Genome-Wide Association Study And *In-Vivo* Functional Validation Implicate The Adaptive Immune System In Chronic Postsurgical Pain

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INTRODUCTION

Chronic postsurgical pain (CPSP) is considered a major complication after surgical procedure and it is defined as pain lasting at least three months after surgery. CPSP has negative impact on the patients and the operative team as well. The prevalence of CPSP varies with the type of surgery from 5% to 85%. Some surgeries are at higher risk of causing CPSP, such as inguinal hernia repair, breast and thoracic surgery, leg amputation, and coronary artery bypass surgery. The etiology and prognosis of CPSP are still not very clear. However, epidemiological studies have been identified various risk factors for CPSP include clinical (pain history, type of surgery, anesthesia, acute pain severity), psychological factor, demographic and genetic predisposition (Fig.1). Few genetics studies have been done in CPSP; however no significant genome-wide risk loci have been identified yet. In this study we aimed to uncover genetic variants associated with CPSP severity in different surgery types, as well as to determine the genes, pathways, tissues and cells which are implicated in this pain development.

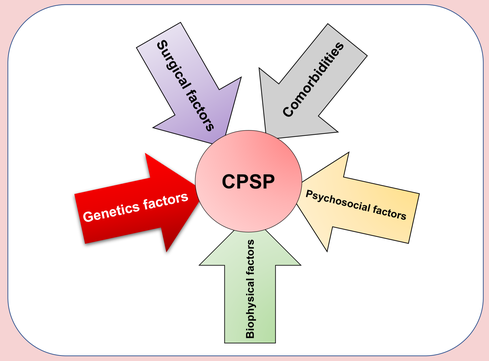


Figure 1. Potential risk factors for CPSP development

OBJECTIVE

In this study we aimed to uncover genetic variants associated with CPSP severity in different surgery types, as well as to determine the genes, pathways, tissues and cells which are implicated in this pain development.

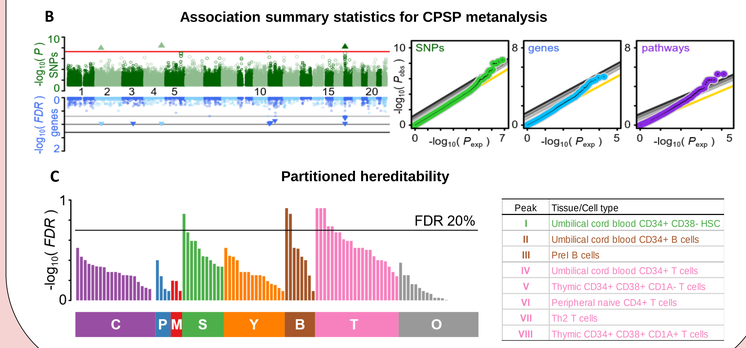
METHODS & RESULTS

Genetic study in CPSP

Genome-wide association studies (GWASs) have been performed in post-surgical cohorts of five surgery types: hysterectomy, mastectomy, abdominal, hernia, and knee. Total participants in these cohorts was 1350 individuals. Additionally, and for further analysis, the cohorts were combined via a GWAS meta-analysis framework. Then, gene- and pathway-level statistics have been obtained by MAGNA analysis using the summary SNP p-values from the GWASs and the meta-analysis (Fig.2).

A **CPSP GWAS cohorts**

Surgery	Acronym	Enrolled	Country	Genotyping Array
Hysterectomy	HYS	345	Netherlands	Illumina PsychArray
Abdomen & knee	ABKNEE	190	Italy	Illumina PsychArray
Mastectomy	PMPS	665	USA	Axiom UK Biobank
Knee replacement	TKR	100	USA	Axiom Precision Medicine
Hernia	HRN	226	Denmark	Axiom Precision Medicine
Knee arthroplasty	TANK	180	USA	Axiom Precision Medicine



