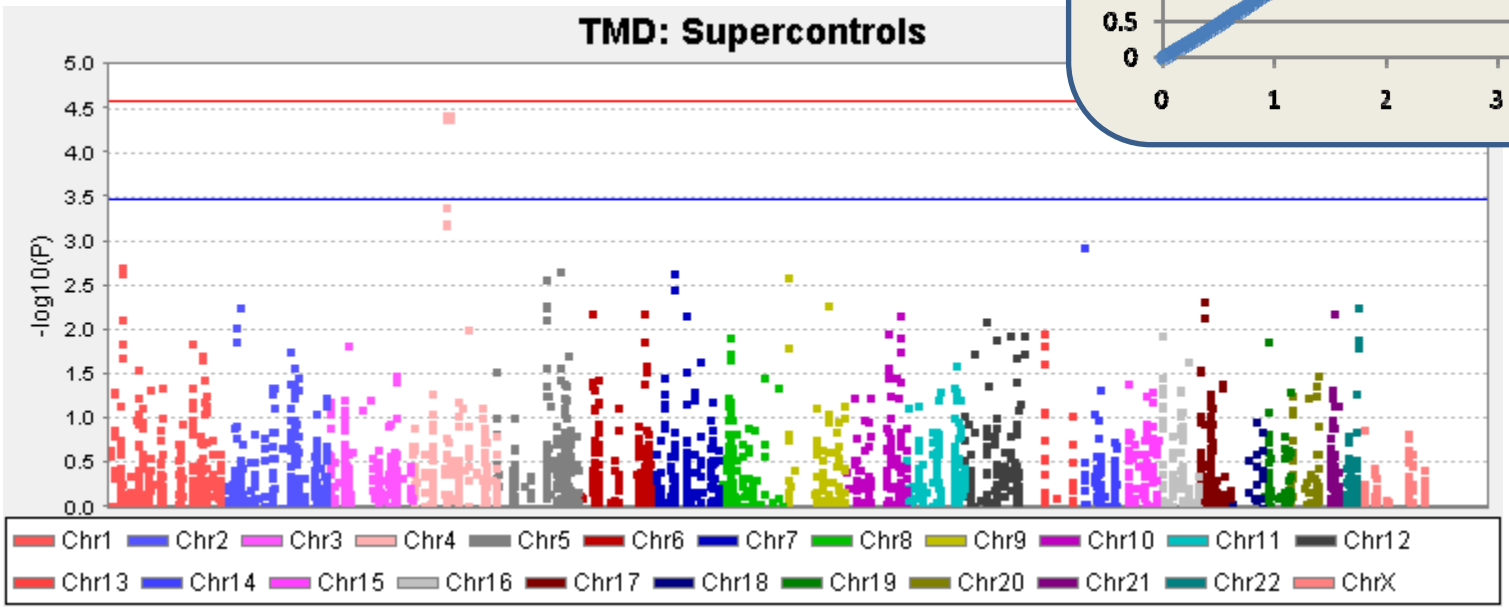
Gene	Protein	Function
NR3C1	Glucocorticoid receptor gene	HPA Axis Function and inflammation
HTR2A	Serotonin 2A receptor	Pain transmission , TMD, CWP
CAMK4	Calcium/calmodulin-dependent protein kinase 4 gene	Pain transmission and opioid analgesia
CHRM2	Muscarinic cholinergic receptor 2	Mood and inflammation
IFRD1	Interferon-related developmental regulator 1	Induced by NFG, neutrophil function
GRK5	G protein-coupled receptor kinase 5	Regulation of G protein-coupled receptors including ADRB2
COMT	Catecholamine-O-transferase	Pain transmission, TMD and opioid function
ADRA2C	Alpha-2C	Pain transmission
OPRD	Delta opioid receptor	Pain transmission
IL10	Interleukin 10	Inflammation and Pain
GRIN2A	Ionotropic N-methyl-D-aspartate (NMDA) receptor 2A	LTP, Pain transmission

