Supplementary Information

1. PARTICIPANTS

1.1. Recruitment.

Participants for the current study were recruited from Cincinnati Children's Hospital, the University of Cincinnati (UC) and the surrounding areas using study flyers posted on bulletin boards around the hospital, digital flyers posted on CCHMC's and UC's digital research pages, and from referrals from other research studies in the lab. Participants contacted study staff and were screened for initial eligibility. All study procedures were approved by the Cincinnati Children's Institutional Review Board (IRB#2015-4992).

1.2. Inclusion/exclusion criteria.

Participants with TMD and healthy controls were eligible if they were between the ages 18-50, English-speaking, not pregnant, not using opioids, had no recent hospitalization or surgery within the past six months. Participants were ineligible if they had history of cancer, diabetes, thyroid disorder, hypertension, pulmonary disease, neurological disorders, irregular menstrual cycles (>40 days) that cause pain and interfere with daily activities, or psychiatric disorders requiring hospitalization in the past year. In addition to at standardized clinical exam (DC-TMD) additional eligibility for the TMD cohort is the presence of pain five or more days in the last month and having a history of facial pain for more than six months.

1.3. Parent study.

Sixty-seven participants were consented for the primary study (IRB #2015-4992). Of these, five were withdrawn at the first session when it was discovered that they had pulmonary disease (n=1), cardiovascular disease (n=2), or irregular menstrual cycles (n = 2). The final sample of the parent study consisted of 40 participants with TMD and 22 healthy controls.

1.4. Current study.

Of these participants, Tempus Tubes were collected in nineteen participants with TMD and seventeen pain-free healthy controls during the later part of the study.

1.5. Collection and analysis of covariates.

Information about sociodemographic characteristics including age (years) sex (Male, Female), race (White, African American, Asian), BMI, and history of smoking and alcohol consumption were collected by self-report (Table 1). Descriptive statistics and *t*-tests were used to compare

differences between TMD and HCs. Effect sizes were calculated using Cohen's D (0.2 = small effect; 0.5 = medium effect, 0.8 = large effect) for paired samples *t*-tests.

2. METHODS

2.1. Blood collection.

Blood samples were collected with Tempus Blood RNA Tube (Applied Biosystems, ThermoFisher, Catalog Number: 4342792), which was used for the stabilization of RNA from whole blood for gene expression profiling. Samples were collected during the morning (range - 8:00 am to 10:30 am) to control for circadian changes in pain and inflammation. Tubes were stored at -80°C until processing.

2.2. RNA processing.

When all samples were collected, RNA was extracted and isolated with Tempus Spin RNA Isolation Kit (Invitrogen, ThermoFisher, Catalog Number: 4380204) per recommended protocols. Quality checks were conducted for the RNA including mass (>200 ng by Nano Drop Spectrophotometry).

2.3. Transcriptome analysis.

Samples were then sent to the UCLA Social Genomics Core Laboratory. (C) After re-tested for suitable RNA mass (Thermo-Fisher Quant-it Ribo Green) and quality (Agilent TapeStation capillary electrophoresis), genome-wide mRNA profiling using a high-efficiency 3' mRNA-targeted transcript counting assay (Lexogen QuantSeq 3' FWD) with multiplex cDNA sequencing on an Illumina HiSeq 4000 instrument in the UCLA Neuroscience Genomics Core Laboratory. Sequencing targeted >10 million single-stranded 65 nt sequencing reads for each sample (achieved mean 18.2 million), mapped to the hg38 reference human transcriptome sequence using the STAR aligner (achieved mean mapping rate 97.8%).