Peak	Tissue/Cell type
I	Umbilical cord blood CD34+ CD38- HSC
II	Umbilical cord blood CD34+ B cells
III	PreI B cells
IV	Umbilical cord blood CD34+ T cells
V	Thymic CD34+ CD38+ CD1A- T cells
VI	Peripheral naive CD4+ T cells
VII	Th2 T cells
VIII	Thymic CD34+ CD38+ CD1A+ T cells

Figure 2. (A) Study design of GWAS CPSP cohorts (B) Association summary statistics for CPSP meta-analysis. Manhattan plots (left) for SNP-level (top, green) and gene-level (bottom, blue) summaries, as well as QQ plots (right) for SNP-level (green), gene-level (blue), and pathway-level (purple) summaries. **(C) Partitioned heritability in selected tissues in the CPSP meta-analysis**. *To the left,* enrichment tracked in 106 tissues or cell lines from Benita et al. Tissues are grouped by: central nervous system (C, purple, n=21), peripheral nervous system (P, blue, n=4), muscle (M, red, n=3), stem cells (S, green, n=11), myeloid cells (Y, orange, n=16), B cells (B, brown, n=8), T cells (T, pink, n=22), and other tissues or cell lines (O, grey, n=21). Shown are FDR-corrected P-values for enrichment. *To the right*, table showing the tissues and cell lines significantly enriched at the FDR 20% level.

Stem cells, B cells and T cells are involved in CPSP.

In-vivo validation

In order to validate the results obtained by our genetics analysis, we did examine the pain behavior in a mouse plantar incisional model of postoperative pain. Two groups of mice (six C57BL/6 WT and four Rag1 knock-out) did undergo to plantar incision surgery in the right hind paw. Then, the pain behavior has been studied using Von Frey filament after surgery at day 1, 4, 7, then every week till day 105. The Rag1 knock-out mice, which don't produce mature B and T cells, showed significant hypersensitivity even after 2 months from the plantar incision surgery, while wide-type mice resolved their post-incisional hypersensitivity in 5 days (Fig.3A).

With the aim of determining which of the immune cells are the responsible for recovering form CPSP, we did inject four groups of Rag1 -/- mice by B cells, T cells B&T cells together. Two weeks later these mice did undergo to plantar incision, then the pain behavior has been measured by Von Frey. We observed that the mice injected by B&T cells together have been recovered from pain after 7 days. However, Rag1 -/- mice injected by T or B cells showed hypersensitivity after pain recovering at day 7 and day 10 respectively (Fig.3B).

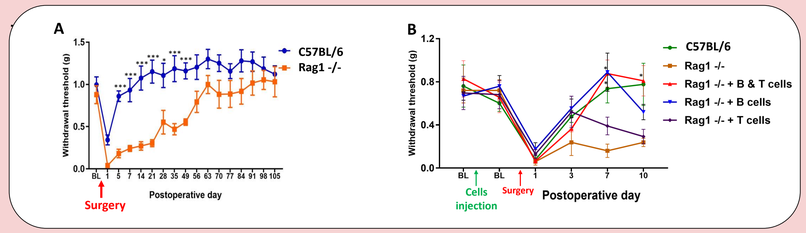


Figure 3. Pain behavior of the WT C57BL/6 mice, Rag 1-/- with or with no B/T cells injection. **(A)** Von Frey comparing the pain behavior of C57BL/6 and Rag 1 -/- mice after plantar incision (10 mice in each group). **(B)** Vo n Frey comparing the pain behavior of C57BL/6 and Rag 1 -/- mice injected by B and/ or T cells (5 mice in each group). **P< 0.05, **P< 0.01, ***P<0.001, t.test compared to Rag 1 -/- group/*

Rag 1-/- mice show higher hypersensitivity behavior than WT mice after plantar incision. B and T cells together accelerate the Rag 1 -/- mice recovery from CPSP.

CONCLUSION

Taken altogether, our results suggest a crucial role for the adaptive immune system in CPSP, especially for B cells. and T cells. *in-vivo* functional study showed the importance of B and T cells together for recovering from chronic postsurgical pain.