Association Tests

127 TMD cases and 231 "supercontrols"

CHR	SNP	GENE	Call Rate	MAF (W)	MAF (B)	OR	P
4	rs1563826	EREG	100.0%	0.21	0.51	0.41	3.66E-05
14	rs10498313	PRKD1	97.48%	0.21	0.15	1.89	0.00011
1	rs2236857	OPRD1	99.93%	0.27	0.32	1.83	0.0019
5	rs2963155	NR3C1	99.98%	0.22	0.24	0.52	0.0021
7	rs1140475	EGFR	100.0%	0.12	0.08	2.08	0.0023





Logistic Regression model:

$y = \beta\emptyset + \beta1(\text{allele dosage}) + \beta2\text{-}4(4 \text{ sites}) + \beta5\text{-}6(2 \text{ race eigenvectors}) + e$

EREG/EGFR Pathways and Pain

- EREG
  - Epiregulin is a mitogenic peptide that binds to EGFR
  - Active in multiple cell types, including fibroblasts, macrophages, keratinocytes

- EGFR
  - Anti-ErbB antibody treatment has been shown to be analgesic in cancer treatment
  - EGFR regulates DOR and MOR via tyrosyl phosphorylation and activation of GRK2

Future Directions

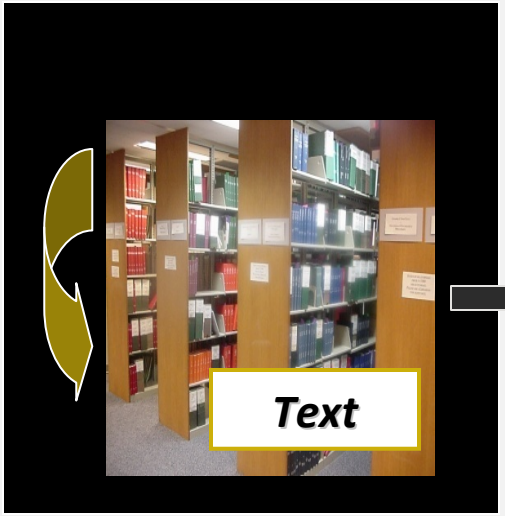
Deconstructing Heterogeneous TMD Cases into Homogenous Subgroups

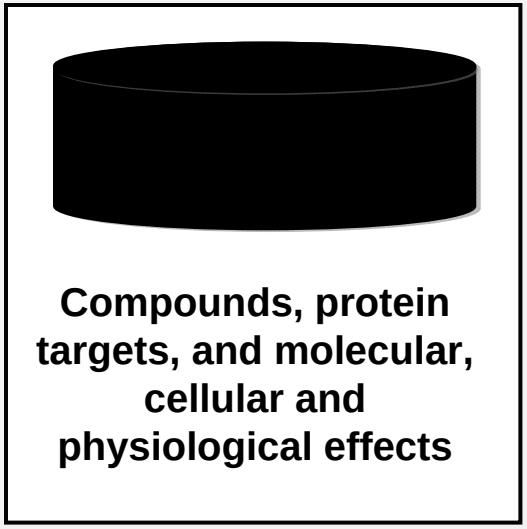
- Not all pain patients are created equal – several pathways to pain and suffering
- Need to assess intermediate phenotypes associated with causal pathways
- Statistical approaches that identify relatively homogenous patient subgroups or clusters based on intermediate phenotypes and clinical signs and symptoms.
  - Principal Component Analysis to determine latent constructs
  - Clustering of latent constructs
  - Machine Learning
- Integrate the information associated with many polymorphisms - each expressing a relatively small effect on the phenotype or cluster of interest.

