

**TITLE:** Genetic Interaction between Central Pair Apparatus Genes *CFAP221*, *CFAP54*, and *SPEF2* in Mouse Models of Primary Ciliary Dyskinesia

**AUTHORS:** Casey W. McKenzie<sup>1</sup> and Lance Lee<sup>1,2,\*</sup>

**AFFILIATIONS:**

<sup>1</sup>Pediatrics and Rare Diseases Group, Sanford Research, 2301 E. 60<sup>th</sup> Street N., Sioux Falls, SD 57104

<sup>4</sup>Department of Pediatrics, Sanford School of Medicine of the University of South Dakota, 1400 W. 22<sup>nd</sup> Street, Sioux Falls, SD 57105

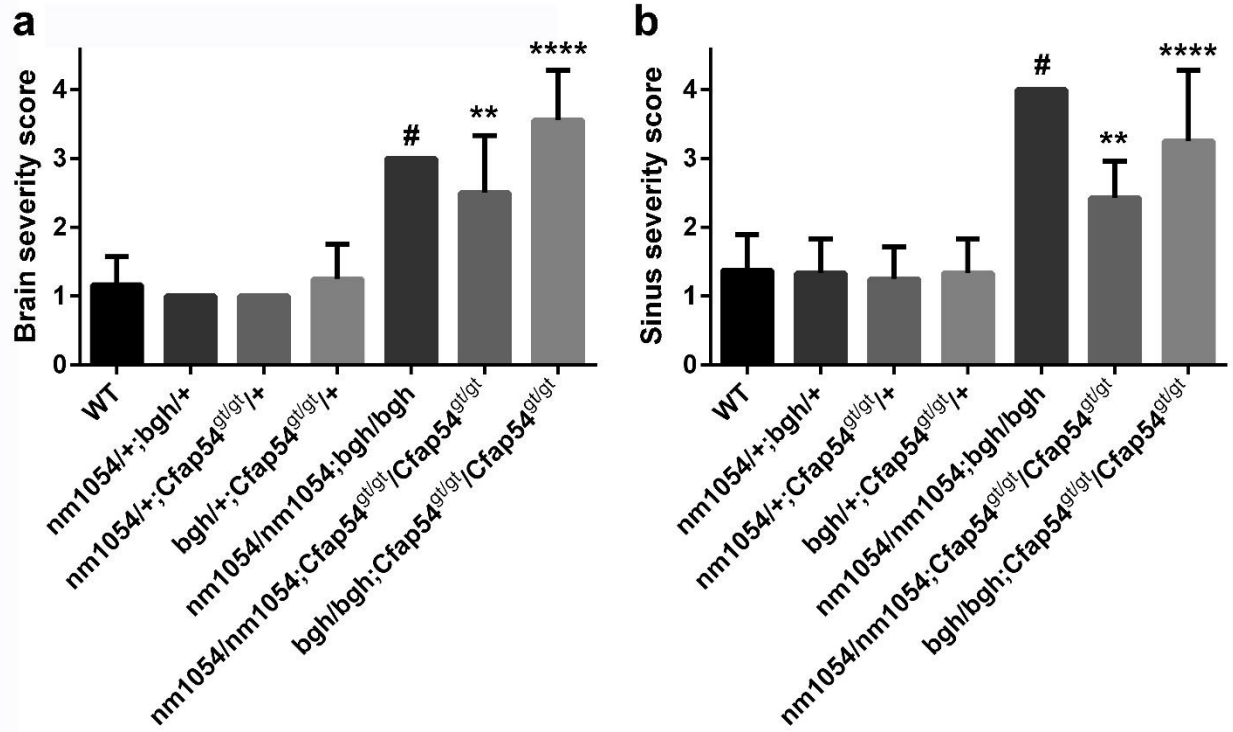
**Supplementary Table 1. Mouse Numbers**