2.4. Bioinformatic analysis of transcriptome outcomes.

Gene expression was quantified as transcripts per million mapped reads (i.e., the STAR transcript count for each gene / total number of STAR-mapped transcripts for all genes), floored at 1 to suppress spurious low-range variability, log2-transformed to stabilize variance, and screened to exclude transcripts showing minimal variation in expression (SD < .5 log2 unit). All genes showing a point estimate of >1.2-fold differential expression in TMD vs. Controls (adjusted by covariate control for age, sex, race, BMI, and history of smoking, and history of heavy alcohol consumption) served as input into Transcription Element Listening System

(TELiS) promoter-based bioinformatic analyses conducted as previously described [1] using the TRANSFAC position-specific weight matrix V$NFKB_C to indicate NF-κB activity, V$IRF2_01 to indicate IRF activity, and V$CREBP1_01 to indicate CREB activity.

This study focused *a priori* on bioinformatic measures of CTRA-related transcription factor activity (i.e., NF-kB, IRF, and CREB) and was not powered for any gene-specific hypothesis testing or unbiased genome-wide discovery analysis (which would require a substantially greater sample size to achieve adequate power for such analyses). As in previous research, point estimates of differential expression served as input into TELiS bioinformatics analyses because gene lists based on biological effect sizes have been found to produce more reliable downstream bioinformatics results than gene lists derived from statistical p-/q-values [2-4]. Thus, no *p*-values were computed for individual genes, and statistical testing was restricted to a single *p*-value computed to assess the statistical significance of asymmetry in the prevalence of transcription factor-binding motifs (e.g., NF-κB, IRF, or CREB) within the core promoter DNA sequences of two groups of genes selected on the basis of differential expression effect size (i.e., genes up-regulated >1.2-fold in TMD vs. controls and genes down-regulated >1.2-fold in TMD vs. controls).

2.5. Components of the CTRA profile.

We examined whether any observed empirical differences in genes were attributed to differential activity of inflammatory-, interferon-, and sympathetic-related transcription factors using a bioinformatics database, Transcription Element Listening System (TELiS). All analyses were conducted over 9 parametric combinations of core promoter length (-300, -600, and -1000 to +200 nucleotides relative to the RefSeq gene transcription start site) and transcription factor-binding motif (TFBM) detection stringency (TRANSFAC mat_sim values of .80, .90, and .96), with the average (log2) TFBM ratio for > 1.2-fold up- vs. down-regulated promoters tested for statistically significant difference from 0 using standard errors derived from bootstrap resampling of linear model residual vectors (which controls for dependence across genes).

2.6. References

[1] Cole SW, Yan W, Galic Z, Arevalo J, Zack JA. Expression-based monitoring of transcription factor activity: The TELiS database. Bioinformatics (Oxford, England) 2005;21(6):803-810.

[2] Fredrickson BL, Grewen KM, Coffey KA, Algoe SB, Firestine AM, Arevalo JM, Ma J, Cole SW. A functional genomic perspective on human well-being. Proc Natl Acad Sci U S A 2013;110(33):13684-13689.

[3] Shi L, Jones WD, Jensen RV, Harris SC, Perkins RG, Goodsaid FM, Guo L, Croner LJ, Boysen C, Fang H, Qian F, Amur S, Bao W, Barbacioru CC, Bertholet V, Cao XM, Chu TM, Collins PJ, Fan XH, Frueh FW, Fuscoe JC, Guo X, Han J, Herman D, Hong H, Kawasaki ES, Li QZ, Luo Y, Ma Y, Mei N, Peterson RL, Puri RK, Shippy R, Su Z, Sun YA, Sun H, Thorn B, Turpaz Y, Wang C, Wang SJ, Warrington JA, Willey JC, Wu J, Xie Q, Zhang L, Zhang L, Zhong S, Wolfinger RD, Tong W. The balance of reproducibility, sensitivity, and specificity of lists of differentially expressed genes in microarray studies. BMC Bioinformatics 2008;9 Suppl 9:S10.

[4] Witten DM, Tibshirani R. A comparison of fold-change and the t-statistic for microarray data analysis. Stanford University Technical Report: Stanford, 2007. pp. 1-17.

