

## **Whole genome sequencing reveals exonic variation of *ASIC5* gene results recurrent pregnancy loss**

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**S1 Table. Autorecessive model analysis results of whole genome sequence of trio samples.**

| <b>Features</b>         | <b>Autorecessive model analysis results</b> |
|-------------------------|---------------------------------------------|
| S_zyg                   | hom                                         |
| M_zyg                   | het                                         |
| F_zyg                   | het                                         |
| Model                   | ARH                                         |
| chromosome              | chr4                                        |
| start pos               | 156773374                                   |
| end pos                 | 156773374                                   |
| context                 | GCTCAAACCTCTTCCAGAGAC                       |
| DP_Ref                  | 0(Rfw=0)                                    |
| DP_All                  | 0(Afw=0)                                    |
| reference               | C                                           |
| observed                | A                                           |
| quality                 | 1488.77                                     |
| filter                  | PASS                                        |
| zygosity                | hom                                         |
| refGene function        | exonic                                      |
| refGene gene            | ASIC5                                       |
| refGene exonic function | nonsynonymous SNV                           |

|                           |                                                            |
|---------------------------|------------------------------------------------------------|
| refGene AA change         | ASIC5:NM_017419:exon4:c.680G>T;p.R227I                     |
| ensGene function          | exonic                                                     |
| ensGene gene              | ENSG00000256394                                            |
| ensGene exonic function   | nonsynonymous SNV                                          |
| ensGene AA change         | ENSG00000256394:ENST00000537611:<br>exon4:c.680G>T;p.R227I |
| knownGene function        | exonic                                                     |
| knownGene gene            | <i>ASIC5</i>                                               |
| knownGene exonic function | nonsynonymous SNV                                          |
| knownGene AA change       | ASIC5:uc003ipe.1:exon4:c.680G>T;p.R227I                    |
| cytoBand                  | 4q32.1                                                     |
| dbSNP                     | -                                                          |
| SGP777X2                  | 0                                                          |
| SGP777X2HOM               | 0                                                          |
| SGP777X2(Exome)           | 0                                                          |
| SGP777X2(Exome)HOM        | 0                                                          |
| Screenee                  | 2379                                                       |
| AC                        | 0                                                          |
| AC_hom                    | 0                                                          |
| SGPFreq                   | 0                                                          |

|                                    |          |
|------------------------------------|----------|
| SGPFreqHom                         | 0        |
| SGP_AF_Strict                      | 0        |
| 1000g2015aug_all                   | 0        |
| ExAC_pLI                           | 2.26E-07 |
| REVEL                              | 0.326    |
| Eigen                              | -0.053   |
| SIFT_score                         | 0.013    |
| SIFT_converted_rankscore           | 0.538    |
| SIFT_pred                          | D        |
| Polyphen2_HDIV_score               | 0.239    |
| Polyphen2_HDIV_rankscore           | 0.291    |
| Polyphen2_HDIV_pred                | B        |
| Polyphen2_HVAR_score               | 0.348    |
| Polyphen2_HVAR_rankscore           | 0.41     |
| Polyphen2_HVAR_pred                | B        |
| MutationTaster_score               | 1        |
| MutationTaster_converted_rankscore | 0.588    |
| MutationTaster_pred                | D        |
| MetaSVM_score                      | -0.641   |
| MetaSVM_rankscore                  | 0.631    |

|                     |                                                 |
|---------------------|-------------------------------------------------|
| MetaSVM_pred        | T                                               |
| M-CAP_score         | 0.051                                           |
| M-CAP_rankscore     | 0.646                                           |
| M-CAP_pred          | D                                               |
| CADD_raw            | 5.542                                           |
| CADD_raw_rankscore  | 0.753                                           |
| CADD_phred          | 26.4                                            |
| GTEx_V6_gene        | .                                               |
| GTEx_V6_tissue      | .                                               |
| OMIM Gene Id        | 51802                                           |
| OMIM type           | Gene (*)                                        |
| OMIM MimNumber      | 616693                                          |
| OMIM Title          | ACID-SENSING ION CHANNEL FAMILY MEMBER 5; ASIC5 |
| OMIM Alt Titles     | HUMAN INTESTINE SODIUM CHANNEL; HINAC           |
| OMIM Comment        | -                                               |
| OMIM Disease Gene   | ASIC5, HINAC                                    |
| OMIM Disorders      | Acid-sensing ion channel family member 5        |
| OMIM Pred           | H                                               |
| OMIM Confidence     | (C)onfirmed                                     |
| OMIM Mapping Method | REc, H                                          |

