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1 # A *de novo* paradigm for male infertility

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Introduction

De novo mutations (DNMs) are known to play a prominent role in sporadic disorders with reduced fitness[1]. We hypothesize that DNMs play an important role in male infertility and explain a significant fraction of the genetic causes of this understudied disorder. To test this hypothesis, we performed trio-based exome-sequencing in a unique cohort of 185 infertile males and their unaffected parents. Following a systematic analysis, 29 of 145 rare protein altering DNMs were classified as possibly causative of the male infertility phenotype. We observed a significant enrichment of Loss-of-Function (LoF) DNMs in LoF-intolerant genes (p-value=1.00x10-5) as well as predicted pathogenic missense DNMs in missense-intolerant genes (p-value=5.01x10-4). One DNM gene identified, RBM5, is an essential regulator of male germ cell pre-mRNA splicing[2]. In a follow-up study, 5 rare pathogenic missense mutations affecting this gene were observed in a cohort of 2,279 infertile patients, with no such mutations found in a cohort of 5,784 fertile men (p-value=0.009). Our results provide the first evidence for the role of DNMs in severe male infertility and point to many new candidate genes affecting fertility.

Main

Male infertility contributes to approximately half of all cases of infertility and affects 7% of the male population. For the majority of these men the cause remains unexplained[3]. Despite a clear role for genetic causes in male infertility, there is a distinct lack of diagnostically relevant genes and at least 40% of all cases are classified as idiopathic[3–6]. Previous studies in other conditions with reproductive lethality, such as neurodevelopmental disorders, have demonstrated an important role for *de novo* mutations (DNMs) in their etiology[1]. In line with this, recurrent *de novo* chromosomal abnormalities play an important role in male infertility. Both azoospermia Factor (AZF) deletions on the Y chromosome as well as an additional X chromosome, resulting in Klinefelter syndrome, occur *de novo.* Collectively, these *de novo* events explaining up to 25% of all cases of non-obstructive azoospermia (NOA)[3,6]. Interestingly, in 1999 a DNM in the Y-chromosomal gene USP9Y was reported in a man with azoospermia[7]. Until now, however, a systematic analysis of the role of DNMs in male infertility had not been attempted. This is partly explained by a lack of

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74 basic research in male reproductive health in general[6,8], but also by the practical challenges of collecting
75 parental samples for this disorder, which is typically diagnosed in adults.

76 In this study, we investigated the role of DNMs in 185 unexplained cases of oligozoospermia (<5 million sperm
77 cells/ml; n=74) and azoospermia (n=111) by performing whole exome sequencing (WES) in all patients and
78 their parents (see Supplementary Figure 1 and 2, Supplementary notes and tables for details on methods and
79 clinical description). In total, we identified and validated 192 rare DNMs, including 145 protein altering DNMs.
80 All *de novo* point mutations were autosomal, except for one on chromosome X, and all occurred in different
81 genes (Supplementary Table 1). Two *de novo* copy number variations (CNVs) were also identified affecting a
82 total of 7 genes (Supplementary Figure 3).

83 None of the 145-protein altering DNMs occurred in a gene already known for its involvement in autosomal
84 dominant human male infertility. This is not unexpected as only 4 autosomal dominant genes have so far been
85 linked to isolated male infertility in humans[5,9]. Broadly speaking, across genetic disorders, dominantly acting
86 disease genes are usually intolerant to loss-of-function (LoF) mutations, as represented by a high pLI score[10].
87 The median pLI score of genes with a LoF DNM (n=17) in our cohort of male infertility cases was significantly
88 higher than that of genes with 181 LoF DNMs identified in a cohort of 1,941 control cases from denovo-db
89 v1.6.1[11] (pLI male infertility=0.80, pLI controls=3.75×10^{-5}, p-value=1.00×10^{-5}) (Figure 1). This observation
90 indicates that LoF DNMs likely play an important role in male infertility, similar to what is known for
91 developmental disorders and severe intellectual disability[12,13]. As an example, a heterozygous likely pathogenic
92 frameshift DNM was observed in the LoF intolerant gene *GREB1L* (pLI=1) of Proband_076. Homozygous *Greb1L*
93 knock-out mice appear to be embryonic lethal, however, typical male infertility phenotypic features such as
94 abnormal fetal testis morphology and decreased fetal testis volume are observed[14]. Interestingly, this patient
95 has a reduced testis volume and severe oligospermia (Supplementary Notes Table 1). Nonsense and missense
96 mutations in *GREB1L* in humans are known to cause renal agenesis[15] (OMIM: 617805), not known to be
97 present in our patient. Of note, all previously reported damaging mutations in *GREB1L* causing renal agenesis
98 are either maternally inherited or occurred *de novo.* This led the authors of one of these renal agenesis studies
99 to speculate that disruption to *GREB1L* could cause infertility in males[14]. A recent WES study involving a cohort
100 of 285 infertile men also noted several patients presenting with pathogenic mutations in genes with an
101 associated systemic disease where male fertility is not always assessed[16]. We also assessed the damaging

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102 effects of the two *de novo* CNVs by looking at the pLI score of the genes involved. Proband_066 presented with
103 a large 656 kb *de novo* deletion on chromosome 11, spanning 6 genes in total. This deletion partially
104 overlapped with a deletion reported in 2014 in a patient with cryptorchidism and NOA[17]. Two genes affected in
105 both patients, *QSER1* and *CSTF3*, are extremely LOF-intolerant with pLI scores of 1 and 0.98, respectively. In
106 particular, *CSTF3* is highly expressed within the testis and is known to be involved in pre-mRNA 3′ end cleavage
107 and poylyadenylation[18].

