Rare Loss-of-function Variants Analyses Identified SLC13A1 Associated with Chronic Pain

Xiang Ao[1], Marc Parisien[1], Luda Diatchenko[1,*]

1. Faculty of Dental Medicine and Oral Health Sciences; Department of Anesthesia, Faculty of Medicine and Health Sciences; Alan Edwards Centre for Research on Pain; McGill University, Montreal, Canada

***. Corresponding author**

Poster Number: PFR151

INTRODUCTION

- Whole-exome sequencing (WES) is a cost-effective method to capture genomic coding regions at a deep level [1].
- WES has significantly reformed studies in human genetics, identified enormous disease-causing variants and therapeutic targets.
- Chronic pain is a worldwide common unpleasant condition, incurring appreciable healthcare and socioeconomic costs.
- Pain genetics studies have unfolded various common variants related to pain, yet missing heritability consistently appeared [2,3].
- Rare variant association study offers opportunity and advantages for explaining the missing heritability as it implicates more precise genetic factors.
- Rare variant is defined as a genetic variant with its minor allele frequency (MAF) less than a threshold, commonly MAF < 0.01.
- Loss-of-function (LoF) variants alter gene function, resulting a reduced or abolished protein function.
- Large-scale biobanks provide accessibility and reliability to rare variants.

OBJECTIVES

1. To identify genes and rare variants that contribute to the development of eight chronic pain conditions in UK biobank (UKBB) [4], including back pain, facial pain, general pain, headaches, hip pain, knee pain, neck or shoulder pain, stomach or abdominal pain, and multisite pain.
2. To study the expression pattern of the genes in mice pain assays.
3. To investigate the functional consequence of the identified genes and rare variants.

METHODS

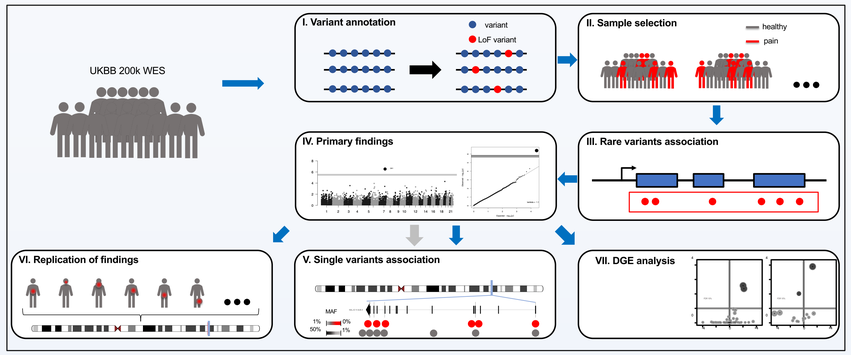

Figure 1. Schematic flow chat of the study. Analyses were done on cohorts UKBB 200k. Step I. Variant annotation. Step II. Sample selection. Step III. Gene-based rare variants association test. Step IV. Primary results investigation. Step V. Single variant association tests. Step VI. Replication analyses in pain conditions. Step VII. Differential Gene Expression (DGE) analysis in animal pain models. Blue arrows indicate primary analyses and grey arrows indicate replication analyses.

- Back pain, facial pain, general pain, headaches, hip pain, knee pain, neck or shoulder pain, stomach or abdominal pain, and multisite pain samples were selected from UK biobank 200k WES cohort.
- Variant annotation was done with Ensembl Variant Effect Predictor (VEP, release 99) [5] and Combined Annotation Dependent Depletion (CADD, v1.6) [6]
- Gene-based rare variant association was done with SAIGE-GENE(v0.44.2) [7]
- Differential gene expression (DGE) analysis was performed with R package DESeq2 [8]

