# Kv6.4 as a Selective Therapeutic Target for Uterine Pain: Functional Consequences of a Rare KCNG4 Variant

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## BACKGROUND

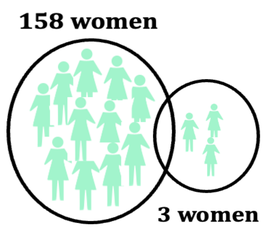
Female pain remains significantly under-researched and frequently under-treated. Chronic primary pain conditions, such as migraine, fibromyalgia, and complex regional pain syndrome, affect women up to four times more often than men. Uterine-specific conditions like dysmenorrhea and endometriosis further exacerbate this disparity, significantly impacting quality of life and contributing to a growing socioeconomic burden.

In 2020, the Woods lab identified a rare genetic variant (rs140124801, $K_V$6.4-Met419) associated with reduced labor pain. Healthy women who did not request analgesia during their first delivery, despite normal sensory thresholds, were significantly enriched for this variant in the *KCNG4* gene.

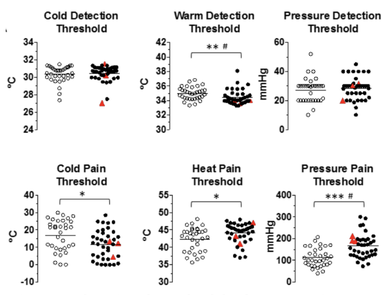
*KCNG4* encodes Kv6.4, a silent voltage-gated potassium channel subunit that modulates sensory neuron excitability through interaction with Kv2.1, a key delayed-rectifier potassium channel expressed in the central and peripheral nervous systems.

## RESULTS

### 1. Clinical Discovery: rs140124801 & Labor Pain




Among a cohort of 158 women who did not request analgesia during their first delivery, 3 were carriers of the rs140124801 variant.



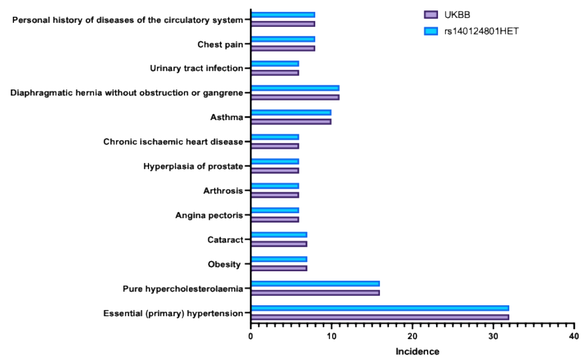

**Sensory detection and pain threshold phenotyping**

Clear circles indicate individual in control cohort and filles circles indicate those in the test cohort. The three individuals with KV6.4 p.Val419Met are indicated by red triangles.

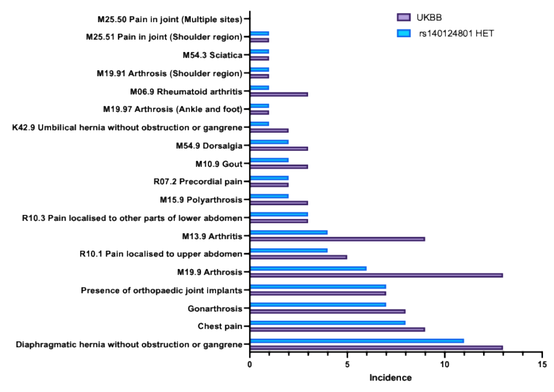
Women carrying the Kv6.4-Met419 mutation have normal sensory and psychometric test results, except for significantly higher cuff pressure pain