108 To systematically evaluate and predict the likelihood of these DNMs causing male infertility and identify novel
109 candidate disease genes, we assessed the predicted pathogenicity of all DNMs using three prediction methods
110 based on SIFT[19], MutationTaster[20] and PolyPhen2[21]. Using this approach, 84/145 protein altering DNM were
111 predicted to be pathogenic, while the remaining 61 were predicted to be benign. To further analyse the impact
112 of the variants on the genes affected, we looked at the missense Z-score of all 122 genes affected by a
113 missense variant, which indicates the tolerance of genes to missense mutations[22]. Our data highlights a
114 significantly higher missense Z-score in genes affected by a missense DNM predicted as pathogenic (n=63)
115 when compared to genes affected by predicted benign (n=59) missense DNMs (p-value=5.01×10^{-4}, Figure 2,
116 Supplementary Figure 4). Furthermore, using the STRING database[23], we found a significant enrichment of
117 protein interactions amongst the 84 genes affected by a protein altering DNM predicted to be pathogenic (PPI
118 enrichment p-value = 2.35×10^{-2}, Figure 3). No such enrichment was observed for the genes highlighted as
119 likely benign (n=61, PPI enrichment p-value=0.206) or those affected by synonymous DNMs (n=35, PPI
120 enrichment p-value=0.992, Supplementary Figure 5). These two findings suggest that (1) the predicted
121 pathogenic missense DNMs detected in our study affect genes sensitive to missense mutations, and (2) the
122 proteins affected by predicted pathogenic DNMs share common biological functions.

123 The STRING network analysis also highlighted a central module of interconnected proteins with a significant
124 enrichment of genes required for mRNA splicing (Supplementary Figure 6). The genes *U2AF2*, *HNRNPL*, *CDC5L*,
125 *CWC27* and *RBM5* all contain predicted pathogenic DNMs and likely interact at a protein level during the
126 mRNA splicing process. Pre-mRNA splicing allows gene functions to be expanded by creating alternative splice
127 variants of gene products and is highly elaborated within the testis[24]. One of these genes, *RBM5* has been
128 previously highlighted as an essential regulator of haploid male germ cell pre-mRNA splicing and male fertility[2].
129 Mice with a homozygous ENU-induced allele point mutation in *RBM5* present with azoospermia and germ cell

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130 development arrest at round spermatids. Whilst in mice a homozygous mutation in *RBM5* is required to cause
131 azoospermia, this may not be the case in humans as is well-documented for other genes[25], including the
132 recently reported male infertility gene *SYCP2*[9]. Of note, *RBM5* is a tumour suppressor in the lung[26], with
133 reduced expression affecting RNA splicing in patients with non-small cell lung cancer[27]. *HNRNPL* is another
134 splicing factor affected by a possible pathogenic DNM in our study. One study implicated a role for *HNRNPL* in
135 patients with Sertoli cell only phenotype[28]. The remaining three mRNA splicing genes have not yet been
136 implicated in human male infertility. However, mRNA for all three is expressed at medium to high levels in
137 human germ cells and all are widely expressed during spermatogenesis[29]. Specifically, CDC5L is a component of
138 the PRP19-CDC5L complex that forms an integral part of the spliceosome and is required for activating pre-
139 mRNA splicing[30], as is CWC27[31]. U2AF2 plays a role in pre-mRNA splicing and 3'-end processing[32]. Interestingly,
140 *CSTF3,* one of the genes affected by a *de novo* CNV in Proband_066, affects the same mRNA pathway[17].

141 Whilst DNMs most often cause dominant disease, they can contribute to recessive disease, usually in
142 combination with an inherited variant on the trans allele. This was observed in Proband_060, who carried a
143 DNM on the paternal allele, in trans with a maternally inherited variant in Testis and Ovary Specific PAZ
144 Domain Containing 1 (*TOPAZ1*) (Supplementary Figure 7). *TOPAZ1* is a germ-cell specific gene which is highly
145 conserved in vertebrates[33]. Studies in mice revealed that *Topaz1* plays a crucial role in spermatocyte, but not
146 oocyte progression through meiosis[34]. In men, *TOPAZ1* is expressed in germ cells in both sexes[29,35,36]. Analysis
147 of the testicular biopsy of this patient revealed a germ cell arrest in early spermiogenesis (Figure 4).

