**Table S1: WES data analysis of Family PK-INF-1148**

**Whole exome sequencing of the patients (IV:1 and IV:3) & their father (III:1)**

**13889 variants**

**980 variants**

**Variant type**

Variants affecting protein sequences (frameshift substitution, stop-gain, splicing, nonsynonymous SNVs) were kept

**Other information**

* Variants within genes that relatively highly expressed in testis were kept.
* selected

**Segregation Data**

Variants following autosome recessive inherence pattern were kept

**554 variants**

**426 variants**

***QRICH2: c.4618C>T, p.R1540W; Missense***

**4 variants in 3 genes**

**Population Data (MAF)**

Variants with MAF>0.01 in the 1000 Genomes, ESP6500, ExAC or GnomAD were excluded, variants homozygous in our in-house 578 fertile men (41 Pakistanis, 254 Chinese and 283 Europeans) were excluded

**Computational &Predictive Data**

Variants predicted to be non-deleterious by >50% software covering them were excluded

**Functional Data**

Variants within genes that are non-function in spermatogenesis based on FertilityOnline or KO mouse phenotype recorded by MGI were excluded

Table S2: Information of Antibodies

|  |
| --- |
| **Antibodies used for IF** |
| **Antibodies** | **Reference** | **Dilution for IF** |
| anti-QRICH2 | Sigma HPA021935 | 1:100 |
| anti-AKAP4 | Sigma HPA020046 | 1:100 |
| anti-ODF2 | Proteintech 12058-1-AP | 1:100 |
| anti-α-tubulin | Sigma T6074 | 1:200 |
| Goat anti-Mouse-488 | Invitrogen A21121 | 1:100 |
| Donkey anti-Rabbit-555 | Invitrogen A31572 | 1:100 |
| **Antibodies used for WB** |
| **Antibodies** | **Reference** | **Dilution for WB** |
| anti-QRICH2 | Sigma HPA021935 | 1:10000 |
| anti-AKAP4 | Sigma HPA020046 | 1:10000 |
| anti-ODF2 | Proteintech 12058-1-AP | 1:10000 |
| anti-α-tubulin | Sigma F2168 | 1:10000 |

**Table S3: Details of previously identified mutations in the *QRICH2***

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **cDNA variation** | **Amino acid variation** | **Variant type** | **Allelic status** | **Origin** | **Main phenotype** |
| **QRICH2** | c.192G>A | p.Trp64**\*** | Nonsense | Homozygous | China | No progressive motility, absence of central pair, Regular arrangements of MTDs and ODF in mid-piece, while in principal piece some MTDs and ODF were disorganized and some were absent |
| c.3037C>T | p.Arg1013**\*** | Nonsense | Homozygous | No progressive motility, absence of central pair, the typical 6+0 arrangement of microtubules in mid-piece while in principal piece MTDs and ODF were disorganized  |
| c.3501C>G | p.Tyr1167**\*** | Nonsense | Homozygous | North Africa | 7% total motility was observed |
| c.4614C>G | p.Tyr1538**\*** | Nonsense | Homozygous | No progressive motility was observed |

Supplementary Table 4: Effect of identified mutation on the structural stability of the QRICH2 protein

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene**  | **Protein alteration**  | **Mutant Pro** | **I-Mutant2.0** |
| *QRICH2* | p. R1540W | DDG Kcal/Mol\* | Stability | DDG Kcal/Mol | RI\* | pH | Stability |
|  |  | -1.23369 | Decrease | -0.54 | 5 | 7 | Decrease |

DDG Kcal/Mol\*: Free energy change value

DDG Kcal/Mol\*: DG (New Protein)-DG (Wild Type) in Kcal/mol

DDG<0: Decrease Stability

DDG>0: Increase Stability

RI\*: Reliability Index