### 2. No Pain Association in UKBB Carriers

**Rs140124801 Shows No Adverse Disease Associations in Population-Scale UKBB Data**

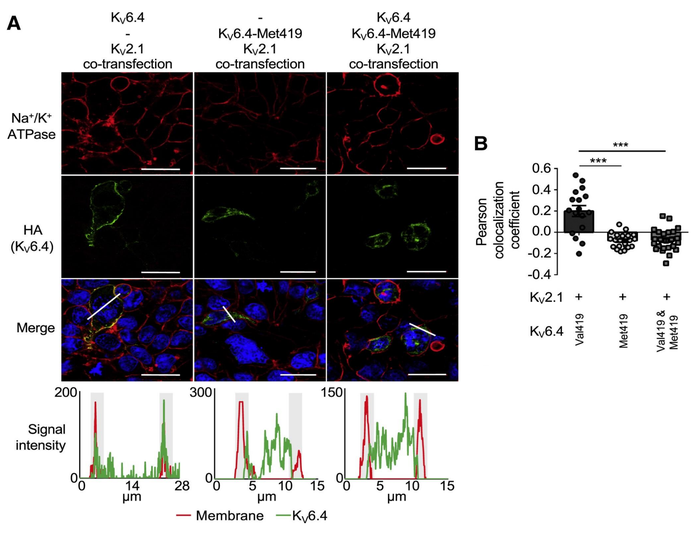



**No Enrichment of Pain-Associated Diagnoses in Carriers of Rs140124801**

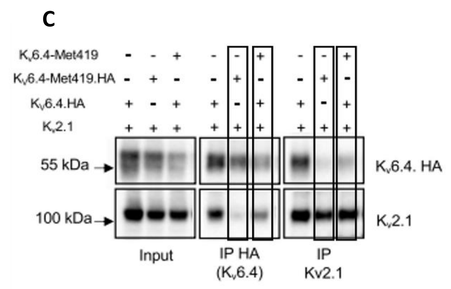



### 3. rs140124801 Disrupts Kv2.1 Modulation by Kv6.4

**Kv6.4-Met419 fails to reach the plasma membrane, doesn't interact with Kv2.1 and exerts a dominant-negative effect**

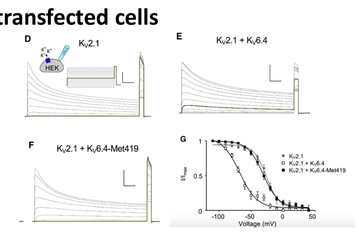



**A and B. Immunofluorescence imaging and Pearson colocalization analysis of HA-Kv6.4 (green) and $Na^+/K^+$-ATPase (red)**, as a plasma membrane marker. HEK293 cells were transfected with Kv2.1 and either HA-Kv6.4 or HA-Kv6.4-Met419 or both. The results show that Kv6.4-Met419 fails to reach the plasma membrane and exerts a dominant-negative effect when co-expressed with wild-type Kv6.4-Val19.



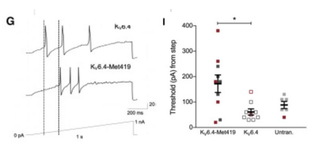

**C. Wild-Type $K_V$6.4 co-immunoprecipitates with $K_V$2.1 when co-expressed in HEK293 cells** (pulling down with $K_V$2.1 or HA-tagged $K_V$6.4). $K_V$6.4-Met419 disrupts binding to $K_V$2.1, and there is significantly reduced binding of HA-tagged $K_V$6.4 to $K_V$2.1 when co-expressed with untagged $K_V$6.4-Met419

