**Toward the pathogenicity of the *SLC26A4* p.C565Y variant using a genetically driven mouse model**

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Running title: Mouse model with *Slc26a4* p.C565Y variant


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Supplementary Table 1. Criteria of assessment for pathogenic variants and verdicts for *SLC26A4*:p.C565Y from Varsome platform

| | Supporting (PP) | Moderate (PM) | Strong (PS) | Very Strong (PVS) |
|---|---|---|---|---|
| **Allele frequency** | | Absent in population database. ✓ (PM2) | Significantly increased prevalence in affected population compared to control group. (PS4) | |
| **Theoretical or predictive results** | Multiple predictive algorithms to support damages caused by variants. ✓ (PP3) | Novel missense change at reported pathogenic locus. ✓ (PM5) Protein lengths change by in-frame insertion/deletion. (PM4) | Variants cause same reported pathogenic amino acid changes. (PS1) | Variants bring about null gene expression (e.g. abnormal splicing, termination, frameshifts). (PVS1) |
| **Functional evidence** | High rate of reported pathogenic missense variants in a gene are commonly involved in mechanism of the disease. ✓ (PP2) | Variants locating at hot spots or functional domain without recorded benign variation in the gene. ✓ (PM1) | Well-established functional studies as evidence for the deleterious effects caused by variants. (PS3) | |
| **Segregation extent** | Co-segregation of causative variants in multiple affected family members. (PP1) | | | |
| **De novo pathogenic mutation** | | De novo mutation but not confirmed by paternity testing. (PM6) | De novo mutation and verified by paternity testing. (PS2) | |
| **In trans with pathogenic variants** | | Detected trans-variant on opposing allelic locus of another pathogenic variant. (PM3) | | |
| **Records from reputable database** | Same locus annotated as pathogenic variants in reputable database. ✓ (PP5) | | | |
| **Specific phenotype** | Symptoms in patients are high specific for a genetic etiology. (PP4) | | | |

§ Above table modified from guideline criteria of American College of Medical Genetics and Genomics (ACMG)

Supplementary Table 2. The list of explanations for corresponding criteria for *SLC26A4*:p.C565Y from Varsome platform

| Criteria | Explanation |
|---|---|
| PM1 | Hot-spot of length 61 base-pairs has 11 non-VUS* coding variants (11 pathogenic and 0 benign), pathogenicity = 100.0%, qualifies as hot-spot. |
| PM2 | Variant not found in gnomAD exomes (good gnomAD exomes coverage = 63.4).<br>GnomAD genomes homozygous allele count = 0 is less than 3 threshold for recessive gene *SLC26A4* (good gnomAD genomes coverage = 29.5). |
| PM5 | Alternative variant chr7:107340606:T>G (Cys565Gly) is classified "Likely Pathogenic" with 1 star, by ClinVar (and confirmed using ACMG). |
| PP2 | ◆ 177 out of 194 non-VUS missense variants in gene *SLC26A4* are pathogenic = 91.2% which is more than threshold of 51.0%.<br>◆ 327 out of 593 clinically reported variants in gene *SLC26A4* are pathogenic = 55.1% which is more than threshold of 12.0%. |
| PP3 | Pathogenic computational verdict based on 7 pathogenic predictions (DANN, FATHMM-MKL, M-CAP, MVP, MutationTaster, REVEL and SIFT) vs 4 benign predictions (DEOGEN2, EIGEN, MutationAssessor and PrimateAI). |
| PP5 | ◆ ClinVar classifies this variant as "Likely Pathogenic", rated 2 stars, with 3 submissions, 16 publications and no conflicts.<br>◆ UniProt classifies this variant as "Pathogenic", associated with Pendred syndrome (PS) and rare genetic deafness. Its related publications: PMID 14679580, 19204907 and 9618166. |

§ All the above contents are quoted from Varsome platform (https://varsome.com/). (Data quated at 2020/07/10)

* VUS: variants of uncertain significance.

Supplementary Table 3 Classification rule for various combination of multiple ACMG criteria and pathogenicity verdicts for *SLC26A4*:p.C565Y

| Pathogenicity verdict | Combination of various ACMG criteria | | | |
|---|---|---|---|---|
| Pathogenic | (1) | 1 PVS1 | And | ≥1 PS<br>≥2 PM<br>1 PM & 1 PP<br>≥2 PP |
| | (2) | ≥2 PS | | |
| | (3) | 1 PS | And | ≥ 3 PM<br>2 PM & ≥2 PP<br>1 PM & ≥4 PP |
| Likely Pathogenic | (1) | 1 PVS1 & 1 PM | | |
| | (2) | 1 PS & 1-2 PM | | |
| | (3) | 1 PS & ≥2 PP | | |
| | (4) | ≥3 PM   (for *SLC26A4*:p.C565Y) ✔ | | |
| | (5) | 2 PM & ≥2 PP | | |
| | (6) | 1 PM & ≥4 PP | | |

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§ PVS: pathogenic very strong; PS: pathogenic Strong (PS1-PS4), PM: pathogenic Moderate (PM1-PM6); pathogenic supporting (PP1-PP5)