Gene	Adjusted Fold Difference (Higher: TMD)
MYOM2	2.03
CCZ1B	1.74
SLC35E2B	1.60
TMEM176B	1.54
AC067852.2	1.53
SMIM24	1.51
TAS2R4	1.44
ULK4	1.41
EIF1AX	1.40
MTND1P23	1.39
ESPN	1.37
FAM118A	1.37
RUNDC3A	1.37
KDM8	1.36
TTC26	1.36
MT-TY	1.35
SCNN1D	1.34
TMPRSS9	1.34
HBG1	1.34
HLA-J	1.33
PWAR6	1.32
FOLR3	1.32
MC1R	1.32
AC087343.1	1.31
PAXIP1-AS1	1.31
KLC3	1.31
NACAP1	1.31
TMEM176A	1.30
MTRNR2L1	1.30
LAT	1.29
KLF1	1.29
AC064807.1	1.28
CHI3L1	1.27
AL683813.1	1.27
TNFRSF4	1.27
CCDC144B	1.27
NR2F6	1.26
AC114980.1	1.26
DCP1B	1.26
B3GALT2	1.26

NFXL1	1.25
PHLDA1	1.25
Y_RNA	1.25
AC011498.8	1.24
LINC00877	1.24
SNORD104	1.24
AC011477.2	1.24
LINC00402	1.23
AMN	1.23
AL356356.1	1.23
PNMA3	1.23
DCAKD	1.23
FCGR2C	1.23
AL158071.2	1.23
AL139246.5	1.23
ZFP41	1.22
AC068724.1	1.22
AC008741.2	1.22
PDE6G	1.22
SLC1A7	1.22
EPS8L2	1.22
CACNG8	1.22
RIPOR3	1.22
LINC00106	1.22
GPER1	1.22
AL592183.1	1.22
DBH-AS1	1.22
MFSD8	1.22
Z83843.1	1.21
ZC2HC1A	1.21
SMIM1	1.21
ACTA2	1.21
AL928654.5	1.21
TNFRSF9	1.21
RAB11FIP3	1.20
NID1	1.20
ECHDC2	1.20
NFKBIL1	1.20
SLC22A1	1.20
AC015871.3	1.20

Gene	Adjusted Fold Difference (Lower: TMD)
IGLC3	0.40
TUBB2A	0.44
S100B	0.51
RNF182	0.52
IGHA2	0.55
HLA-DRB5	0.55
FCGR2B	0.55
SCAMP5	0.57
TPST1	0.59
GUCY1B3	0.59
IL18RAP	0.60
EPHB1	0.60
ANKRD13C	0.60
GP1BA	0.60
TTC33	0.61
EEF1DP5	0.61
PPP4C	0.62
DTD1	0.62
CHCHD3	0.62
FASLG	0.62
MTND2P28	0.62
LHFPL2	0.62
AQP10	0.62
TSPAN9	0.62
DUSP2	0.62
AC245884.12	0.62
COL18A1	0.63
MARCO	0.63
B4GALT4	0.63
RNF4	0.64
BRPF3	0.64
KIAA0556	0.64
IFNGR1	0.64
IRX3	0.64
C2CD3	0.64
LGALSL	0.64
PTPRS	0.64
EPB41L2	0.64
NT5C3B	0.64
NCAPG2	0.65

CHUK	0.65
IFITM1	0.65
S1PR5	0.65
SCGB3A1	0.65
BCAT1	0.65
CTSW	0.66
C1QBP	0.66
AC022400.7	0.66
GOLIM4	0.66
USP21	0.66
PLEKHG1	0.66
FLVCR2	0.67
RTKN2	0.67
IL15RA	0.67
DNAJB9	0.67
ARSK	0.67
TRPM6	0.67
GPRC5C	0.67
CD300C	0.67
SMIM13	0.67
MYO1E	0.67
SMCO4	0.67
DEFA4	0.68
TRAF4	0.68
NET1	0.68
ZNF227	0.68
SH2D1B	0.68
FEN1	0.68
USP6NL	0.68
IGHG3	0.68
NCAPD2	0.68
WRNIP1	0.68
MGST1	0.69
U91328.2	0.69
FADS2	0.69
RARA-AS1	0.69
AL162424.1	0.69
NCAM1	0.69
ABHD5	0.69
ACSL6	0.69
GFM2	0.69
MAD2L1BP	0.69
YIF1B	0.69

