# Signs of chronic itch in the mouse imiquimod model of psoriasiform dermatitis: sex differences and roles of TRPV1 and TRPA1

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## Abstract

Plaque psoriasis is a chronic inflammatory skin disease that affects a substantial proportion of the world population. This disorder is characterized by scaly, thick skin, intense ongoing itch, and itch from light touch (such as clothing contacting skin, called "alloknesis"). Imiquimod is a topical treatment for basal cell carcinomas and warts that has been used to create a mouse model of plaque psoriasis. Imiquimod-treated male, but not female, wildtype B6 mice showed significant increases in spontaneous scratching, while both sexes exhibited increased alloknesis, indicative of chronic itch. TRPV1 and TRPA1 knockout (KO) mice all exhibited numeric increases in spontaneous scratching which were significant for TRPV1KO mice and TRPA1KO males. Female TRPV1KO and TRPA1KO mice exhibited imiquimod-induced increases in alloknesis scores that did not significantly differ from wildtypes, while alloknesis scores in imiquimod-treated male TRPV1KO and TRPA1KO mice were significantly lower compared with wildtypes, suggesting that these ion channels are necessary for the development of alloknesis in males but not females in this model. Curiously, none of the groups exhibited any significant overall change in chloroquine-evoked scratching following imiquimod treatment, indicating that hyperknesis does not develop in this mouse model. Overall, the data indicate that there are sex differences in this mouse model of psoriasis, and that TRPV1 and TRPA1 ion channels have a small role in promoting the development of itch sensitization. This contrasts with the far greater role these channels play in the manifestation of skin changes in psoriatic dermatitis.

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Psoriasis is one of the most common chronic inflammatory skin disorders, and affects upwards of 11.4% of the population[1]. In the United States, the prevalence of psoriasis in adults ranged between 0.5% and 5.1%[2–5]. Most previous epidemiological studies do not report a major sex difference in the prevalence of psoriasis in populations of all ages (summarized in Michalek et al[1]). The prevalence of itch in psoriasis patients is 60%–90% [6–9] (reviewed in Théréné et al[10]), and many psoriasis patients consider itch to be the most bothersome symptom[11]. A recent meta-analysis has revealed that some treatments for the itch of psoriasis have beneficial antipruritic effects, including anti-IL17, JAK inhibitors, adulimumab, apremilast, UVB photo-therapy (reviewed in Théréné et al[10]) and ixekizumab[12].

A murine model of plaque psoriasis has been developed that involves topical application of 5% imiquimod to the skin[13–18]. This results in symptoms consistent with plaque psoriasis in humans, including skin changes, and dependence on the IL-17/23 axis (reviewed in Flutter and Nestle[13]). A recent study[17] reported that imiquimod treatment in mice induced a transcriptional profile that varied by strain and sex, and best matched the human condition in C57Bl/6 (B6) mice.

Cardinal symptoms of chronic itch include ongoing (spontaneous) itch, itch elicited by light mechanical stimulation (alloknesis) such as contact with clothing[19], and increased itch to stimuli that normally elicit weak itch (hyperknesis). The transient receptor potential (TRP) channels TRPV1 and TRPA1 have been implicated in histaminergic and nonhistaminergic itch, respectively[20–23]. A recent study of imiquimod-treated B6 mice reported increases in spontaneous and touch-evoked scratching (alloknesis), with no change in skin mRNA expression of TRPV1 or TRPA1[15]. The main aims of the present study were to behaviorally assess if B6 mice exhibit increased scratching, alloknesis and hyperknesis following the development of imiquimod-induced psoriasiform dermatitis, if such signs are altered in knockout (KO) mice lacking TRPV1 or TRPA1, and if there is a sex difference in the behavioral manifestations of itch in this model.

A role for thermosensitive TRP channels in psoriatic itch was recently reported. Imiquimod treatment normally produces IL-23-mediated psoriasis-like inflammation. However, following neurotoxic ablation of TRPV1-expressing nociceptors, imiquimod elicited a significantly reduced inflammatory response, with decreased recruitment of leukocytes and acanthosis[24]. TRPV1-expressing nociceptive endings were observed in close contact with IL-23-releasing dermal dendritic cells, and it was hypothesized that nociceptors activate dendritic cells to drive psoriasiform inflammation. TRPV1 and TRPA1 are extensively coexpressed in sensory neurons[25], suggesting that nociceptors coexpressing both TRPV1 and TRPA1 may participate in the initial induction of psoriasis. Indeed, TRPV1-expressing sensory neurons are activated by imiquimod[26]. In contrast, another recent study has reported that imiquimod-induced psoriasiform dermatitis was enhanced in TRPA1KO mice and by a TRPA1 antagonist, suggesting that TRPA1 protects against, rather than promotes,

the development of dermatitis[27]. One aim of the present study was to address these discrepant findings by assessing if the behavioral manifestations of imiquimod-induced psoriasiform dermatitis are increased or reduced in KO mice lacking TRPV1 or TRPA1 compared with wildtype counterparts.