148 In addition to all systematic analyses described above, we evaluated the function of all DNM genes to give
149 each a final pathogenicity classification (Table 1, details in Material & Methods). Of all 145 DNMs, 29 affected
150 genes linked to male reproduction and were classified as possibly causative. For replication purposes,
151 unfortunately no other trio-based exome data are available for male infertility, although we note that a pilot
152 study including 13 trios was recently published[37]. While this precluded a genuine replication study, we were
153 able to study these candidate genes in exome datasets of infertile men (n=2,279), in collaboration with
154 members of the International Male Infertility Genomics Consortium and the Geisinger Regeneron DiscovEHR
155 collaboration[38]. The 33 candidate genes selected for this analysis include the 29 genes mentioned above and 4
156 additional LoF intolerant genes carrying LoF DNMs with an ‘unclear’ final pathogenicity classification. For

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157 comparison, we included an exome dataset from a cohort of 11,587 fertile men and women from
158 Radboudumc.

159 In the additional infertile cohorts, we identified only 2 LoF mutations in our DNM LoF intolerant genes
160 (Supplementary table 2). Next, we looked for an enrichment of rare predicted pathogenic missense mutations
161 in these cohorts (Table 2). A burden test revealed a significant enrichment in the number of such missense
162 mutations present in infertile men compared to fertile men in the *RBM5* gene (adjusted p-value=0.009). In this
163 gene, 5 infertile men were found to carry a distinct rare pathogenic missense mutation, in addition to the
164 proband with a *de novo* missense mutation (Supplementary figure 8, Supplementary table 3). Importantly, no
165 such predicted pathogenic mutations were identified in men in the fertile cohort. In line with these results,
166 *RBM5*, already highlighted above as an essential regulator of male germ cell pre-mRNA splicing and male
167 infertility[2], is highly intolerant to missense mutations (missense Z-score 4.17).

168 Given the predicted impact of these DNMs on spermatogenesis, we were interested in studying the parental
169 origin of DNMs in our trio-cohort. We were able to phase 29% of all our DNMs using a combination of short-
170 read WES and targeted long-read sequencing (Supplementary Table 4). In agreement with literature[39–42], 72%
171 of all DNMs occurred on the paternal allele. Interestingly, phasing of 8 likely causative DNMs showed that 6 of
172 these were of paternal origin (75%). This suggests that DNMs with a deleterious effect on the future germline
173 can escape negative selection in the paternal germline. This may be possible because the DNM occurred after
174 the developmental window in which the gene is active, or the DNM may have affected a gene in the gamete's
175 genome that is critical for somatic cells supporting the (future) germline. Transmission of pathogenic DNMs
176 may also be facilitated by the fact that from spermatogonia onwards, male germ cells form cysts and share
177 mRNAs and proteins[43]. As such, the interconnectedness of male germ cells, which is essential for their
178 survival[44], could mask detrimental effects of DNMs occurring during spermatogenesis.

179 In 2010, we published a pilot study pointing to a *de novo* paradigm for mental retardation[45] (now more
180 appropriately termed developmental delay or intellectual disability). This work contributed to the widespread
181 implementation of patient-parent WES studies in research and diagnostics for neurodevelopmental
182 disorders[46], accelerating disease gene identification and increasing the diagnostic yield for these disorders. The
183 data presented here suggest that a similar benefit could be achieved from trio-based sequencing in male

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184 infertility. This will not only help to increase the diagnostic yield for men with infertility but will also enhance
185 our fundamental biological understanding of human reproduction and natural selection.
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189 **Data access**
190 Raw and processed exome sequencing data of our 185 patient-parent trios is available under controlled access
191 and requires a Data Transfer Agreement from the European Genome-Phenome Archive (EGA) repository:
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206
207 **Author contributions**

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208 This study was designed by MSO, LELMV, LR and JAV. RMS, JG, HT and GWvdH provided all clinical data and
209 performed the TESE histology and cytology analysis under supervision of LR, DDMB, ES, KF, KDH and KM. JC
210 performed the exome sequencing with support from BA, and bioinformatics support was provided by MJX, GA,
211 CG and SC. Sanger sequencing was performed by PFdV, HI, HES, LEB and BKSA. MSO and HES performed the
212 SNV analyses with support from MJX, FKM performed CNV analysis with support from AM and MSK, and GSH
213 and LEB performed the phasing. DJE, HS, BJH and MKOB provided support on the functional interpretation of
214 mutations. DFC, LN, CF, SK, FT, KIA, ARE, CK, and CG-J were involved in the replication study. The first draft of
215 the manuscript was prepared by MSO, HES, RMS, MJX, GWvdH, and JAV. All authors contributed to the final
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218 **References**

