LacZ Reporter Gene Expression in 81 KOMP Heterozygous Mutants: Sensitivity, Staining Patterns and Functional Inferences

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Introduction

As of part of the Knockout Mouse Project (KOMP), 81 unique KO mutant lines were generated carrying KOMP alleles with the promoter of the targeted gene driving a LacZ (bacterial beta-galactosidase) reporter. LacZ enzyme activity was evaluated by histochemical staining in HET mutants and wildtype controls in order to characterize gene expression patterns, evaluate the frequency of specific and non-specific staining, and to identify unique anatomical structures expressing the targeted gene.

Methods

- KO mice were produced with several KOMP alleles **(Fig 1)**;

Fig 1. KOMP Alleles Driving LacZ

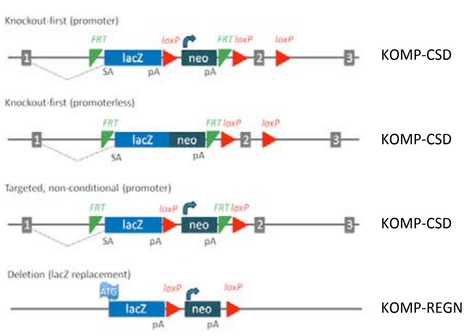

Fig 1. Alleles most frequently used in KOMP knockout mice. KOMP-CSD alleles: LacZ is fused with protein coded by more proximal exons (usually only Exon1); KOMP-REGN allele: LacZ coding sequence is introduced in frame at the translational start site of the targeted gene. For all alleles, LacZ expression is driven by the promoter of the targeted gene.

- LacZ enzyme activity was measured histochemically using X-gal as the substrate in tissues harvested at 50 days-of-age from ~ 2 HET mice for whole-mount, and ~2 HET mice for frozen section staining;
- Wildtype littermate controls were processed to identify patterns of non-specific staining due to endogenous galactoside and resident bacterial enzyme activity;
- Specific and non-specific staining was photo-documented in ~50 whole-mount stained tissues, and ~ 42 organs/tissues in frozen sections, for each animal.

Results

- Annotated LacZ images for these mutants are available on the project webpage (www.kompphenotype.org);

- For the 81 mutants described here, ~80% have specific LacZ staining, only a small % are brain specific, and 29% have specific LacZ staining in 6 or more organs/ tissues **(Fig 2)**;

Fig 2. Patterns of LacZ Staining in KOMP Mutants

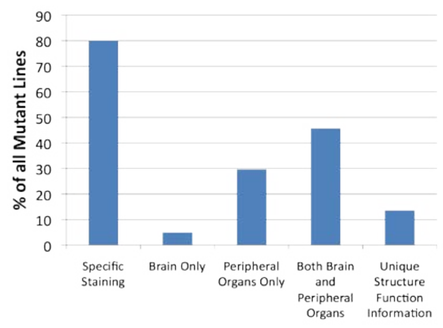

Fig 2. LacZ staining patterns in KOMP mutants. Brain exclusive expression is rare and ~ 15% of mutants have a staining pattern that reveals unique functional information.

- ~15% of the mutants have LacZ staining that reveal previously unappreciated anatomical distribution and functions of the gene **(Fig 3)**:

Fig 3. Structure-Function from LacZ Studies

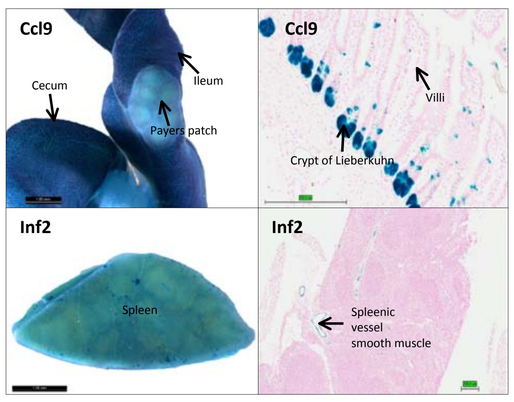

Fig 3a.
→ Ccl9, chemokine (C-C motif) ligand 9: not previously reported that this gene is expressed in the GI tract and the intestinal crypts;
→ Inf2, inverted formin, FH2 and WH2 domain containing: LacZ staining confirms ubiquitous expression and provides anatomical detail for this gene of unknown function

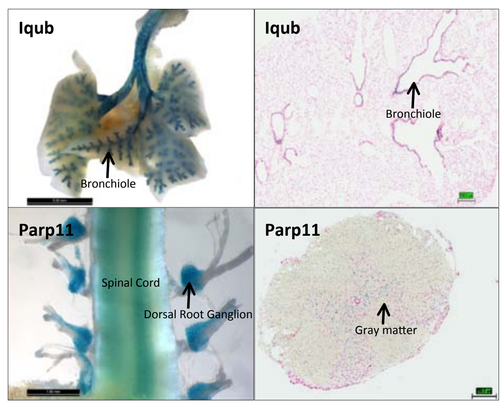

Fig 3b.
→ Iqub, IQ motif and ubiquitin domain containing gene: LacZ staining reveals epithelium-specific expression in brain ventricles (ependyma) and peripheral organs including lung shown here, not previously reported;
→ Parp11, poly (ADP-ribose) polymerase family, member 11: expression in dorsal root ganglion and spinal cord not previously reported