Analysis of Signaling Pathways

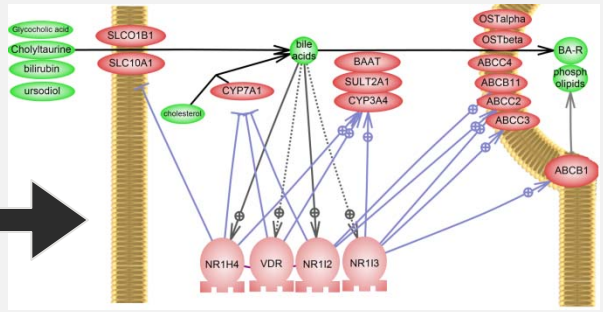
Creating Biological Pathways Using SNP Data from the Pain Research Panel

ResNet Database

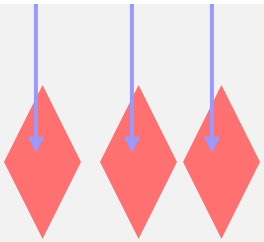




Compounds, protein targets, and molecular, cellular and physiological effects

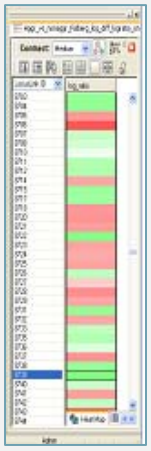


Mechanistic Model



New biomarkers and drug targets

Pain Panel SNP Data



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OPPERA TMD Pathways Data (Extreme Phenotypes)

Pathway	# related pathways	P Values
EGFR -> signaling pathways	7	0.00129
GFR ->signaling pathways	3	0.00516
TGFBR -> signaling pathways	3	0.01026
AdenosineR -> AP-1 signaling		0.01361
FibronectinR -> AP-1/ELK-SRF/SREBF signaling		0.01423
DopamineR2 -> AP-1/CREB/ELK-SRF signaling		0.01727
NeurotensinR -> ELK-SRF/AP-1/EGR signaling		0.01904
VasopressinR2 -> CREB/ELK-SRF/AP-1/EGR signaling		0.02339
EndothelinRa -> AP-1/CREB signaling		0.03353
ICAM1 -> AP-1/CREB/ELK-SRF signaling		0.03353
TLR -> AP-1 signaling		0.04296
NGFR -> AP-1/CEBPB/CREB/ELK-SRF/TP53 signaling		0.04310
T-cell receptor -> AP-1 signaling		0.04467
EctodysplasinR -> AP-1 signaling		0.04467
VEGFR -> ATF/CREB/ELK-SRF signaling		0.04533
CCR5 -> TP53 signaling		0.04889