219 1. Veltman, J. A. & Brunner, H. G. De novo mutations in human genetic disease. *Nat. Rev. Genet.* **13**, 565–
220 575 (2012).
221 2. O'Bryan, M. K. *et al.* RBM5 Is a Male Germ Cell Splicing Factor and Is Required for Spermatid
222 Differentiation and Male Fertility. *PLoS Genet.* **9**, e1003628 (2013).
223 3. Krausz, C. & Riera-Escamilla, A. Genetics of male infertility. *Nat. Rev. Urol.* **15**, 369–384 (2018).
224 4. Tüttelmann, F., Ruckert, C. & Röpke, A. Disorders of spermatogenesis. *medizinische Genet.* **30**, 12–20
225 (2018).
226 5. Oud, M. S. *et al.* A systematic review and standardized clinical validity assessment of male infertility
227 genes. *Hum. Reprod.* **34**, 932–941 (2019).
228 6. Kasak, L. & Laan, M. Monogenic causes of non-obstructive azoospermia: challenges, established
229 knowledge, limitations and perspectives. *Hum. Genet.* **140**, 135–154 (2021).
230 7. Sun, C. *et al.* An azoospermic man with a de novo point mutation in the Y-chromosomal gene USP9Y.
231 *Nat. Genet.* **23**, 429–432 (1999).
232 8. De Jonge, C. & Barratt, C. L. R. The present crisis in male reproductive health: an urgent need for a
233 political, social, and research roadmap. *Andrology* **7**, 762–768 (2019).
234 9. Schilit, S. L. P. *et al.* SYCP2 Translocation-Mediated Dysregulation and Frameshift Variants Cause
235 Human Male Infertility. *Am. J. Hum. Genet.* **106**, 41–57 (2020).
236 10. Lek, M. *et al.* Analysis of protein-coding genetic variation in 60,706 humans. *Nature* **536**, 285–291
237 (2016).
238 11. denovo-db, Seattle, WA (denovo-db.gs.washington.edu) [Aug 2020].
239 12. Gu, Y. *et al.* Three intellectual disability-associated de novo mutations in MECP2 identified by trio-WES
240 analysis. *BMC Med. Genet.* **21**, 99 (2020).
241 13. Fritzen, D. *et al.* De novo FBXO11 mutations are associated with intellectual disability and behavioural
242 anomalies. *Hum. Genet.* **137**, 401–411 (2018).
243 14. De Tomasi, L. *et al.* Mutations in GREB1L Cause Bilateral Kidney Agenesis in Humans and Mice. *Am. J.*
244 *Hum. Genet.* **101**, 803–814 (2017).
245 15. Brophy, P. D. *et al.* A Gene Implicated in Activation of Retinoic Acid Receptor Targets Is a Novel Renal

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246 Agenesis Gene in Humans. *Genetics* **207**, 215–228 (2017).

247 16. Alhathal, N. *et al.* A genomics approach to male infertility. *Genet. Med.* **22**, 1967–1975 (2020).

248 17. Seabra, C. M. *et al.* A novel Alu-mediated microdeletion at 11p13 removes WT1 in a patient with
249 cryptorchidism and azoospermia. *Reprod. Biomed. Online* **29**, 388–391 (2014).

250 18. Grozdanov, P. N., Li, J., Yu, P., Yan, W. & MacDonald, C. C. Cstf2t Regulates expression of histones and
251 histone-like proteins in male germ cells. *Andrology* **6**, 605–615 (2018).

252 19. Vaser, R., Adusumalli, S., Leng, S. N., Sikic, M. & Ng, P. C. SIFT missense predictions for genomes. *Nat.*
253 *Protoc.* **11**, 1–9 (2016).

254 20. Schwarz, J. M., Rödelsperger, C., Schuelke, M. & Seelow, D. MutationTaster evaluates disease-causing
255 potential of sequence alterations. *Nat. Methods* **7**, 575–576 (2010).

256 21. Adzhubei, I. A. *et al.* A method and server for predicting damaging missense mutations. *Nat. Methods*
257 **7**, 248–249 (2010).

258 22. Samocha, K. E. *et al.* A framework for the interpretation of de novo mutation in human disease. *Nat.*
259 *Genet.* **46**, 944–950 (2014).

260 23. Szklarczyk, D. *et al.* The STRING database in 2017: quality-controlled protein–protein association
261 networks, made broadly accessible. *Nucleic Acids Res.* **45**, D362–D368 (2017).

262 24. Song, H., Wang, L., Chen, D. & Li, F. The Function of Pre-mRNA Alternative Splicing in Mammal
263 Spermatogenesis. *Int. J. Biol. Sci.* **16**, 38–48 (2020).

264 25. Elsea, S. H. & Lucas, R. E. The Mousetrap: What We Can Learn When the Mouse Model Does Not Mimic
265 the Human Disease. *ILAR J.* **43**, 66–79 (2002).

266 26. Jamsai, D. *et al.* In vivo evidence that RBM5 is a tumour suppressor in the lung. *Sci. Rep.* **7**, 16323
267 (2017).

268 27. Liang, H. *et al.* Differential Expression of RBM5, EGFR and KRAS mRNA and protein in non-small cell
269 lung cancer tissues. *J. Exp. Clin. Cancer Res.* **31**, 36 (2012).

270 28. Li, J. *et al.* HnRNPL as a key factor in spermatogenesis: Lesson from functional proteomic studies of
271 azoospermia patients with sertoli cell only syndrome. *J. Proteomics* **75**, 2879–2891 (2012).

272 29. Wang, M. *et al.* Single-Cell RNA Sequencing Analysis Reveals Sequential Cell Fate Transition during
273 Human Spermatogenesis. *Cell Stem Cell* **23**, 599-614.e4 (2018).

274 30. Ajuh, P. Functional analysis of the human CDC5L complex and identification of its components by mass

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275 spectrometry. *EMBO J.* **19**, 6569–6581 (2000).

276 31. Brea-Fernández, A. J. *et al.* Expanding the clinical and molecular spectrum of the CWC27-related
277 spliceosomopathy. *J. Hum. Genet.* **64**, 1133–1136 (2019).