CD27-AS1	0.69
BCDIN3D	0.69
EPB41L3	0.69
CBX8	0.70
MIF	0.70
JKAMP	0.70
GLB1L	0.70
STARD3NL	0.70
C5orf15	0.70
SRP19	0.70
AL139020.1	0.70
RNY4	0.70
MRPL32	0.70
MZB1	0.70
BCL10	0.70
FAF1	0.70
NIPA1	0.70
PANX1	0.70
AC130895.1	0.70
EFNB1	0.70
BCL9	0.70
UNC119B	0.70
LILRA4	0.70
FCGBP	0.70
TAF5L	0.70
WRB	0.70
ARID5B	0.70
MKI67	0.71
RMI2	0.71
GP9	0.71
ASB7	0.71
PLB1	0.71
THBS1	0.71
DZIP3	0.71
ST8SIA1	0.71
ITM2C	0.71
ACADSB	0.71
UAP1L1	0.71
SDHB	0.71
NR2C2AP	0.71
FAAH2	0.71
QSOX2	0.71
ZNF503	0.71

DRAXIN	0.71
RBFA	0.71
CMC1	0.71
SLC37A1	0.71
BLK	0.71
AL049873.1	0.71
BACE1	0.71
EEF1E1	0.71
PGM1	0.71
VPS37A	0.71
NUP37	0.71
CLIC4	0.71
NIP7	0.72
ALAS1	0.72
TIFA	0.72
ZNF107	0.72
CHAMP1	0.72
PEAR1	0.72
AC245100.8	0.72
GPR160	0.72
FBXO4	0.72
TBK1	0.72
SCD	0.72
ROPN1L	0.72
GABPA	0.72
GLA	0.72
RPS23P8	0.72
METTL25	0.72
TNFRSF17	0.72
VNN1	0.72
H1F0	0.72
LRRCC1	0.72
C1GALT1C1	0.72
ADCY3	0.72
TBC1D12	0.72
CALHM2	0.72
C16orf87	0.72
SLC16A5	0.73
GP5	0.73
SAMD8	0.73
ZNF440	0.73
DLGAP1-AS1	0.73
TRIT1	0.73

ANKRD40	0.73
BICRA	0.73
DNAJC14	0.73
HMGXB3	0.73
AAED1	0.73
CERS2	0.73
TMEM126A	0.73
RCC1	0.73
KIAA1324	0.73
C1orf174	0.73
PLXNA4	0.73
C1D	0.73
CROCCP2	0.73
PDCL3	0.73
ZBTB10	0.73
TXNDC9	0.73
TRUB1	0.73
ACTL10	0.73
LRFN4	0.73
CRLS1	0.73
TBX19	0.73
NUDT14	0.73
DFFB	0.73
INTS13	0.73
PRPS2	0.73
ITPKC	0.73
ZMPSTE24	0.73
ACAT1	0.73
LY96	0.73
ARSB	0.73
SVIL-AS1	0.73
WDR89	0.74
HAUS1	0.74
RBMS2	0.74
NCOA5	0.74
SAMD14	0.74
BCL7B	0.74
SGMS1	0.74
LRRK1	0.74
EBNA1BP2	0.74
LZTFL1	0.74
REV3L	0.74
PSMD5-AS1	0.74

PTBP2	0.74
MEGF6	0.74
CDK8	0.74
PXDN	0.74
TCEAL1	0.74
LATS1	0.74
FBXO28	0.74
KCTD5	0.74
SLC36A4	0.74
C4orf33	0.74
REV1	0.74
MFSD2B	0.74
SVBP	0.74
FH	0.74
PAPSS1	0.74
FAM213B	0.74
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BAG3	0.74
MCRIP2	0.74
POLR2K	0.74
FPR3	0.74
CLEC10A	0.74
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LGR6	0.74
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LATS2	0.74
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MAP2K1	0.74
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FCRL3	0.75
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INTS14	0.75
CPNE2	0.75