|                  |                                                                                                                                                                                                                          |
|------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| VCF INFO         | AC=2;AF=1.00;AN=2;BaseQRankSum=-8.540e-01;ClippingRankSum=-1.239e+00;DP=46;FS=0.000;GQ_MEAN=59.00;MLEAC=2;MLEAF=1.00;MQ=60.00;MQ0=0;MQRankSum=0.083;NCC=0;QD=33.08;ReadPosRankSum=0.303;SOR=0.836;VQSLOD=6.08;culprit=FS |
| VCF FORMAT key   | GT:AD:DP:GQ:PL                                                                                                                                                                                                           |
| VCF FORMAT value | 1/1:2,43:45:59:1517,59,0                                                                                                                                                                                                 |

**S2 Table.** List of heterozygous mutations observed in the proband.

| #<br>Header<br>= child | mother | father | other info (from<br>child record if<br>exists | otherwise from mother) |           |           |                                 |          |          |               |          |
|------------------------|--------|--------|-----------------------------------------------|------------------------|-----------|-----------|---------------------------------|----------|----------|---------------|----------|
| S_zyg                  | M_zyg  | F_zyg  | Model                                         | Chromosome             | start pos | end pos   | context                         | DP_Ref   | DP_All   | reference     | observed |
| het                    | het    | F_NA   | NVL;NVR;CMP                                   | chr20                  | 2729253   | 2729253   | TGCGGGGAATCCCAGAGACAT           | 0(Rfw=0) | 0(Afw=0) | C             | A        |
| het                    | het    | F_NA   | NVL;NVR;CMP                                   | chr20                  | 2729254   | 2729254   | GCGGGGAATCCCAGAGACATG           | 0(Rfw=0) | 0(Afw=0) | C             | T        |
| het                    | het    | F_NA   | NVR;CMP                                       | chr12                  | 53343059  | 53343059  | GCGCGGCCAGCGTCTATGCAG           | 0(Rfw=0) | 0(Afw=0) | C             | A        |
| het                    | het    | F_NA   | NVR;CMP                                       | chr12                  | 53343069  | 53343069  | CGTCTATGCAGGCGCTGGGGG           | 0(Rfw=0) | 0(Afw=0) | G             | T        |
| het                    | het    | F_NA   | CMP                                           | chr12                  | 53343084  | 53343084  | TGGGGGCTCTGGTTCCCGGAT           | 0(Rfw=0) | 0(Afw=0) | G             | C        |
| het                    | M_NA   | het    | CMP                                           | chr1                   | 144830505 | 144830506 | TATTCTTCGTGAGTGTGAGTGT          | 0(Rfw=0) | 0(Afw=0) | GA            | -        |
| het                    | M_NA   | het    | CMP                                           | chr1                   | 144830511 | 144830512 | TCGTGAGTGTGAGTGTGTGTGT          | 0(Rfw=0) | 0(Afw=0) | GA            | -        |
| het                    | M_NA   | het    | CMP                                           | chr9                   | 97080923  | 97080925  | AAGAGAGGTCGCTTCTTGACTT          | 0(Rfw=0) | 0(Afw=0) | GCT           | -        |
| het                    | M_NA   | het    | CMP                                           | chr9                   | 97080930  | 97080932  | GTCGCTTCTTGACTTGCTGGCA          | 0(Rfw=0) | 0(Afw=0) | GGA           | -        |
| het                    | het    | F_NA   | CMP                                           | chr12                  | 40874774  | 40874774  | ATCAACTGAAGGATCAGGGGC           | 0(Rfw=0) | 0(Afw=0) | -             | T        |
| het                    | het    | F_NA   | CMP                                           | chr12                  | 40874777  | 40874777  | AACTGAAGGATCAGGGGCAAC           | 0(Rfw=0) | 0(Afw=0) | T             | -        |
| het                    | M_NA   | het    | CMP                                           | chr14                  | 94845825  | 94845826  | GGGGTGCCTCCTCTGTGACCCC          | 0(Rfw=0) | 0(Afw=0) | CT            | -        |
| het                    | M_NA   | het    | CMP                                           | chr14                  | 94845829  | 94845829  | TGCCTCCTCTGTGACCCCGGA           | 0(Rfw=0) | 0(Afw=0) | -             | AC       |
| het                    | het    | F_NA   | CMP                                           | chr14                  | 95566098  | 95566098  | CACACACACACACACACAC             | 0(Rfw=0) | 0(Afw=0) | C             | -        |
| het                    | het    | F_NA   | CMP                                           | chr14                  | 95566100  | 95566108  | CACACACACACACACACA<br>AACTTACCA | 0(Rfw=0) | 0(Afw=0) | CACA<br>CACAC | -        |
| het                    | het    | F_NA   | CMP                                           | chr17                  | 39254152  | 39254152  | ACAGCAGGTGGGCTGGCAGCA           | 0(Rfw=0) | 0(Afw=0) | -             | A        |
| het                    | het    | F_NA   | CMP                                           | chr17                  | 39254156  | 39254156  | CAGGTGGGCTGGCAGCACACA           | 0(Rfw=0) | 0(Afw=0) | G             | -        |
| het                    | het    | F_NA   | CMP                                           | chr20                  | 46279865  | 46279865  | CAGCAGCAGCAACAGCAACAG           | 0(Rfw=0) | 0(Afw=0) | -             | GC       |
| het                    | het    | F_NA   | CMP                                           | chr20                  | 46279866  | 46279866  | AGCAGCAGCAACAGCAACAGC           | 0(Rfw=0) | 0(Afw=0) | -             | GCAG     |