278 32. Millevoi, S. *et al.* An interaction between U2AF 65 and CF Im links the splicing and 3′ end processing
279 machineries. *EMBO J.* **25**, 4854–4864 (2006).

280 33. Baillet, A. *et al.* TOPAZ1, a Novel Germ Cell-Specific Expressed Gene Conserved during Evolution across
281 Vertebrates. *PLoS One* **6**, e26950 (2011).

282 34. Luangpraseuth-Prosper, A. *et al.* TOPAZ1, a germ cell specific factor, is essential for male meiotic
283 progression. *Dev. Biol.* **406**, 158–171 (2015).

284 35. Guo, F. *et al.* The Transcriptome and DNA Methylome Landscapes of Human Primordial Germ Cells. *Cell*
285 **161**, 1437–1452 (2015).

286 36. Li, L. *et al.* Single-Cell RNA-Seq Analysis Maps Development of Human Germline Cells and Gonadal
287 Niche Interactions. *Cell Stem Cell* **20**, 858-873.e4 (2017).

288 37. Hodžić, A. *et al.* De novo mutations in idiopathic male infertility—A pilot study. *Andrology* **9**, 212–220
289 (2021).

290 38. Dewey, F. E. *et al.* Distribution and clinical impact of functional variants in 50,726 whole-exome
291 sequences from the DiscovEHR study. *Science (80-.).* **354**, aaf6814 (2016).

292 39. Francioli, L. C. *et al.* Genome-wide patterns and properties of de novo mutations in humans. *Nat.*
293 *Genet.* **47**, 822–826 (2015).

294 40. Rahbari, R. *et al.* Timing, rates and spectra of human germline mutation. *Nat. Genet.* **48**, 126–133
295 (2016).

296 41. Goldmann, J. M. *et al.* Parent-of-origin-specific signatures of de novo mutations. *Nat. Genet.* **48**, 935–
297 939 (2016).

298 42. Jónsson, H. *et al.* Parental influence on human germline de novo mutations in 1,548 trios from Iceland.
299 *Nature* **549**, 519–522 (2017).

300 43. Braun, R. E., Behringer, R. R., Peschon, J. J., Brinster, R. L. & Palmiter, R. D. Genetically haploid
301 spermatids are phenotypically diploid. *Nature* **337**, 373–376 (1989).

302 44. Greenbaum, M. P., Iwamori, T., Buchold, G. M. & Matzuk, M. M. Germ Cell Intercellular Bridges. *Cold*
303 *Spring Harb. Perspect. Biol.* **3**, a005850–a005850 (2011).

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304 45. Vissers, L. E. L. M. *et al.* A de novo paradigm for mental retardation. *Nat. Genet.* **42**, 1109–12 (2010).

305 46. Vissers, L. E. L. M., Gilissen, C. & Veltman, J. A. Genetic studies in intellectual disability and related
306 disorders. *Nat. Rev. Genet.* **17**, 9–18 (2016).

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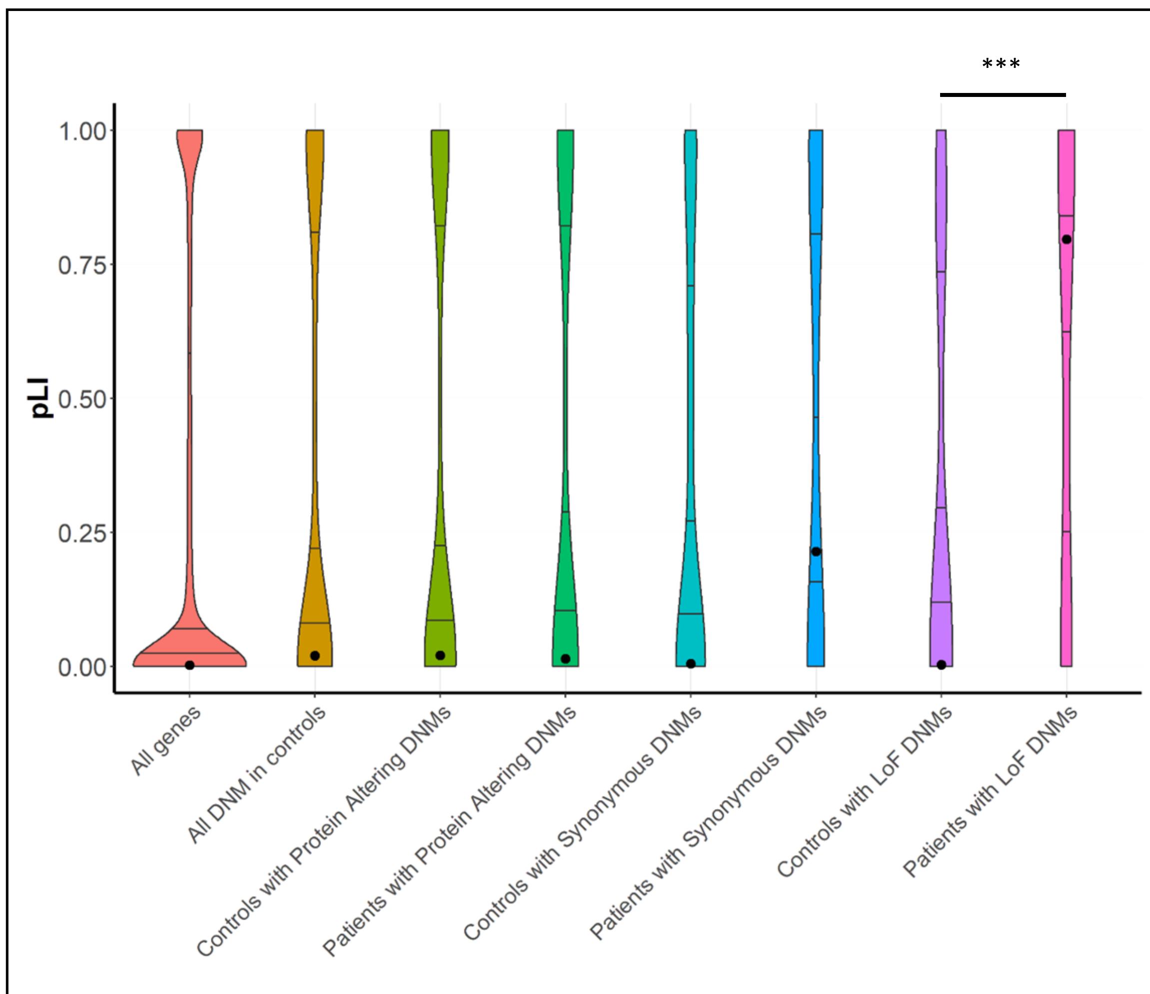
333 **Figures and Tables**



