|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | pLI Score | MERGE Cohort of Infertile Men (n=901) | GEMINI Cohort of NOA Men (n=926) | Regeneron Cohort of Infertile Men (n=88) | Italian Cohort of NOA Men (n=48) | Total infertile cohorts (n=1,963) | Fertile Dutch Men (n=5,784) | Fertile Dutch Women (n=5,803) |
| ATP1A1 | 1 | N/A | 0 | N/A | 0 | 0 | 0 | 0 |
| CSTF3 | 0.98 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| FBXO5 | 0.97 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| GREB1L | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| HTT | 1 | N/A | 0 | 0 | 0 | 0 | 3 | 3 |
| PPP1R7 | 0.99 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| QSER1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 |
| SOGA1 | 1 | 1 | 0 | 0 | 0 | 1 | 2 | 6 |
| TENM2 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| ZFHX4 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |

**Supplementary table 2: Rare loss-of-function (LoF) mutations observed in additional cohorts of infertile men and fertile control cohorts.**

These 10 genes were selected because of a LoF DNM present in the original discovery cohort in these LoF intolerant genes (as defined by a pLI score >0.9). Exome data from four additional cohorts of infertile men as well as control cohorts of fertile men and women were investigated for the presence of LoF mutations in these genes. N/A – Data not available for this gene in WES data.

**Supplementary Table 3: Clinical details of individuals with *RBM5* pathogenic mutations** **described in this study.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cohort Name | Patient ID | Age of Patient\* | Karyotype | Y deletions | Conclusion semen analysis  | Conclusion testis histology | Testicular volume left (ml) | Testicular volume right (ml) | Semen conc. (x106) | Semen volume (ml) | Semen pH | FSH (U/L) | Testicular sperm retrieved | Urological history  |
| NIJ/NLC Cohort of Patient-Parent Trios  | Proband\_108 | 40 | 46, XY | None | Severe oligo-zoospermia | No biopsy | NA | NA | 0.5 | 3.2 | 7.5 | 0 | N/A | Unknown |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| NIJ/NLC Cohort of Infertile Men | Proband00282 | 32 | 46, XY | None | Azoospermia | Biopsy compromised | 15 | 15 | 0 | 3.1 | 7.7 | 6.7 | Yes | Cryptorchism with orchidopexy (unknown if unilateral/bilateral) |
| NIJ/NLC Cohort of Infertile Men  | Proband00524 | 36 | 46, XY | None | Azoospermia | Hypo-spermatogenesis | 11 | 6 | 0 | 3.4 | 7.5 | 47 | Yes | None |
| MERGE Cohort of Infertile Men | M248 | 43 | 46, XY | None | Azoospermia | Complete SCO | 14 | 27 | 0 | 7.3 | 7.7 | 31.7 | No | None |
| MERGE Cohort of Infertile Men | M2013 | 28 | 46, XY | None | Azoospermia | No biopsy | 7 | 8 | 0 | 3.2 | 8.3 | 27.9 | N/A | None |

\* At diagnosis of infertility.

Multiple infertile men from different cohorts were found with a rare pathogenic mutation on RBM5 in addition to the Proband\_108 where a DNM on RBM5 was initially identified. Detailed clinical information for the patient from the Geisinger-Regeneron DiscovEHR cohort of infertile men with a rare pathogenic RBM5 mutation is currently not available and was therefore no included in this table.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Possibly Causative | Unclear | Unlikely Causative | Not Causative | Total |
| Maternal | 1 | 5 | 2 | 2 | 10 |
| Paternal | 6 | 5 | 20 | 12 | 43 |
| Post-Zygotic | 1 | 1 | 3 | 1 | 6 |
| Total | 8 | 11 | 25 | 15 | 59 |

**Supplementary Table 4: Phasing of de novo mutations.**

So far, 59 out of 192 variants have undergone phasing to determine the parent of origin. These can then be categorised based on their final classification to investigate the distribution of pathogenic variants with paternal or maternal origin.

**Supplementary table 5:** Long range PCR reaction mixes and running conditions.

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