**Kv6.4-Met419 fails to modulate Kv2.1 currents in HEK293 transfected cells**



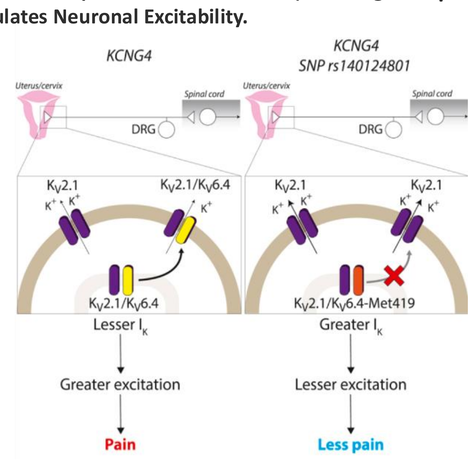

**Representative current recordings to determine $K_V$2.1 (D), $K_V$2.1/$K_V$6.4 (E), and $K_V$2.1/$K_V$6.4-Met419 (F) steady-state inactivation properties.** The applied voltage protocol is illustrated above (D). Vertical scale bar, 10 nA; horizontal scale bar, 0.5 s. Green traces indicate currents recorded during the −40 mV conditioning step. (G) Voltage dependence of steady-state inactivation of $K_V$2.1 (gray filled circles, n = 9), $K_V$2.1/$K_V$6.4 (white squares, n = 12), and $K_V$2.1/$K_V$6.4-Met419

**Kv6.4-Met419 reduces the excitability of mouse sensory neurons by increasing action potential thershold**



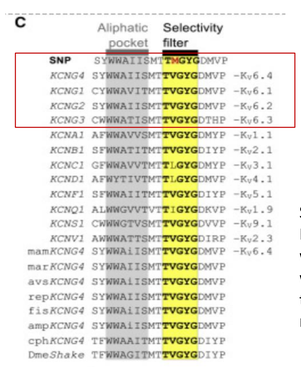

Current clamp recordings and action potential threshold analysis of mouse neurons transfected with $K_V$6.4 or $K_V$6.4-Met419, showing action potentials evoked by ramp injection of current (0–1 nA, 1 s). The thresholds for action potential discharge are annotated with light dashed ($K_V$6.4) or heavy dashed ($K_V$6.4-Met419) lines.

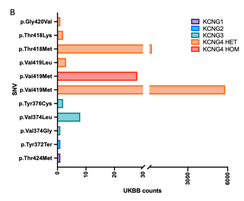
**Schematic of the Mechanism by which the Rare Allele SNP rs140124801 p.Val419Met in *KCNG4* (Encoding the $K_V$ Subunit $K_V$6.4) Regulates Neuronal Excitability.**




In most individuals (left panel), visceral nociceptors capable of transducing labor pain possess a combination of homomeric $K_V$2.1 channels and heteromeric $K_V$2.1/$K_V$6.4 channels, whereas in individuals with the rare allele SNP rs140124801 p.Val419Met in *KCNG4* (right panel), $K_V$2.1/$K_V$6.4-Met419 heteromers fail to traffic from the cytoplasm to the plasma membrane, resulting in a greater proportion of $K_V$2.1 homomeric channels. Because of their steady-state inactivation properties, $K_V$2.1/$K_V$6.4 heteromers have reduced availability at more depolarized membrane potentials compared with $K_V$2.1 homomers, and, thus, in nociceptors expressing $K_V$6.4-Met419, there is greater $K_V$2.1 homomer-mediated current at depolarized membrane potentials, which reduces neuronal excitability.