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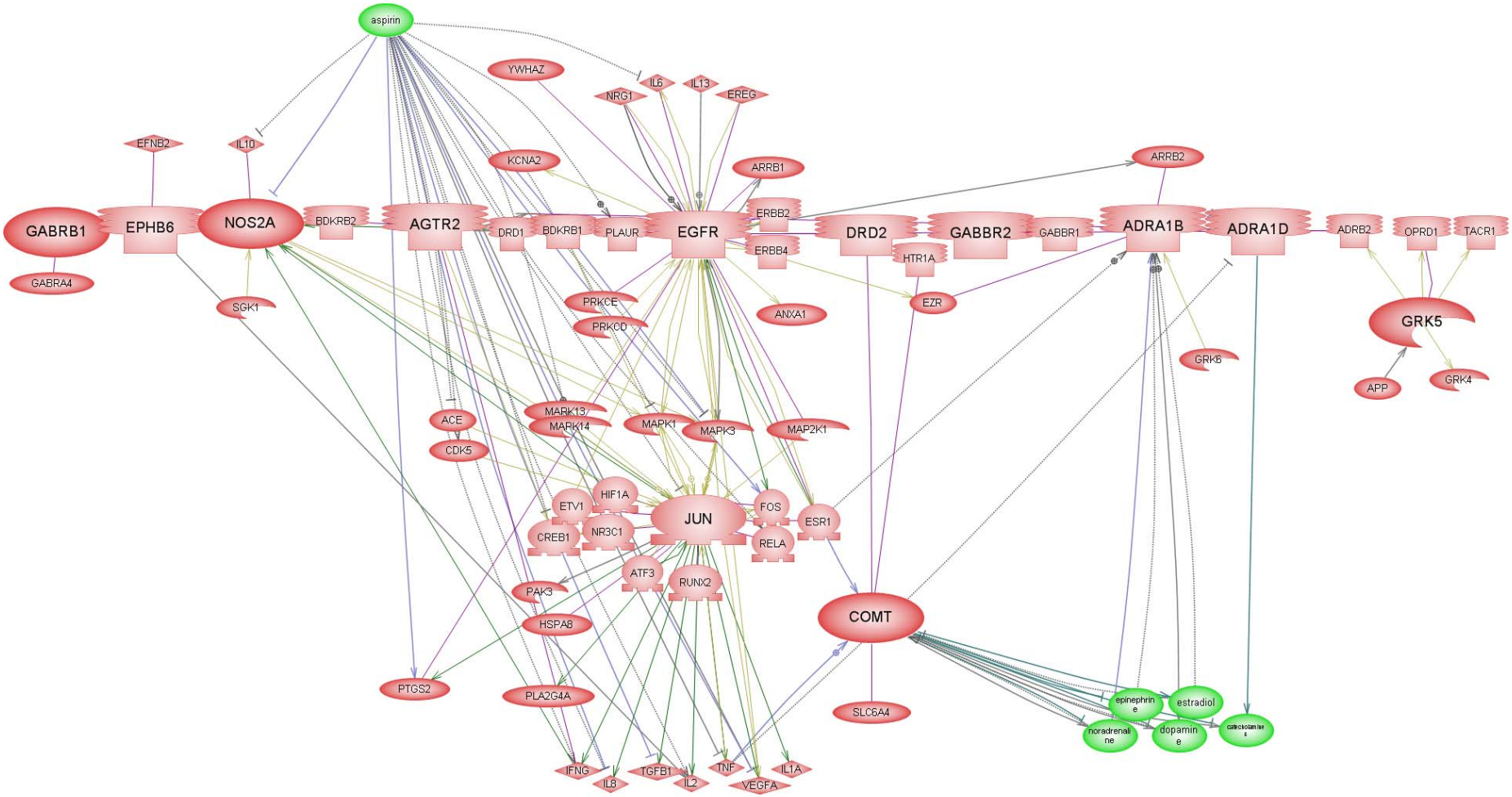
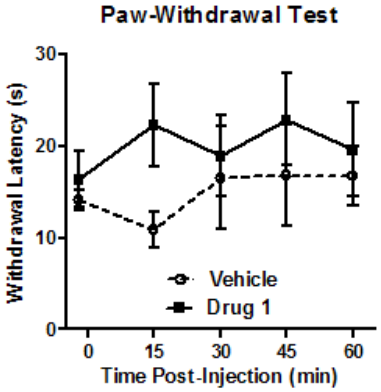
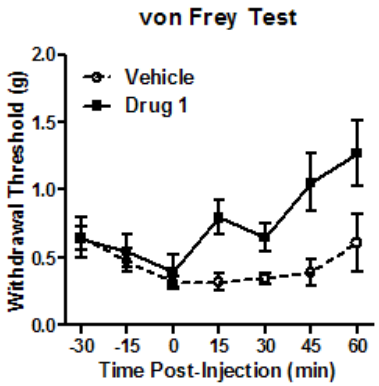
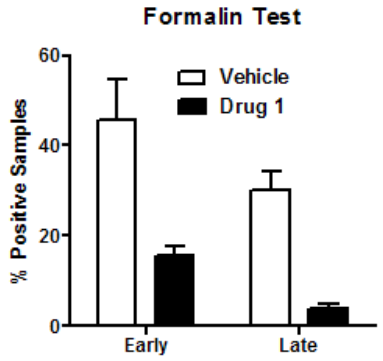
Pathway Analysis – Human Pain Sensitivity