The other main aim of the study was to assess if there are sex differences in manifestations of itch in the imiquimod model. There is a large literature on sex and gender differences in relation to pain, with females frequently exhibiting greater nociceptive responses than males[28–32]. Fewer studies have specifically investigated sex differences for functional TRP channel expression in relation to pain. Prolactin significantly enhanced responses to capsaicin, allyl isothiocyanate and menthol in DRG cells from females, while having no effect on DRG cells from males[33]. There were significantly elevated levels of TRPV1 in the peritoneum of women with endometriosis[34] and was higher in female than male bladder muscle[35]. Female rats exhibited significantly greater nocifensive behaviors to intradermal capsaicin injection in a manner that was reversed following ovariectomy[36]. TRPV1 was upregulated in the trigeminal nucleus of rats following a high but not low dose of estradiol[37]. Kumar et al[38] reported a downregulation of TRPV1 expression in trigeminal ganglia following ovariectomy in rats. There were sex-dependent differences in TRPV1 expression in a chronic inflammatory pain model[39].

Much less is known regarding sex differences in animal models of itch. Female MRL/lpr mice that are deficient in Fas-mediated apoptosis and develop autoimmune diseases[40], or ICR-derived glomerulonephritis mice[41], exhibited significantly more spontaneous scratching behavior accompanied by dermal abrasions than males. Female mice exhibited overall 23% more acute chloroquine-evoked scratching compared with males; there were significant strain differences[42]. Female mice exhibited more chloroquine-evoked scratching versus males[43]. To our knowledge, only one study investigated sex differences in the imiquimod model[17]. There were some significant, but generally minor, sex differences for percent weight change, epidermal thickness, and spleen weight across 7 different mouse strains; itch-related behavior was not assessed. We therefore presently investigated sex differences for signs of itch (spontaneous scratching, alloknesis, hyperknesis) in the imiquimod model of psoriasiform dermatitis in wildtype and TRPV1 and TRPA1 KO mice.

## Methods

The study was approved by the University of California, Davis Institutional Animal Care and Use Committee. Experiments were conducted using male and female wildtype C57BL6/J (B6) mice, as well as KO mice lacking TRPV1 (purchased from Jackson Labs) or TRPA1 (a generous gift from David Julius, University of California, San Francisco). The rostral back was shaved before the beginning of treatment. Imiquimod cream (5%; Aldara, 50 mg; 3 M Health Care Limited, UK) or vehicle (Vanicream; Pharmaceutical Specialties, Rochester, MN) was applied topically to the shaved area on the rostral back once per day for 5 consecutive days. Treatment groups consisted of age-matched male and female wildtype, TRPV1KO and TRPA1KO mice (n = 6 per group). Imiquimod treatment induced clear signs of skin pathology including skin scaling and erythema.

Skin barrier function was assessed by measuring transepidermal water loss (TEWL; Vapometer, Delfin Technologies) before treatment began, and after the fifth day of imiquimod treatment.

Spontaneous scratching behavior was measured before the first day of treatment (day 0), and again on treatment days 1, 3, and 5. All videotaping of spontaneous scratching, and assessment of alloknesis, was conducted 23 hours following imiquimod treatment the previous day. Mice were videotaped and tested between 10 AM and 5 PM, with each individual mouse tested at the same time each day. The mice were habituated to glass cylinders for 3 successive days before recording. Animals were videotaped from above for 30 minutes. Behavioral videos were analyzed by 2 blinded observers. Only discrete bouts of hindlimb scratches directed toward the application site were counted, as described previously[44]. Scratch bouts were measured and summed over the entire 30 minutes period.

Alloknesis was assessed as previously described[45]. The mouse was placed in an enclosed area and a 0.07 g von Frey monofilament was applied to the perimeter of the imiquimod application area 5 consecutive times. The alloknesis score consisted of the number of immediately occurring hindlimb scratch bouts directed to the stimulus site.