### 4- rs140124801 effect on Kv6 family subunits subcellular localization

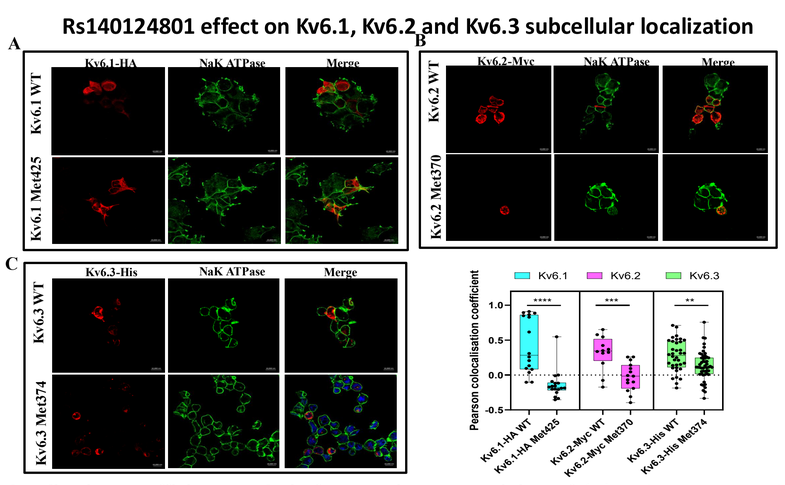



Single nucleotide variants (SNVs) identified in the UK Biobank within the TVGYG motif across all KCNG genes within a cohort of middle aged (57-87 years old) women. KCNG4 shows the highest number of SNVs in this conserved region compared to other family members, suggesting a higher tolerance for mutation

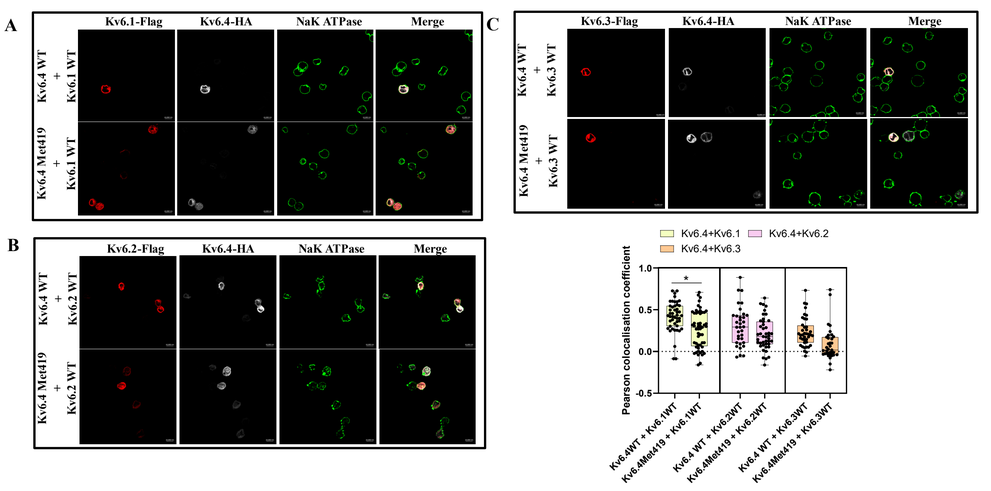
**Evolutionary conservation of human Kv6.4 positions 408–426 across Kv6 subunits and other Kv channel classes.** The rs140124801 variant and representative proteins from each human Kv class, as well as Kv6.4 in vertebrates, are shown. Invariant amino acids are capitalized. The selectivity filter TVGYG is highlighted in yellow, and the conserved aliphatic region in gray

**Rs140124801 effect on Kv6.1, Kv6.2 and Kv6.3 subcellular localization**



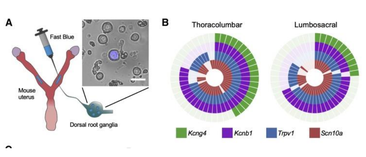

**Pearson colocalization coefficient analysis from co-immunostaining of (A) Kv6.1 WT or Kv6.1-Met425, (B) Kv6.2 WT or Kv6.2Met370, and (C) Kv6.3 WT or Kv6.3Met374, along with $Na^+/K^+$-ATPase as a plasma membrane marker in SH-SY5Y cells**. The analysis shows that mutant Kv6.1, Kv6.2, and Kv6.3 variants don't reach the plasma membrane compared to their respective wild-type subunits, indicating that the rs140124801-like mutation disrupts trafficking across the Kv6 family