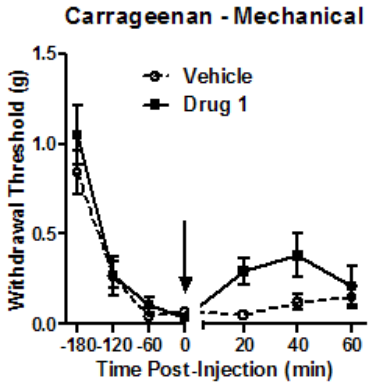


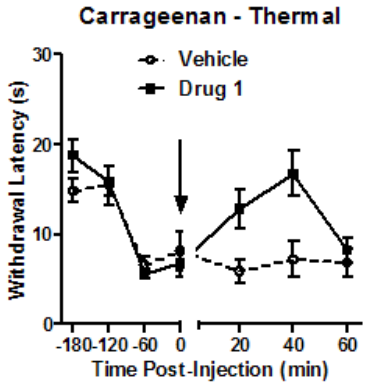
EGFR Receptor Antagonist Produces Analgesia in Multiple Pain Assays Assessed in Mice

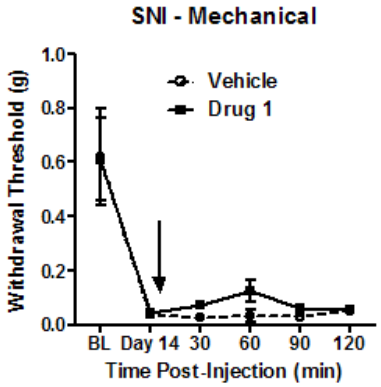








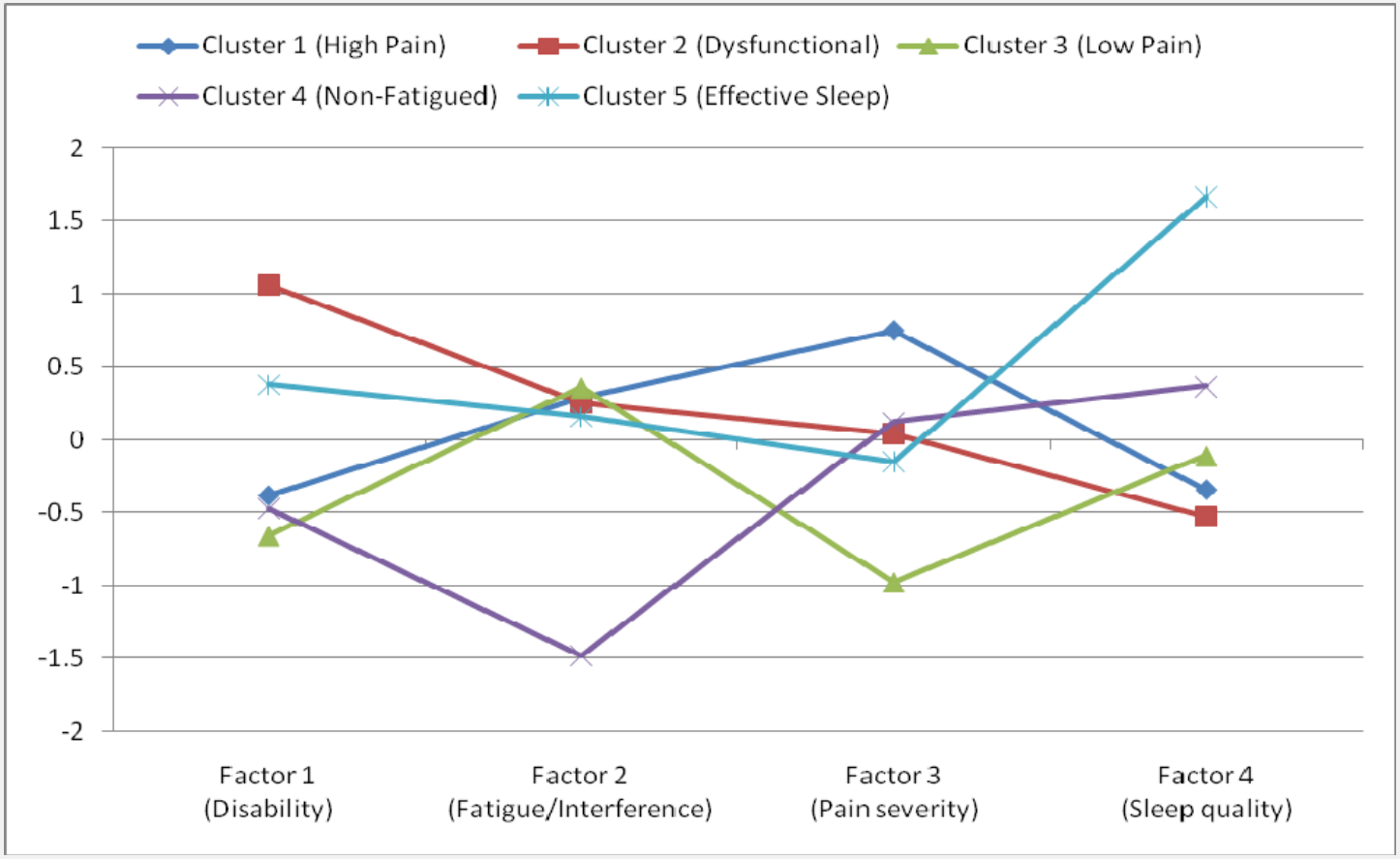




Data provided by Jeff Mogil – McGill University

Clustering and Pathway Analysis – Mechanism Based Diagnostic Classification and Therapeutic Target Identification Based on Pain Signatures

Factor and Cluster Analysis



- **Overlap of factors, with single factor predominating in each cluster**
- **Multiple factors represent challenge for therapy selection**

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Clusters Contrasted Against Healthy Controls to Detect SNPs Associate with 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