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ACKR3	0.75
ZBED3	0.75
TSPAN13	0.75
KRT23	0.75
NBPF3	0.75
TDP2	0.75
TMTC2	0.75
PSMD12	0.75
ATP2B1-AS1	0.75
ORC2	0.75
IMMP1L	0.75
MIR4458HG	0.75
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GTF2H1	0.75
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AKTIP	0.75
SNX9	0.75
SYT11	0.75
NAT9	0.75
KYNU	0.75
RNF6	0.75
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SLC35A5	0.75
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GALNT11	0.75
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ZNF230	0.75
SAP30	0.75
CLDND1	0.75

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CCL3	0.76
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TSFM	0.76
TDRD7	0.76
CCR5	0.76
ZNRF2	0.76
ZNF746	0.76
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TMEM99	0.76
AUTS2	0.76
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IKZF2	0.76
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AC104695.3	0.76

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SLFN12	0.76
UHRF1BP1L	0.76
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SLC35B3	0.77
LYPD3	0.77
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CKAP2	0.77
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GZMH	0.78
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RRM2	0.78
CLK2	0.78
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IFNLR1	0.78
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FANCG	0.78
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TMSB4XP4	0.78
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CAAP1	0.78
C17orf80	0.78

KATNB1	0.78
FAM122A	0.78
FN3KRP	0.78
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CEP83	0.78
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CHEK2	0.79
MB21D1	0.79
RMI1	0.79
CENPBD1P1	0.79
CEP44	0.79
S100Z	0.79
TMEM38B	0.79
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COL3A1	0.79
KBTBD4	0.79
ZNF92	0.79
PLAGL1	0.79
MED7	0.79
ZNF528-AS1	0.79
TSPYL4	0.79
GPR157	0.79
ZSWIM3	0.79
THAP6	0.79
HS3ST3B1	0.79
AP4E1	0.79
ZNF366	0.79
HPSE	0.79
USP31	0.79
CXCL1	0.79
SLC23A2	0.79
MRPS28	0.79
RAB9A	0.79

SIDT1	0.79
CFL2	0.79
ERMAP	0.79
EVI5L	0.79
TRAPPC3L	0.79
NAB2	0.79
INTS8	0.79
LINC00476	0.79
LCMT2	0.79
C17orf75	0.79
EXOSC8	0.79
HECW2	0.79
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SELP	0.79
PNMA1	0.79
SOCS4	0.79
CUL2	0.79
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OTUD1	0.79
MAP1A	0.79
POLR3G	0.79
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UNC50	0.79
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SATB1-AS1	0.79
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CD9	0.79
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BIK	0.81
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C6orf120	0.82
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CENPV	0.82
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PRKAG2-AS1	0.82
CRY1	0.82
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ADCY9	0.82
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THAP1	0.83
TICAM1	0.83
SEC22A	0.83
AC016739.1	0.83
METTL21A	0.83
SCRIB	0.83
ERMP1	0.83
C5orf30	0.83
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BCAS2	0.83
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HOMEZ	0.83
MCM6	0.83
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CHORDC1	0.83

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BORA	0.83
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AXIN2	0.83
APPL2	0.83
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LTV1	0.83
DHRS4L2	0.83
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CLTCL1	0.83
HIST1H3D	0.83
TRAPPC8	0.83
DPP3	0.83
TUBGCP5	0.83
TMEM97	0.83
UBXN8	0.83
IL2RA	0.83
NT5DC3	0.83
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PRDM11	0.83
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SLAMF6	0.83
CD248	0.83
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AKAP1	0.83
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CDK7	0.83
TREML1	0.83
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ADGRG5	0.83

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SYTL2	0.83
SGPL1	0.83
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INPP5E	0.83
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ENPP4	0.83
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CD200	0.83
BAHCC1	0.83
ABHD6	0.83
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