Hyperknesis was assessed by counting the number of hindlimb scratch bouts elicited by intradermal injection of chloroquine (100 μg/10 μL) in rostral back skin within the treatment area, 1 week before day 1 of imiquimod treatment, and again on day 4 of imiquimod treatment. The animals were habituated to glass cylinders and video recorded from above for 15 minutes before and 60 minutes following chloroquine injection. Scratch bouts were counted as described above.

Within-group and between-group comparisons of TEWL, spontaneous scratch bouts, alloknesis scores, and chloroquine-evoked scratch bouts were statistically assessed using analysis of variance followed by a Bonferroni post hoc test, with $P<0.05$ considered to be significant. To compare sex differences within genotypes a linear mixed model that included an interaction between time, treatment, and sex was used in the lme4 package version 1.1–21 in R version 3.6.0[46].

## Results

### TEWL

All genotype groups receiving imiquimod treatment exhibited a significant increase in TEWL score as well as skin scaling (Figs. 1B, (D) compared with vehicle controls (Figs. 1A, C). TEWL scores were significantly lower in both TRPV1KO and TRPA1KO mice compared with wildtypes, for both female and male groups (Figs. 1B, D). Application of vehicle did not result in any significant increase in TEWL in any genotype (Figs. 1A, C).

### Spontaneous scratching

Imiquimod treatment resulted in a significant increase in the number of spontaneous scratch bouts in wildtype males but not females when compared with vehicle controls (Figs. 2A, B). However, because of behavioral variability the effect size for imiquimod treatment failed to

reach statistical significance when sexes were directly compared ($P$ = 0.0677). Male and female TRPV1KO mice receiving imiquimod treatment exhibited a significant increase over vehicle-treated controls in the number of spontaneous scratch bouts (Figs. 2C, D). There was a significant effect of imiquimod treatment when compared for sex of TRPV1 mice ($P$ = 0.0257). Curiously, imiquimod treatment resulted in a significant increase in spontaneous scratching in male (Fig. 2F), but not in female TRPA1KO groups (Fig. 2E), although the latter groups showed a nonsignificant trend toward increased scratching. There was no significant effect of sex on imiquimod treatment in TRPA1 mice when directly compared ($P$ = 0.3486).

## Alloknesis

Before the start of imiquimod treatment, alloknesis scores were essentially zero in all mice (Fig. 3, Pre). All imiquimod-treated mice exhibited a significant increase in alloknesis scores. There were no significant differences in alloknesis scores among imiquimod-treated female wildtype and KO groups (Fig. 3A). In contrast, imiquimod-treated male TRPV1KO and TRPA1KO mice exhibited significantly lower alloknesis scores compared with wildtypes (Fig. 3B). When effects of imiquimod treatment were directly compared by sex, there was a trending yet nonsignificant effect for TRPA1KO and TRPV1KO ($P$ = 0.0598 and 0.585, respectively). Wildtype mice showed no significant sex differences in imiquimod-mediated alloknesis ($P$ = 0.4729).

## Hyperknesis

Before imiquimod treatment, intradermal injection of chloroquine elicited significant increases in the number of scratch bouts in all mice, including TRPA1KOs. Following treatment with imiquimod or vehicle for 4 days, chloroquine still elicited a significant increase in scratch bouts in all treatment groups, with no significant between-group differences (Fig. 4). This indicates that hyperknesis did not develop as a result of imiquimod treatment and was independent of sex and genotype.

# Discussion

The present results confirm those of Sakai et al[15] in that imiquimod treatment of wildtype B6 mice resulted in a significant increase in spontaneous scratching, at least in males, as well as increased alloknesis. We extend these findings in several ways. We observed a sex difference for spontaneous scratching, and a trend toward a sex difference for alloknesis, following imiquimod treatment. Neither sex exhibited any significant hyperknesis. The roles of TRPV1 and TRPA1 were somewhat complex. Male and female TRPV1KO and male TRPA1KO mice exhibited significant increases in spontaneous scratching, and female TRPA1KO mice exhibited a tendency toward increased scratching which, however, did not reach statistical significance. Alloknesis scores were significantly lower in male, but not female, TRPV1KO and TRPA1KO mice, indicating that TRPV1 and TRPA1 contribute to the development of mechanical psoriatic itch in male, but not female, mice. Finally, following imiquimod treatment TEWL was significantly lower in both male and female TRPV1 and TRPA1KO mice compared with wildtypes, suggesting that these ion channels participate in imiquimod-induced degradation of skin barrier function.