| Genotype                                                                 | Total<br>n | Early<br>Mortality <sup>a</sup> | Sinus<br>Histology | Brain<br>Histology | Testis<br>Histology | Sperm<br>Morphology | CBF <sup>b</sup> |
|--------------------------------------------------------------------------|------------|---------------------------------|--------------------|--------------------|---------------------|---------------------|------------------|
| WT                                                                       | 16         | 0                               | 10                 | 7                  | 8                   | 6                   | 11               |
| <i>nm1054/+; bgh/+</i>                                                   | 14         | 0                               | 9                  | 5                  | 8                   | 7                   | 12               |
| <i>nm1054/+; Cfap54<sup>gt/gt</sup>/+</i>                                | 15         | 0                               | 5                  | 5                  | 7                   | 6                   | 10               |
| <i>bgh/+; Cfap54<sup>gt/gt</sup>/+</i>                                   | 17         | 0                               | 10                 | 5                  | 9                   | 5                   | 15               |
| <i>nm1054/nm1054; bgh/bgh</i>                                            | 11         | 11                              | 1                  | 1                  | 0 <sup>c</sup>      | 0 <sup>c</sup>      | 0                |
| <i>nm1054/nm1054;<br/>Cfap54<sup>gt/gt</sup>/ Cfap54<sup>gt/gt</sup></i> | 8          | 1                               | 7                  | 7                  | 4                   | 3                   | 0                |
| <i>bgh/bgh;<br/>Cfap54<sup>gt/gt</sup>/ Cfap54<sup>gt/gt</sup></i>       | 12         | 7                               | 8                  | 9                  | 3                   | 2                   | 0                |

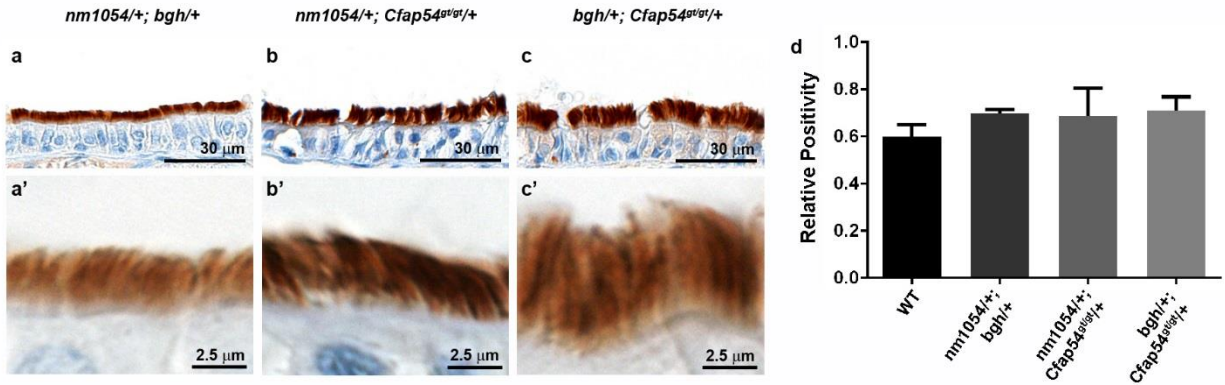
<sup>a</sup>Death prior to 8 weeks, often in the postnatal period preventing collection and analysis of samples

<sup>b</sup>Since so few double homozygous mutants survived to 8 weeks, CBF data is only reported for double heterozygotes and WT controls

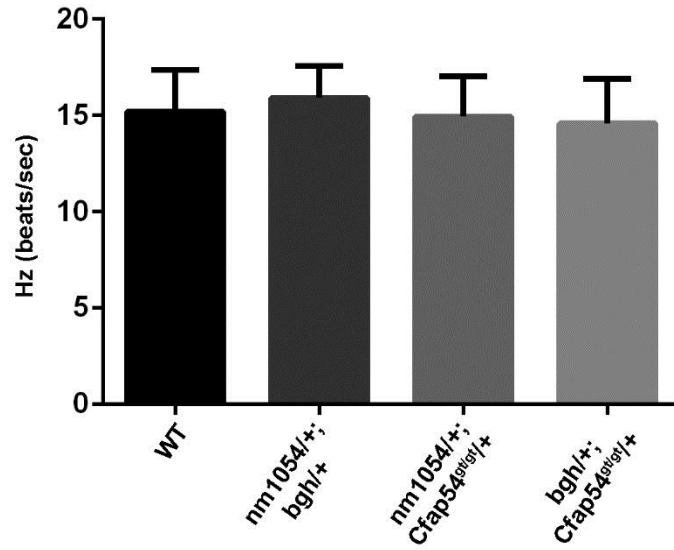
<sup>c</sup>No *nm1054/nm1054; bgh/bgh* mice survived to sexual maturity, and the only mouse that survived long enough for tissue collection was female