RESULTS

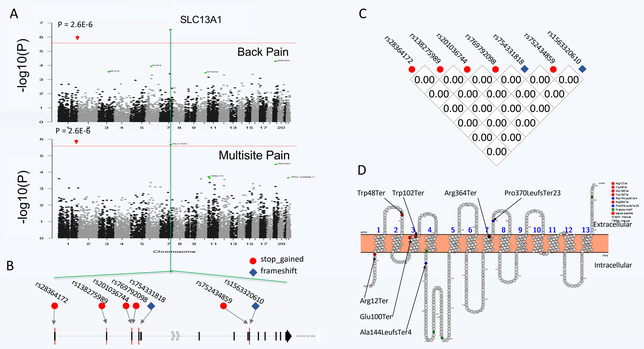

Figure 2. Findings from chronic back pain and multisite pain in the UKBB 200k. Manhattan plot of the gene-based rare LoF variants test in chronic back pain (up) and multisite pain (bottom) in the UKBB 200k WES cohort. The red line represents the genome-wide significance threshold. Gene SLC13A1, i.e. Solute Carrier Family 13 (Sodium/Sulfate Symporters) Member 1, was identified to be associated with the development of chronic back pain and multisite pain. B. Genomic coordinates of the rare LoF variants from the primary findings, red circles represent stop gained variants while blue diamonds represent frameshift variants. C. A linkage disequilibrium plot of the primary LoF variants, the order of variants is the same as in panel B. D. Mutations corresponding to the identified variants in protein SLC13A1.

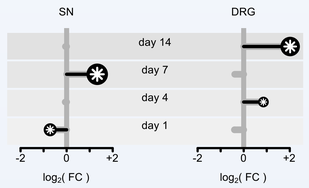

Figure 3. Differential expression of Slc13a1 in a rat model of nerve injury. Plots track fold change (FC) intensities and direction (up- or down-regulation), in sciatic nerve (SN; left) and dorsal root ganglia (DRG; right) tissues, at multiple time points compared to baseline (day 0).

Table 1. Gene-based LoF rare variants association test on SLC13A1 and chronic pain conditions in the UKBB 200k WES. P-values were calculated with three different models: burden test, SKAT test, and SKAT-O test. Results from primary analysis with genome-wide statistic threshold 2.6E-06.

Pain	#LoF	Burden-Pvalue	SKAT-Pvalue	SKAT-O-Pvalue
Back Pain	7	2.0E-07	2.3E-05	2.9E-07
Facial Pain	4	5.6E-02	1.6E-01	8.2E-02
General Pain	4	8.3E-01	6.7E-01	7.9E-01
Headache	5	9.4E-04	9.2E-03	1.2E-03
Hip Pain	6	9.1E-01	2.9E-01	1.3E-01
Knee Pain	7	1.7E-05	4.8E-05	1.7E-05
Neck, Shoulder Pain	7	1.5E-05	6.1E-05	1.7E-05
Stomach, Abdominal Pain	4	4.2E-02	8.8E-02	6.2E-02
Multisite Pain	7	1.0E-07	4.6E-06	1.4E-07

Table 2. LoF rare variants found in chronic back pain and multisite pain samples from UKBB 200k WES.

rsid	Chr:Pos	Mutation	Ref	Alt	Consequence	CADD
rs28364172	7:123199913	Arg12Ter	G	A	stop_gained	34
rs138275989	7:123181057	Trp48Ter	C	T	stop_gained	36
rs201036744	7:123171835	Glu100Ter	C	A	stop_gained	38
rs769792098	7:123171827	Trp102Ter	C	T	stop_gained	38
rs754331818	7:123169270	Ala144LeufsTer4	GC	G	frameshift	15.9
rs752434859	7:123128888	Arg364Ter	G	A	stop_gained	34
rs1563320610	7:123128868	Pro370LeufsTer23	AG	A	frameshift	29.7

Table 3. Association of LoF rare variants found in UKBB 200k WES with chronic back pain and multisite pain.