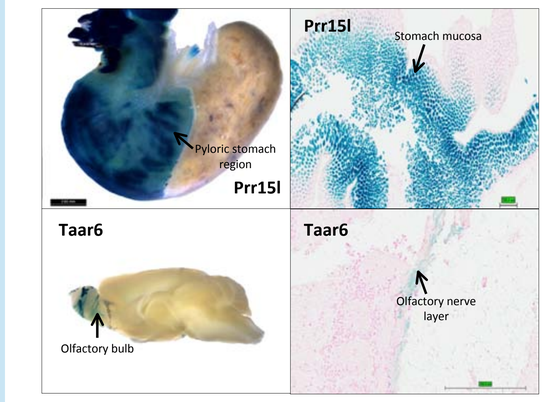

Fig 3c.
→ Prr15l, proline rich 15-like gene: LacZ confirms previously reported expression in GI tract and kidney but also reveals expression in epithelial mucosa of many tissues;
→ Taar6, a trace amine-associated receptor gene: expressed only in the olfactory nerve in the brain; suggesting a role in olfaction not previously reported

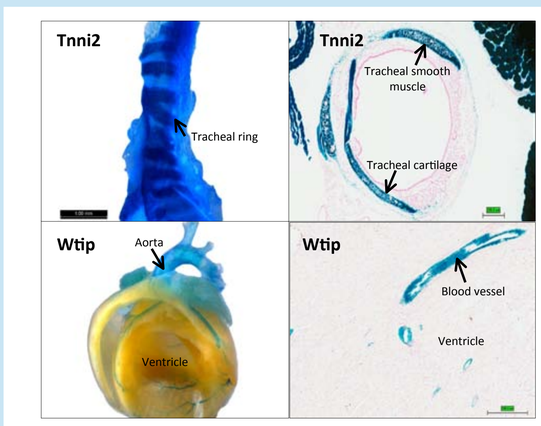

Fig 3d.
→ Tnni2, troponin I, skeletal, fast 2: LacZ identifies gene expression in structures other than skeletal muscle
→ Wtip, Wilms tumor 1 interacting protein: LacZ staining revealed ubiquitous expression in blood vessels, including aorta and coronary arteries shown here, suggesting a role in cardiovascular function not previously described.

- Non-specific staining is observed in both whole-mount and frozen sections due to endogenous enzyme activity or resident bacteria. **(Figs 4, 5)**.

Fig 4. Frequency of Non-specific Staining in Wild-type Whole-mount LacZ Preparations

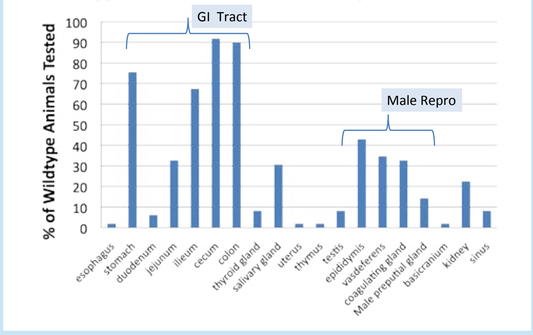

Fig 4. Non-specific staining in different whole-mount tissues. Non-specific staining is most frequently found in the GI tract, male reproductive tract, and the kidney.

Fig 5. Non-specific Staining in Wildtype Controls

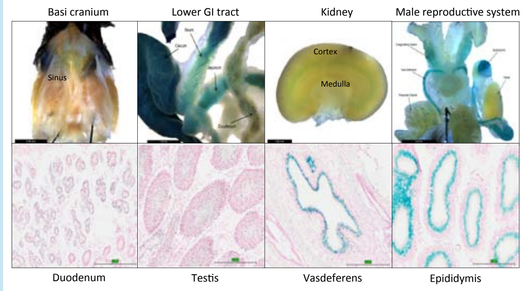

Fig 5. Examples of non-specific LacZ staining observed in wildtype mice. Top (L-R) whole-mount nonspecific staining found in wildtype basicranium, GI tract, kidney and male reproductive organs. Bottom (L-R) frozen-section staining in wildtype duodenum, testis, vas deferens and epididymis.

- Non-specific staining in frozen sections has a similar anatomical distribution and is observed less frequently than that found with whole-mounts.

Significance

These results validate the approach of assessing all KOMP mutants for LacZ expression as part of a broad-based mutant screening program. ~80% of the mutants show specific LacZ staining often revealing patterns of gene expression not previously reported and unique structure-function relationships are identified. The LacZ staining reveals anatomical details regarding organ and tissue substructures expressing the gene. Non-specific staining (i.e., due to endogenous galactoside enzyme activity or bacteria) also is found but this non-specific staining can usually be distinguished from reporter gene enzyme activity based upon intensity and pattern.

Acknowledgements

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