352 **Figure 1: Analysis of the intolerance to loss-of-function variation for DNM genes.** Violin plots represent the distribution of
353 the pLI scores of all genes in gnomAD, all genes affected by DNMs and all LoF DNM in this study and in a control population
354 (http://denovo-db.gs.washington.edu/denovo-db/). The observed median pLI score is displayed for each category as a
355 black circle. The closer the pLI score is to 1, the more intolerant to LoF variation a gene is[10]. Comparison between LoF
356 DNMs in our study and control populations shows a significance difference (p-value=1.00×10^{-5}).

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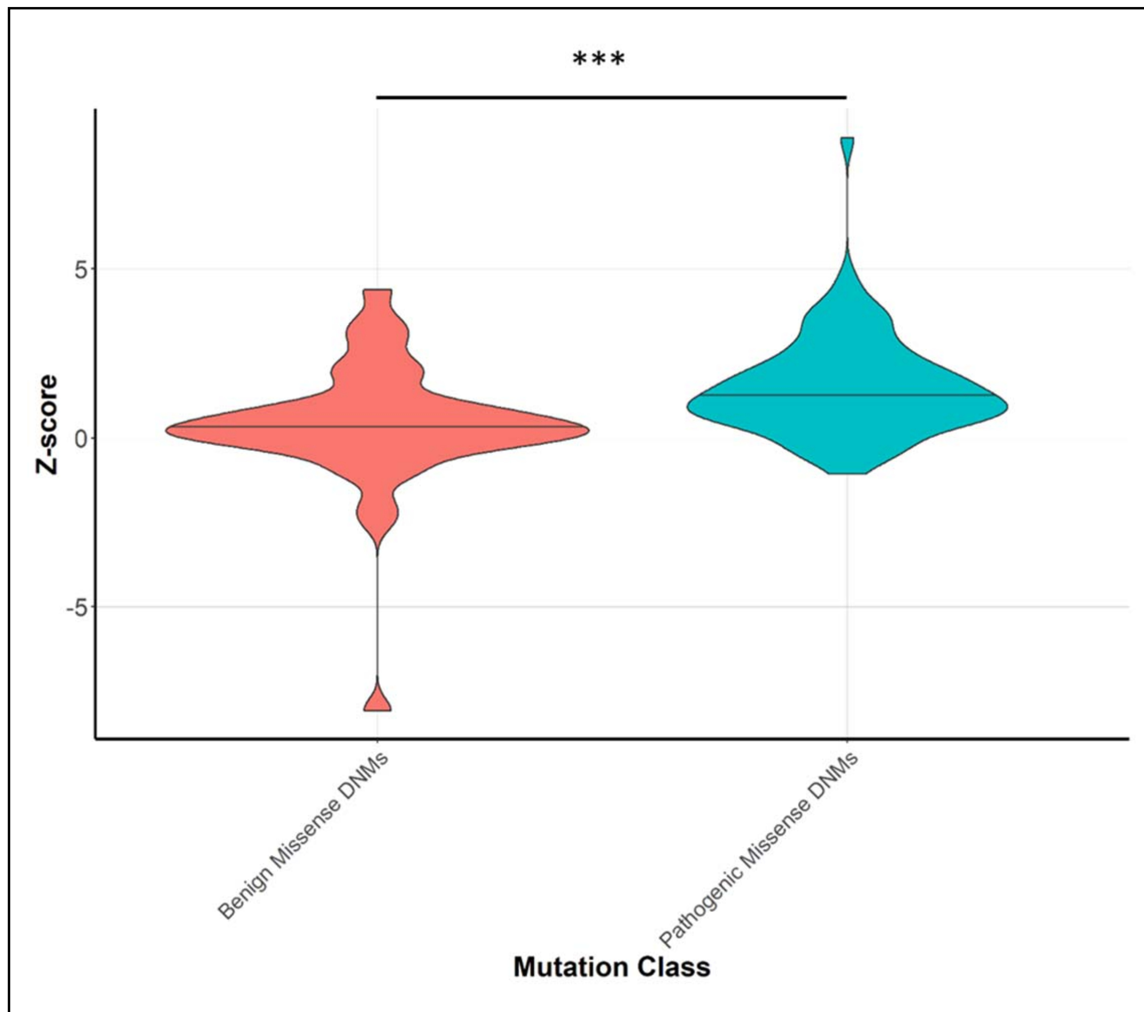