### Spontaneous scratching and alloknesis

Male but not female wildtype mice exhibited a significant increase in spontaneous scratching following imiquimod treatment. TRPV1KO and TRPA1KO mice of both sexes showed increased spontaneous scratching following imiquimod treatment, although this did not reach statistical significance in female TPA1KO mice. These data, at least for male wildtypes, confirm the findings of Sakai et al[15]. However, others reported that male and female B6 wildtype and TRPA1KO mice exhibited variable hindlimb scratching following 4 days of imiquimod treatment that did not significantly differ from vehicle-treated mice[27]. This partly agrees with our present findings that female imiquimod-treated TRPA1KO mice did not exhibit a significant difference in scratching compared with vehicle-treated group, although the male TRPA1KO mice did. Kemény et al[27] also reported a significant increase in other nocifensive behaviors (biting, licking, flinching) in TRPA1KO mice, suggesting that TRPA1 negatively regulates the development of these behaviors in psoriasiform dermatitis.

Both sexes exhibited significant increases in alloknesis scores following imiquimod treatment. Female TRPV1KO and TRPA1KO mice showed imiquimod-induced increases in alloknesis scores that did not differ from wildtypes, whereas male TRPV1KO and TRPA1KO mice showed significantly lower alloknesis scores following imiquimod treatment. These data represent a sex difference, and suggest that TRPV1 and TRPA1 are required for the development of alloknesis, i.e. are positive regulators, in this model for males but not females.

In contrast to the fairly minor differences in itch behavior between the wildtype and KO mice observed presently, we have observed significant reductions in key markers such as skin thickness, transepithelial water loss (TEWL) and pathogenic cytokines characteristic of psoriasiform inflammation[47,48]. The significant reduction in imiquimod-induced skin changes implies a positive regulatory role of TRPV1 and TRPA1 in the manifestation of imiquimid-induced psoriasiform dermatitis, but less so in the behavioral signs of chronic itch. Our findings contrast with a recent report that signs of imiquimod-induced psoriasiform dermatitis (dorsal skin blood flow, skin thickness, Munro microabsesses, inflammatory cytokine levels) were significantly enhanced in TRPA1KO mice[27]. Part of the apparent discrepancy between our data and those of Kemény et al[27] may be attributed to methodological differences between the 2 studies, as discussed elsewhere[47].

### Hyperknesis

Surprisingly, we did not observe an increase in chloroquine-evoked scratching following the imiquimod treatment. This is at odds with previous studies using mouse models of chronic dry skin itch[49] or contact dermatitis[50], which both reported that nonhistaminergic mediators (serotonin, SLIGRL, BAM8–22), but not histamine, elicited significantly enhanced scratching behavior in the treated animals. Our results suggest that hyperknesis is not a major symptom of psoriasiform dermatitis, at least in mice. While itch from light tactile stimulation such as clothing is reported by psoriatic patients to be bothersome[19], it would be interesting to test if mildly itchy stimuli elicit stronger itch in psoriatic patients using quantitative sensory testing.

### Role of TRPA1 in chronic itch

Previous studies have emphasized a role for TRPA1 in mediating acute nonhistaminergic itch[22] as well as certain types of chronic itch[23] in mice. However, our present data indicate that the nonhistaminergic itch mediator, chloroquine, elicited equivalent scratching in TRPA1KO mice compared with wildtypes of both sexes (Fig. 4). It was recently reported that cutaneous afferent nerve fiber responses, as well as scratching behavior, elicited by intradermal injection of chloroquine, was not different between wildtype and TRPA1KO mice[51], consistent with our present data. The latter study speculated that TRPA1 may play a role in itch at a site central to the primary afferent pruriceptive nerve endings in the skin whose response to chloroquine does not require TRPA1[51].