rsid	Pain	AF(alt)	N	BETA	SE	Pvalue	OR(95%CI)
rs28364172	Back	2.8E-03	96058	0.41	0.10	2.7E-05	1.5(1.2-1.8)
rs138275989	Back	1.0E-03	96058	0.18	0.16	2.6E-01	1.2(0.9-1.6)
rs201036744	Back	2.1E-05	96058	0.98	1.10	3.8E-01	2.7(0.3-23.0)
rs769792098	Back	1.6E-05	96058	-1.41	1.31	2.8E-01	0.2(0.02-3.2)
rs754331818	Back	1.8E-04	96058	1.75	0.39	5.8E-06	5.8(2.7-12.4)
rs752434859	Back	2.1E-05	96058	2.06	1.09	5.9E-02	7.9(0.9-66.4)
rs1563320610	Back	3.1E-05	96058	1.85	0.85	2.9E-02	6.4(1.2-33.6)
rs28364172	Multisite	2.9E-03	137168	0.13	0.03	8.6E-06	1.1(1.1-1.2)
rs138275989	Multisite	1.0E-03	137168	0.04	0.05	0.4E-00	1.0(0.9-1.1)
rs201036744	Multisite	1.8E-05	137168	-0.009	0.37	9.8E-01	1.0(0.5-2.0)
rs769792098	Multisite	1.1E-05	137168	-0.74	0.48	1.2E-01	0.5(0.2-1.2)
rs754331818	Multisite	1.6E-04	137168	0.58	0.12	2.5E-06	1.8(1.4-2.3)
rs752434859	Multisite	1.8E-05	137168	1.02	0.37	5.6E-03	2.8(1.3-5.7)
rs1563320610	Multisite	2.2E-05	137168	0.63	0.33	6.0E-02	1.9(0.9-3.6)

REFERENCES

[1] Bao, R., Huang, L., Andrade, J., Tan, W., Kibbe, W. A., Jiang, H., & Feng, G. (2014). Review of current methods, applications, and data management for the bioinformatics analysis of whole exome sequencing. *Cancer informatics*, *13*, CIN-S13779.

[2] Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., ... & Visscher, P. M. (2009). Finding the missing heritability of complex diseases. *Nature*, *461*(7265), 747-753.

[3] Sexton, J. E., Cox, J. J., Zhao, J., & Wood, J. N. (2018). The genetics of pain: implications for therapeutics. *Annual review of pharmacology and toxicology*, *58*, 123-142.

[4] Szustakowski, J. D., Balasubramanian, S., Kvikstad, E., Khalid, S., Bronson, P. G., Sasson, A., ... & Reid, J. G. (2021). Advancing human genetics research and drug discovery through exome sequencing of the UK Biobank. *Nature genetics*, *53*(7), 942-948.

[5] McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R., Thormann, A., ... & Cunningham, F. (2016). The ensembl variant effect predictor. *Genome biology*, *17*(1), 1-14.

[6] Rentzsch, P., Schubach, M., Shendure, J., & Kircher, M. (2021). CADD-Splice—improving genome-wide variant effect prediction using deep learning-derived splice scores. *Genome medicine*, *13*(1), 1-12.

[7] Zhou, W., Zhao, Z., Nielsen, J. B., Fritsche, L. G., LeFaive, J., Gagliano Taliun, S. A., ... & Lee, S. (2020). Scalable generalized linear mixed model for region-based association tests in large biobanks and cohorts. *Nature genetics*, *52*(6), 634-639.

[8] Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, *15*(12), 1-21.

ACKNOWLEDGEMENT

This work was funded by the Canadian Excellence Research Chairs (CERC09), a Pfizer Canada Professorship in Pain Research, and CIHR (SCA-145102) for Health Research's Strategy for Patient-Oriented Research (SPOR) in Chronic Pain to LD.

CONFLICT OF INTEREST STATEMENT

Dr. Luda Diatchenko was/is a consultant for Duke University, ONO PHARMA USA Inc, Releviate Inc, and Orthogen AG. All other authors declare that they have no competing interests.

CONCLUSIONS

- Our study unfolded a novel association from SLC13A1 (Solute Carrier Family 13 Member 1) to chronic back pain and multisite pain.
- Association from SLC13A1 was replicated in chronic headache, chronic knee pain, and chronic neck and shoulder pain.
- Seven loss-of-function rare variants from SLC13A1 were found contributed to chronic back pain and multisite pain.
- Elevated expression of Slc13a1 in pain assays compared to sham after its initial down regulation suggested a positive regulatory role of the gene in pain resolution.
- SLC13A1 is responsible for the regulation of inorganic sulfate levels in serum, mice that lacking Slc13a1 exhibited hyposulfatemia, hypersulfaturia, and a bunch of other disorders, like impaired memory, clonic seizure.