**S3 Table. Significant ASIC5 gene annotations using Enrichr.**

| S. No | Data Base                          | Name                                                                 | P-value  | Adjusted p-value | Z-score | Combined score |
|-------|------------------------------------|----------------------------------------------------------------------|----------|------------------|---------|----------------|
| 1     | KEGG 2019 Human                    | Inflammatory mediator regulation of TRP channels                     | 0.005000 | 0.005000         | -6.54   | 34.66          |
| 2     | Reactome 2016                      | Stimuli-sensing channels_Homo sapiens_R-HSA-2672351                  | 0.005000 | 0.01500          | -1.92   | 10.16          |
|       |                                    | Ion channel transport_Homo sapiens_R-HSA-983712                      | 0.01015  | 0.01523          | -2.03   | 9.31           |
|       |                                    | Transmembrane transport of small molecules_Homo sapiens_R-HSA-382551 | 0.02970  | 0.02970          | -2.11   | 7.44           |
| 3     | Kinase Perturbations from GEO down | FGFR3_knockdown_174_ GSE41035                                        | 0.01500  | 0.01500          | -1.95   | 8.17           |
|       |                                    | IGF1R_druginhibition_47_ GSE14024                                    | 0.01500  | 0.01500          | -1.71   | 7.19           |
|       |                                    | ATM_knockout_16_ GDS1544                                             | 0.01500  | 0.01500          | -1.70   | 7.16           |
|       |                                    | EGFR_druginhibition_82_ GSE27638                                     | 0.01500  | 0.01500          | -1.66   | 6.99           |
| 4     | GWAS Catalog 2019                  | Manganese levels                                                     | 0.002900 | 0.002900         | -1.57   | 9.15           |
| 5     | DSigDB                             | NICKEL_CTD_00006389                                                  | 0.009500 | 0.01900          | -2.32   | 10.81          |
| 6     | Tissue Protein Expression          | HCC-202                                                              | 0.01505  | 0.01720          | -3.06   | 12.83          |



|  |                      |               |         |         |       |      |
|--|----------------------|---------------|---------|---------|-------|------|
|  | from<br>ProteomicsDB |               |         |         |       |      |
|  |                      | BT-474 cell   | 0.01505 | 0.01720 | -2.15 | 9.04 |
|  |                      | CEM cell      | 0.01505 | 0.01720 | -1.83 | 7.69 |
|  |                      | BT-549 cell   | 0.01505 | 0.01720 | -1.73 | 7.28 |
|  |                      | NCI-H292 cell | 0.01505 | 0.01720 | -1.72 | 7.23 |
|  |                      | CCRF-CEM cell | 0.01505 | 0.01720 | -1.71 | 7.18 |
|  |                      | HCC-1937 cell | 0.01505 | 0.01720 | -1.63 | 6.85 |