## Acknowledgments

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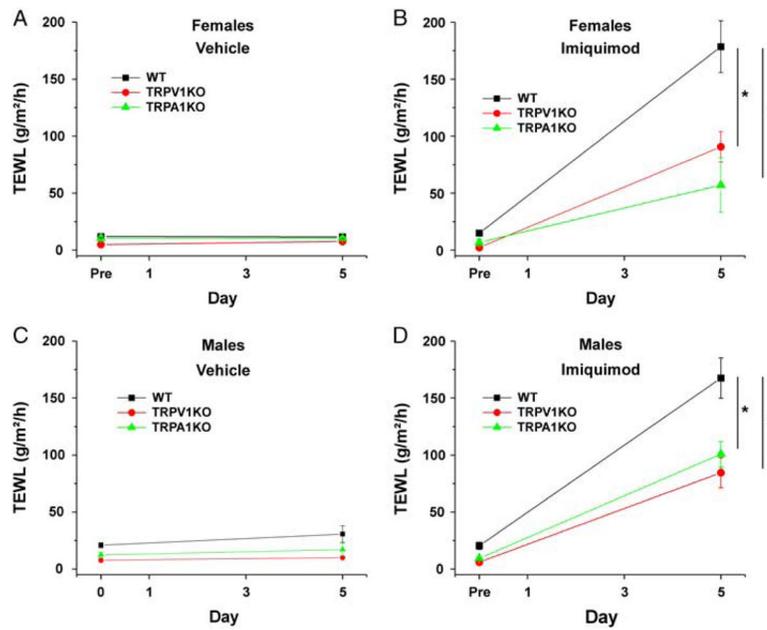
**Figure 1.**

