**Supplemental Files**

**1.Figure 1:** Protein structure and conservation analysis of *ARHGAP29*.

**2.Figure 2:** Gene structure of *ARHGAP29* and mutation spectrum associated with NSCL/P.

**3.Table 1:** Variants detected in NSCL/P family.

**4.Table 2:** Splicing prediction results of the *ARHGAP29* mutation.

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**Figure 1.** Protein structure and conservation analysis of *ARHGAP29*. **A**. The protein domain of Rho GTPase-activating protein 29, the mutation was located in C1 domain. **B**. The mutation nucleotide at c.1920 is highly conserved across species. **C.** The amino acid at position 640 is highly conserved across species.



**Figure 2.** Gene structure of *ARHGAP29* and the mutation spectrum associated with NSCL/P, the blocks indicate the gene exons, all the mutation below were collected from HGMD and the upper mutation was identified in our study. Different mutation types were marked with orange, red, blue and black correspond to missense, nonsense, splicing and frameshift, respectively.

**Table 1.** Variants detected in NSCL/P family.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Variants** | **Variant Type** | **Inheritance** | **I-1** | **I-2\*** | **II-1\*** | **II-2** | **III-1\*** | **III-2\*** | **NO. of normal controls in L-db**  | **ACMG Significance** |
| *ARHGAP29* | c.1920+1G>A [NM\_001328664] | Splice donor | AD | WT | Het | Het | WT | Het | Het | 0 | P(PVS1+PM2+PP1) |
| *CTNND1* | c.2674A>G (p.N892D) [NM\_001085458] | Missense | AD | WT | Het | Het | WT | Het | Het | 21 | VUS(PM1+PP1) |
| *FLNB* | c.2309C>T (p.T770I) [NM\_001164317]  | Missense | AD/AR | WT | Het | Het | WT | Het | Het | 2 | VUS(PM1+PP1+PP3) |
| *TP63* | c.1807G>C (p.D603H) [NM\_003722]  | Missense | AD | WT | Het | Het | WT | Het | Het | 5 | VUS(PM1+PP1+PP3+BS2) |

\* indicates affected; L-db: local database.

**Table 2:** Splicing prediction results of the *ARHGAP29* mutation.

|  |  |  |
| --- | --- | --- |
| **Tools** | **Results** |  |
| dbscSNV | The variant had effect on splicing (ada\_score:0.999; rf\_score:0.936) |  |
| NetGene2 | The original donor splice site disappeared after mutation happens |  |
| Max EntScan | Deleterious (MAXENT score WT=10.06; MUT=1.88) |  |
| HSF | most probably affecting splicing with WT site broken variation score decreased 27.98% |  |