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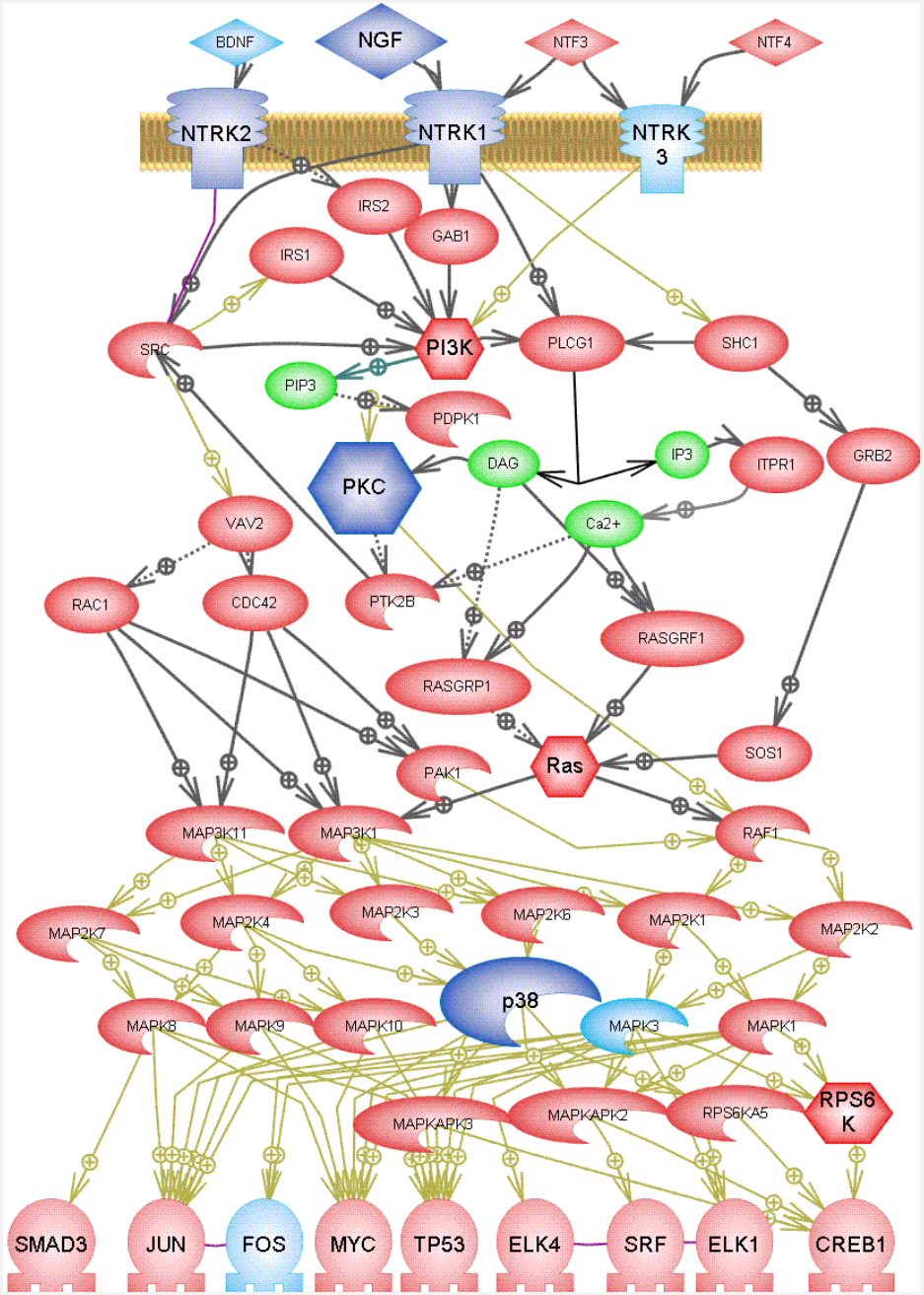