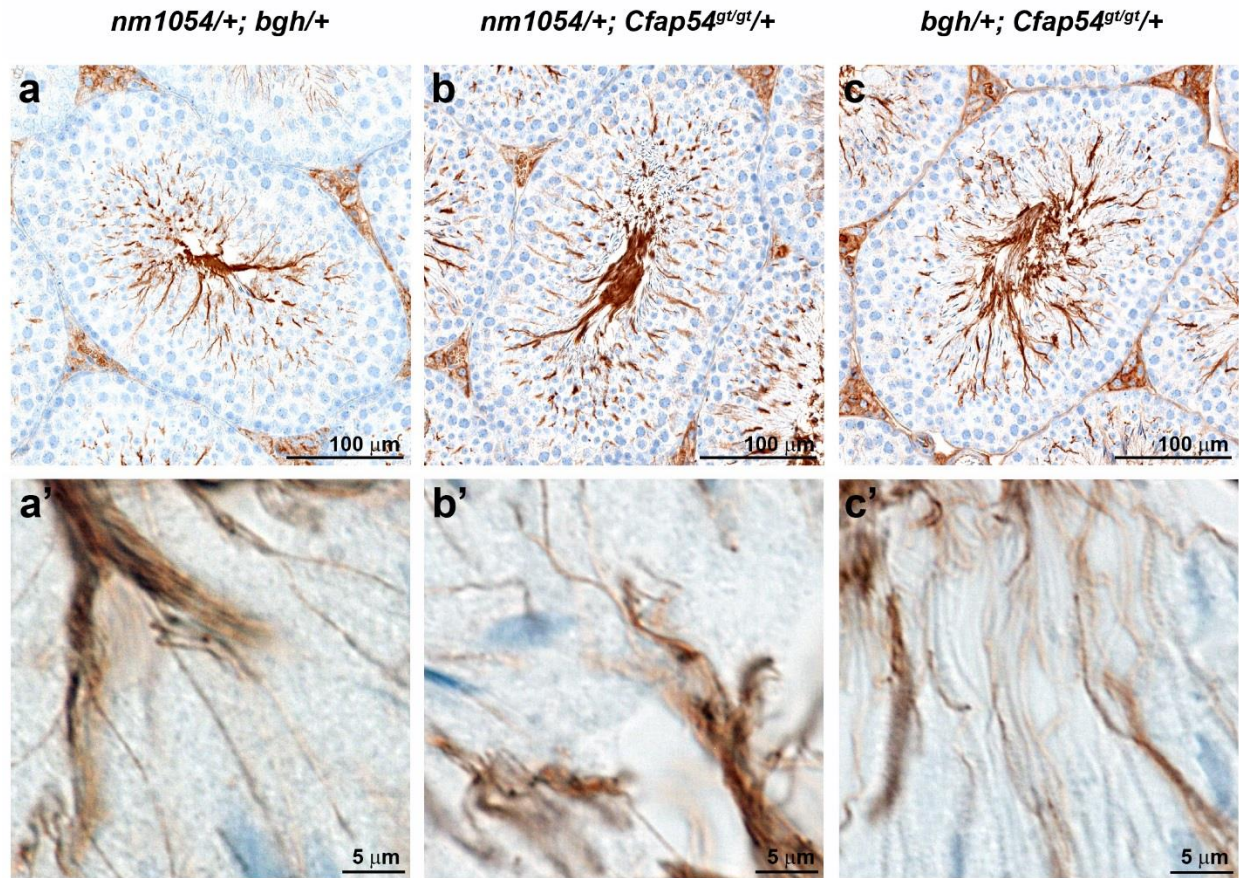
**Supplementary Figure 1. Severity scoring for brain and sinus phenotypes.** Brain (a) and sinus (b) phenotype severity scores for WT, *nm1054/+;bgh/+*, *nm1054/+;Cfap54<sup>gt/gt</sup>/+*, *bgh/+;Cfap54<sup>gt/gt</sup>/+*, *nm1054/nm1054;bgh/bgh*, *nm1054/nm1054;Cfap54<sup>gt/gt</sup>/Cfap54<sup>gt/gt</sup>*, and *bgh/bgh;Cfap54<sup>gt/gt</sup>/Cfap54<sup>gt/gt</sup>* mice. All p values are relative to WT. For brains, p = 0.983 for *nm1054/+;bgh/+*, 0.979 for *nm1054/+;Cfap54<sup>gt/gt</sup>/+*, 0.999 for *bgh/+;Cfap54<sup>gt/gt</sup>/+*, 0.001 for *nm1054/nm1054;Cfap54<sup>gt/gt</sup>/Cfap54<sup>gt/gt</sup>*, and < 0.001 for *bgh/bgh;Cfap54<sup>gt/gt</sup>/Cfap54<sup>gt/gt</sup>* mice. For sinuses, p = 0.999 for *nm1054/+;bgh/+*, 0.994 for *nm1054/+;Cfap54<sup>gt/gt</sup>/+*, 0.999 for *bgh/+;Cfap54<sup>gt/gt</sup>/+*, 0.009 for *nm1054/nm1054;Cfap54<sup>gt/gt</sup>/Cfap54<sup>gt/gt</sup>*, and < 0.001 for *bgh/bgh;Cfap54<sup>gt/gt</sup>/Cfap54<sup>gt/gt</sup>* mice. Statistical significance was determined by one-way ANOVA. Since only one *nm1054/nm1054;bgh/bgh* mouse survived for tissue collection, no statistical analysis was performed (denoted by #).



