Chemokine receptor 2 (CCR2) mediates mechanical and cold hypersensitivity in chronic sickle cell disease pain

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Pain in sickle cell disease (SCD)

To what extent do peripheral inflammatory mediators contribute to sickle cell disease hypersensitivities?
CCL2 elevated in SCD patients and mouse models

Modified from Quari et al. (2012)
One-way ANOVA: main effect of group (p<0.05)
Tukey post-test: **p<0.01

Unpaired t-test: *p<0.05
CCR2 signaling mediates cold behavioral sensitivity

**CCR4 antagonist**

- **Vehicle**
- **C-021**

**Withdrawal Latency (s)**

<table>
<thead>
<tr>
<th>B6;129</th>
<th>Berk SS</th>
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<tbody>
<tr>
<td>6</td>
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<td>5</td>
<td>6</td>
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3 mg/kg, s.c.
Two-way ANOVA: effect of genotype (p<0.05)

**CCR2 antagonist**

- **Vehicle**
- **RS-504393**

**Withdrawal latency (s)**

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3 mg/kg, s.c.
Two-way ANOVA: effect of genotype (p<0.05), drug (p<0.05)
Bonferroni post-test: # p<0.05, ### p<0.001
Assessing contributions of CCR2 to sensory neuron cold sensitivity
CCR2 does not appear to mediate sensory neuron cold sensitivity

10 μM RS 504393
Two-way ANOVA: effect of genotype (p<0.05)
Bonferroni post-test: # p<0.05, ## p<0.01
CCR2 signaling mediates mechanical behavioral sensitivity

Adapted from Hillery et. al (2011) *Blood*
TRPV1 is sensitized in Berk SS neurons

Two-way ANOVA: effect of capsaicin concentration (p<0.05), genotype (p<0.05)

Bonferroni post-test: ## p<0.01, ### p<0.001
TRPV1 neuronal sensitization is mediated by CCR2

5 nM capsaicin + 10 μM CCR2 antagonist

Two-way ANOVA: effect of genotype (p<0.05)
Bonferroni post-test: ### p<0.001

5 nM capsaicin + 100 nM CCL2

Two-way ANOVA: effect of genotype (p<0.05)
Bonferroni post-test: ### p<0.001
CCR2 Signaling Mediates:

- Cold behavior sensitivity
- Mechanical behavior and neuronal sensitivity (TRPV1-dependent)

Future direction: CCR2/TRPV1 coupling mechanism
Ca2+ imaging: Cold ramp responses

**Cold Threshold**

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<th></th>
<th>B6;129</th>
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<th>SS</th>
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<tr>
<td>Cold</td>
<td>130</td>
<td>132</td>
<td>154</td>
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<tr>
<td>Threshold (°C)</td>
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**Response Magnitude**

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<th>SS</th>
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<td>Magnitude response (% baseline)</td>
<td>130</td>
<td>132</td>
<td>154</td>
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Ca2+ Imaging: Capsaicin Responses

Response Magnitude

Magnitude response (% baseline)

B6;129
Berk SS

5 nM
8
22
52
10 nM
41
100 nM
152
264

Percent increase over baseline

Vehicle
RS 504393

B6;129
68
26
SCD
34
26

Two-way ANOVA: effect of genotype (p<0.05)
Bonferroni post-test: # p<0.05, ## p<0.01