**Kv6.4-Met 419 dominant negative effect is specific to the Kv6.4 subunit**




**Pearson colocalization coefficient analysis from co-immunostaining of Kv6.4 Wild Type (WT) or Kv6.4-Met419 with either Kv6.1 WT (A), Kv6.2 WT (B), Kv6.3 WT (C), and NaK-ATPase in SH-SY5Y cells**. The analysis shows that co-transfection of Kv6.1, Kv6.2, and Kv6.3 allows these subunits to reach the plasma membrane when co-transfected with Kv6.4 WT and that Kv6.4Met419 does not affect the membrane localization of Kv6 subunit members. **p = 0.05, t-test.*

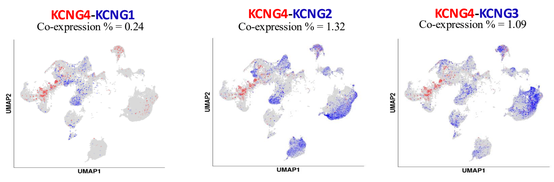
### 5- Kv6.4 is expressed in uterine-innervating sensory neurons and does not interact or colocalize with other Kv6 subunits




***Kcng4* Is Coexpressed with *Kcnb1* in Mouse Uterine Sensory Neurons**

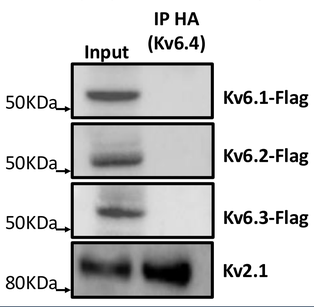
(A) Uterine sensory neurons were retrogradely labeled using fast blue and harvested following dissociation. Scale bar, 40 µm.

(B) Co-expression analysis of thoracolumbar (T12–L2, n = 44 cells) and lumbosacral (L5–S2, n = 45 cells) uterine sensory neurons expressing transcripts for *Kcng4*, *Kcnb1*, *Trpv1*, and *Scn10a*. Each segment in the wheel diagram is representative of a single cell, with a colored segment signifying positive expression.




**Expression pattern of KCNG4 and other KCNG subunits in mouse sensory neurons.**

Single-cell analysis reveals that KCNG4 is expressed in a distinct population of DRG sensory neurons and does not colocalize with other KCNG subunits (Analysis based on Bhuiyan et al., 2024 (http://harmonized.painseq.com)




**Co-immunoprecipitation of Kv6.4-HA WT after co-transfection with either Kv6.1-Flag, Kv6.2-Flag, Kv6.3-Flag, and Kv2.1 in SH-SY5Y cells**. Kv6.4-HA was pulled down using an HA antibody, and the Western blot was probed with a Flag antibody (first, second, and third lanes from top to bottom) or Kv2.1 antibody (fourth lane). Kv6.4 interacts with Kv2.1; however, it does not interact with other Kv6 family members.

### 6- Summary

- rs140124801 (Kv6.4-Met419) is enriched in women who didn't request analgesia
- The variant acts as a dominant-negative, impairing Kv6.4/Kv2.1 function
- This effect is specific to Kv6.4, sparing Kv6.1/2/3
- No other health associations in the UKBB cohort
- All together shows that KCNG4 it a promising therapeutic target for uterus specific pain

### Next step

Can transient silencing of KCNG4 in uterine-innervating sensory neurons via siRNA selectively reduce uterine nociception in mice without impairing general sensory or cognitive function?

References:

- Drissi et al., 2025. Kv6.4 Mutation Confers Painlessness During Labor Without Affecting Kv6 Family Function: A Safe Therapeutic Target to Reduce Epidural Use.
- Bhuiyan et al., 2024. Harmonized cross-species cell atlases of trigeminal and dorsal root ganglia
- Lee et al., 2020. Human Labor Pain Is Influenced by the Voltage-Gated Potassium Channel $K_V$6.4 Subunit
- Bocksteins et al., 2014. The Subfamily-Specific Interaction between Kv2.1 and Kv6.4 Subunits Is Determined by Interactions between the N- and C-termini

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