**Supplementary Figure 2. Immunohistochemical analysis of double heterozygous sinus epithelial cilia.** Sections of *nm1054/+;bgh/+* (a,a'), *nm1054/+;Cfap54<sup>gt/gt</sup>/+* (b,b'), and *bgh/+;Cfap54<sup>gt/gt</sup>/+* (c,c') maxillary sinus airway epithelia stained with an antibody to ciliary marker acetylated tubulin. Quantification of acetylated tubulin staining intensity is shown in (d). All p values are relative to WT.  $p = 0.166$  for *nm1054/+;bgh/+*,  $0.245$  for *nm1054/+;Cfap54<sup>gt/gt</sup>/+*, and  $0.135$  for *bgh/+;Cfap54<sup>gt/gt</sup>/+* mice. Statistical significance was determined by one-way ANOVA.



**Supplementary Figure 3. Double heterozygous tracheal CBF analysis.** Tracheal epithelial CBF from WT, *nm1054/+;bgh/+*, *nm1054/+;Cfap54<sup>gt/gt</sup>/+*, and *bgh/+;Cfap54<sup>gt/gt</sup>/+* mice in beats per second (Hz) shows no statistically significant difference between WT and double heterozygous levels.  $p = 0.767$  for *nm1054/+;bgh/+*,  $0.982$  for *nm1054/+;Cfap54<sup>gt/gt</sup>/+*, and  $0.799$  for *bgh/+;Cfap54<sup>gt/gt</sup>/+* relative to WT. Statistical significance was determined by one-way ANOVA.



**Supplementary Figure 4. Immunohistochemical analysis of double heterozygous testis.** Sections of *nm1054/+;bgh/+* (a,a'), *nm1054/+;Cfap54<sup>gt/gt</sup>/+* (b,b'), and *bgh/+;Cfap54<sup>gt/gt</sup>/+* (c,c') testis stained with an antibody to flagellar marker acetylated tubulin, indicating normal flagellar formation and morphology.