TEWL values are plotted for WT (■), TRPV1KO (●), and TRPA1KO (▲) female c vehicle treatment. No significant effect of treatment and no significant differences among genotypes. B, Females receiving imiquimod treatment. There were significant differences between WTs compared with TRPV1KO and TRPA1KO groups ($P < 0.05$ for both) after imiqimod treatment. C, Males receiving vehicle treatment. No significant effect of treatment and no significant differences among genotypes. D, Males receiving imiquimod treatment. There were significant effects of treatment and genotype, with significant differences between the WT and TRPV1KO or TRPA1KO groups ($P < 0.001$ for both). Some of the data have been reported in a different format separately[47,48]. KO indicates knockout; TEWL, transepidermal water loss; TRP, transient receptor potential; WT, wildtype.
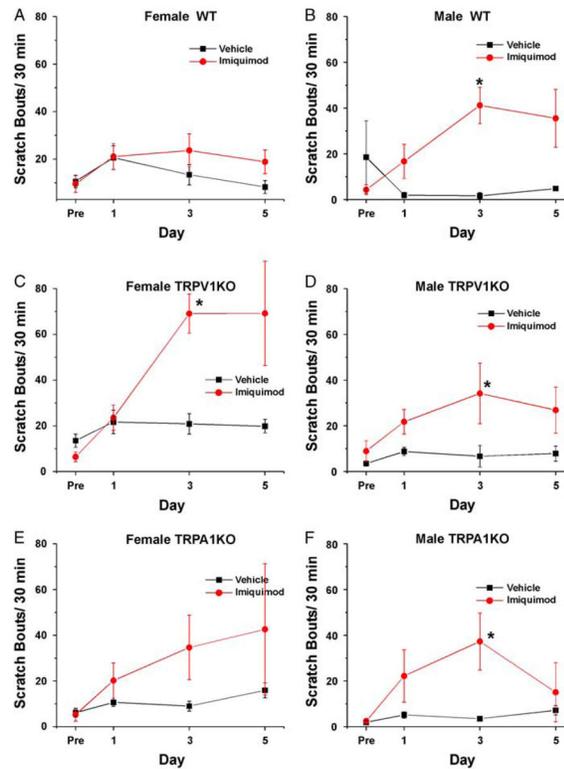
**Figure 2.**
Spontaneous scratching behavior in vehicle-treated (■) and imiquimod-treated (●) female and male mice. A, Female WT. No significant difference in scratching between imiquimod and vehicle groups (2-way analysis of variance, $F = 2.37$, $P > 0.05$). B, Male WTs. There was a significant effect of treatment ($F = 9.04$, $P < 0.01$). Post hoc analysis revealed significantly more scratch bouts in imiquimod-treated males at day 3. C, Female TRPV1KOs. There was a significant effect of treatment ($F = 12.31$, $P < 0.005$), with significantly more scratch bouts in the imiquimod-treated female mice at day 3. D, Male TRPV1KOs. Significant treatment effect ($F = 8.64$, $P < 0.01$). Post hoc analysis revealed significantly more scratch bouts in imiquimod-treated males at day 3. E, Female TRPA1KOs. No significant treatment effect ($F = 3.34$, $P > 0.05$). F, Male TRPA1KOs. Significant treatment effect ($F = 7.57$, $P < 0.05$). Post hoc analysis revealed significantly more scratch bouts in imiquimod-treated males at day 3. KO indicates knockout; TRP, transient receptor potential; WT, wildtype.
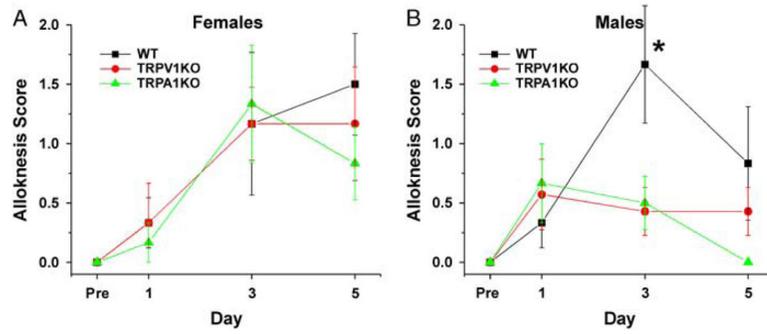
**Figure 3.**
Alloknesis scores are plotted for WT (■), TRPV1KO (●), and TRPA1KO (▲) female and male mice. A, Females. There was a significant effect of treatment ($F = 10.75$, $P < 0.001$) but no difference among genotypes ($F = 0.2$, $P > 0.05$). B, Males. There was a significant effect of treatment ($F = 5.21$, $P < 0.005$) and significant difference among genotypes (*; $F = 3.66$, $P < 0.05$). Post hoc analysis revealed that WT males had significantly higher alloknesis scores than TRPV1KO or TRPA1KO groups at treatment day 3. KO indicates knockout; TRP, transient receptor potential; WT, wildtype.
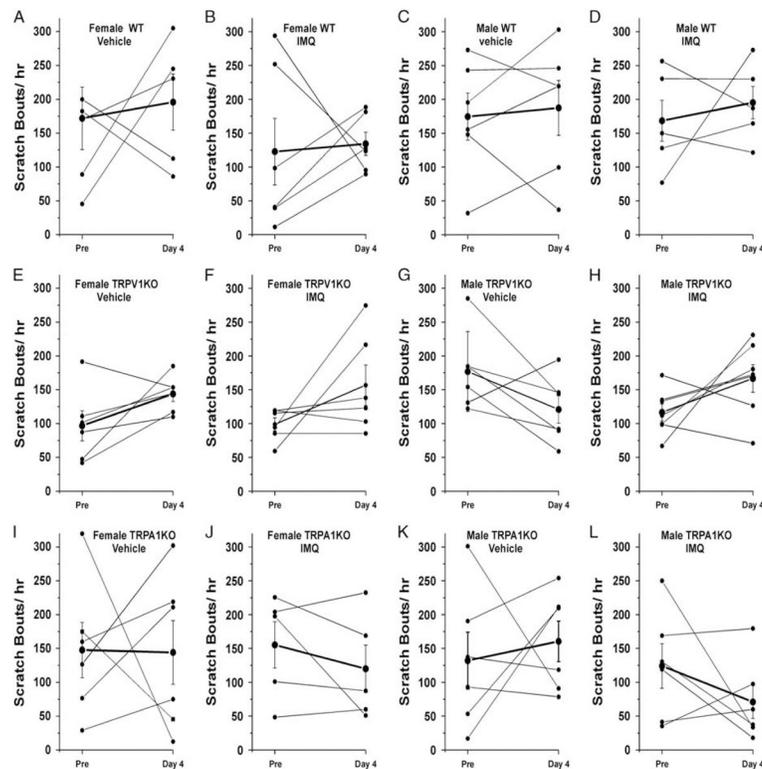
**Figure 4.**
Hyperknesis. A–L, Each graph plots individual (thin lines) and mean (thick lines with error bars = SEM) number of chloroquine-evoked scratch bouts before and after 4 days of treatment with vehicle or imiquimod (IMQ). Graphs are grouped by sex (females: A, B, E, F, I, J; males: C, D, G, H, K, L), treatment (vehicle: A, C, E, G, I, K) and genotype (WT: A–D; TRPV1KO: E–H; TRPA1KO: I–L). There were no significant differences in pre versus day 4 counts of scratch bouts by sex, treatment or genotype. KO indicates knockout; TRP, transient receptor potential; WT, wildtype.