358 **Figure 2: Intolerance to missense variants for genes with a DNM.** Violin plots show the distribution of Z-scores of genes
359 containing a missense DNM in our cohort, where an enrichment can be observed for predicated pathogenic DNMs in genes
360 more intolerant to missense mutations based on their mean z-score with a p-value of 5.01×10^{-4}.

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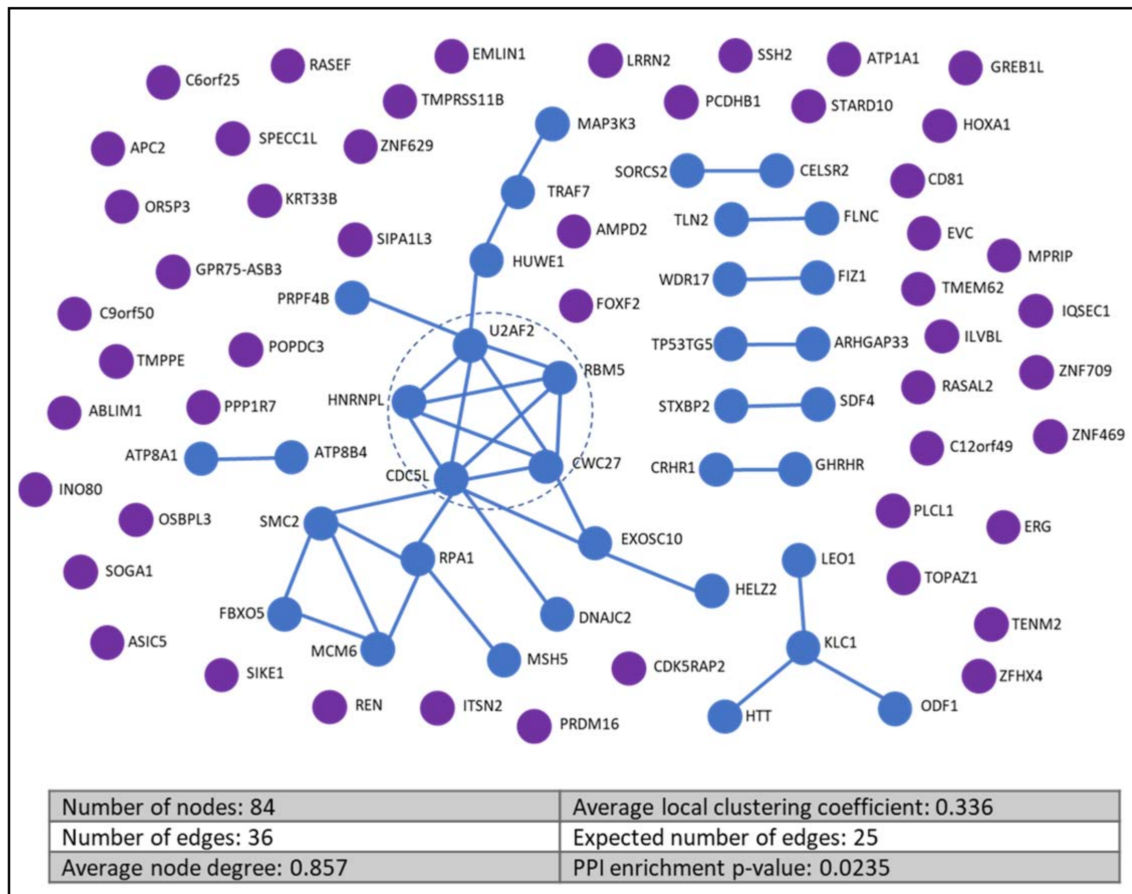

361 **Figure 3: Protein-protein interactions predicted for proteins encoded by damaging DNM genes.** A protein-protein
362 interaction analysis was performed for all 84 genes containing a DNM scored as damaging using the STRING tool[23]. A
363 significantly larger number of interactions is observed between our damaging DNM genes than is expected for a similar
364 sized dataset of randomly selected genes (PPI enrichment p-value 2.35×10^{-2}) with the number of expected edges being 25
365 and the observed being 36. The central module of the main interaction network within the figure contains 5 genes which
366 are all involved in the process of mRNA splicing (Supplementary figure 6)