NTRK -> AP-1 pathways – gene SNPs contributions

dark blue – SNPs contributing to pathway P<0.05

light blue – SNPs contributing to pathways 0.05<P<0.1

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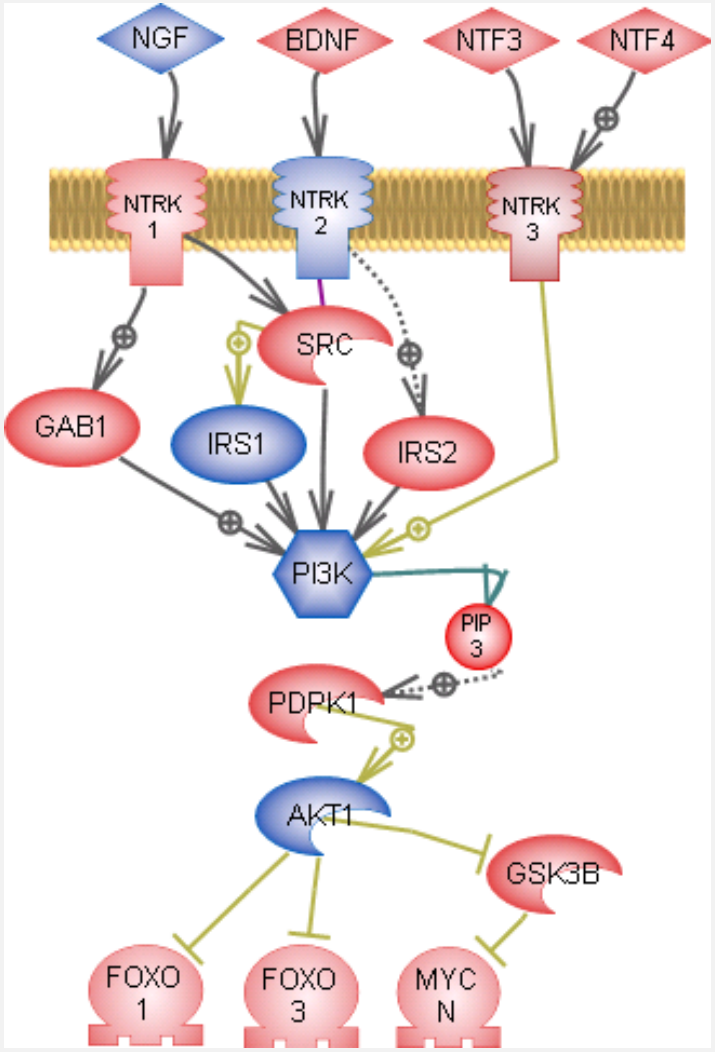
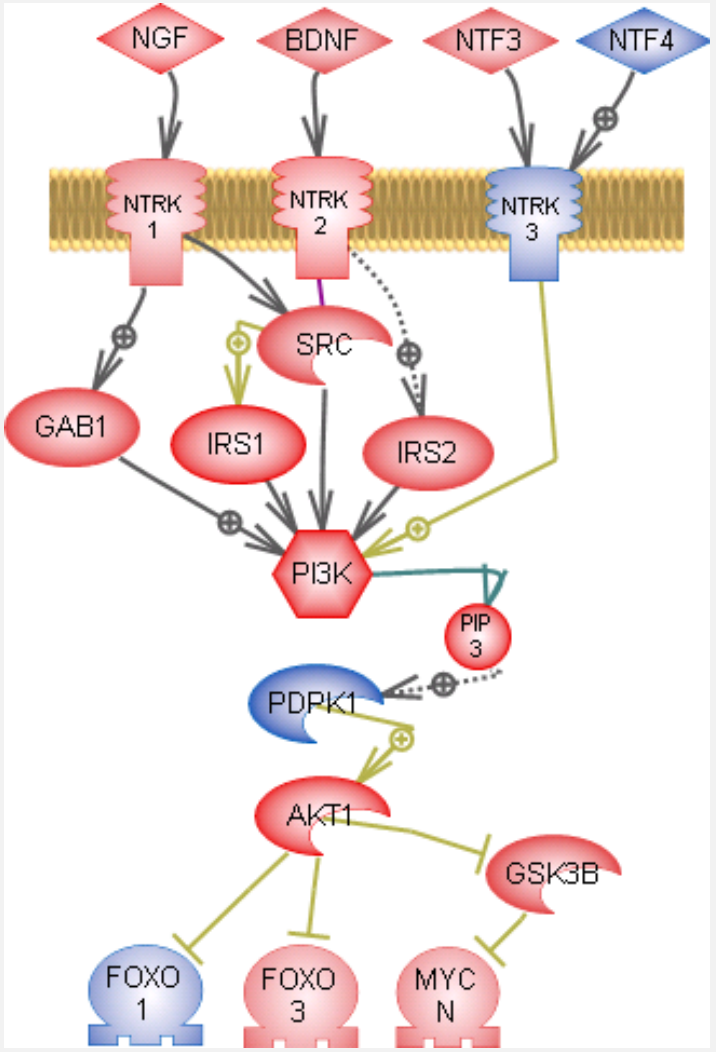
Different Genes – Common Cluster



Patient 1



Patient 2

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Conclusions

1. A set of SNPs, genes and cellular pathways that distinguish cases from controls have been identified

2. Methods that integrate SNPs, genes and cellular pathways that capture the main etiologic constructs for TMD are feasible and are in the process of being developed

3. Future initiatives that will further the diagnosis and treatment of TMD and related conditions require:
   a) Novel informatic tools
   b) Large scale phenotyping and genomic studies (GWAS, sequencing)
   c) Access to large, well characterized TMD populations

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