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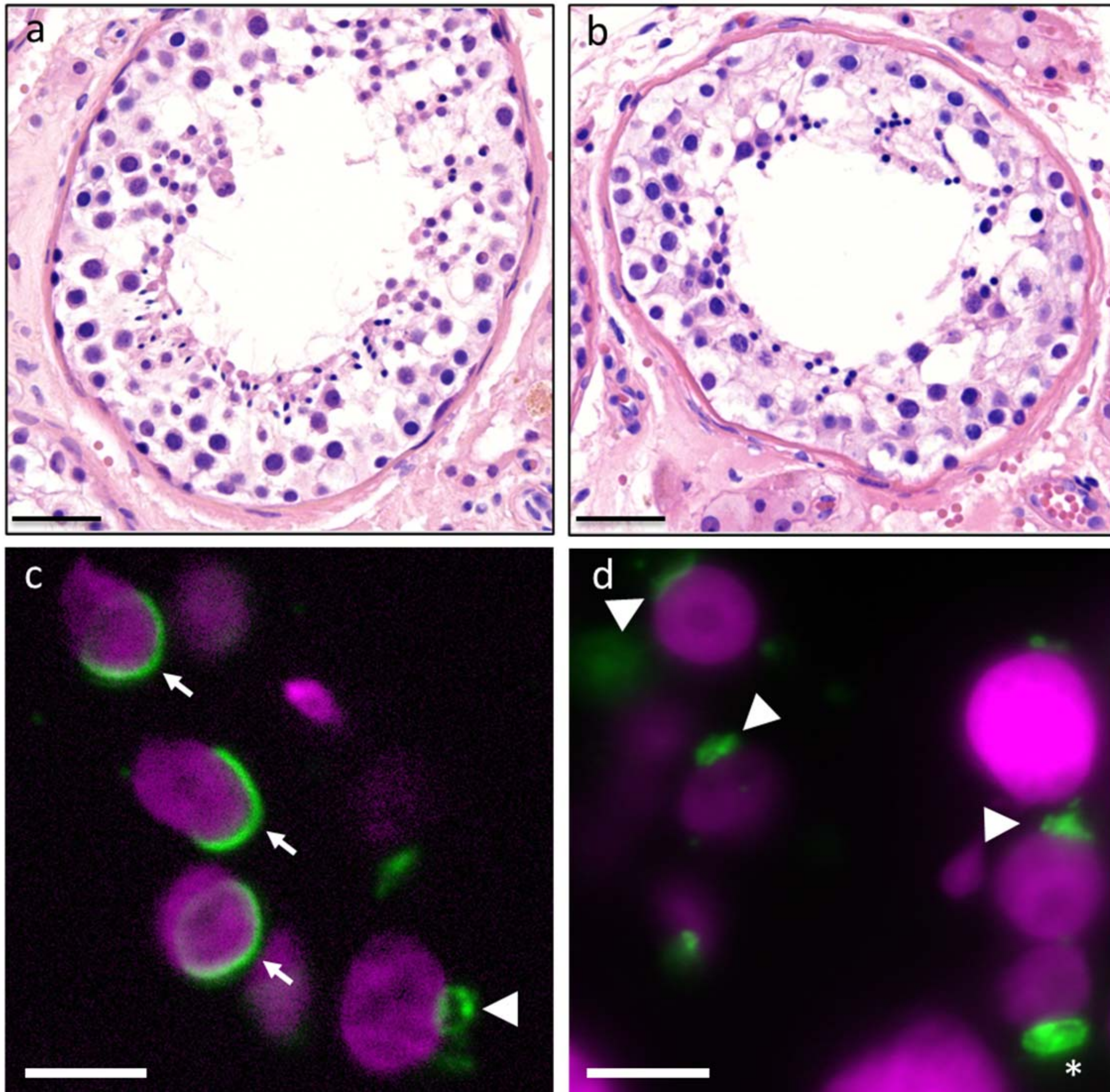

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368 **Figure 4: Description of control and *TOPAZ1* proband testis histology and aberrant acrosome formation: (a,b):** H&E
369 stainings of **(a)** control and **(b)** Proband_060 with DNM in *TOPAZ1* gene. The epithelium of the seminiferous tubules in the
370 *TOPAZ1* proband show reduced numbers of germ cells and an absence of elongating spermatids. **(c,d):** immunofluorescent
371 labelling of DNA (magenta) and the acrosome (green) in control sections **(c)** and *TOPAZ1* proband sections **(d)**. **(c)** The
372 arrowhead indicates the acrosome in an early round spermatid and the arrows the acrosome in elongating spermatids.
373 Spreading of the acrosome and nuclear elongation are hallmarks of spermatid maturation. **(d)** No acrosomal spreading (see
374 arrowheads) or nuclear elongation is observed in the *TOPAZ1* proband. The asterisk indicates an example of progressive
375 acrosome accumulation without spreading. Size bar in a, b: 40 µm, c, d: 5 µm.

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382 **Table 1: *De novo* mutation classification summary.**

	Possibly causative	Unclear	Unlikely causative	Not Causative	Total
Missense	21	38	50	13	122
Frameshift	4	8	1	0	13
Stop gained	1	3	0	0	4
In-frame indels	3	1	1	1	6
Splice site variant	0	0	0	11	11
Synonymous	0	0	0	36	36
TOTAL	29	50	52	61	192

383 A total of 192 rare DNMs were classified based on pathogenicity scores as well as functional data into 4 categories,
384 'Possibly causative', 'Unclear', 'Unlikely Causative' and 'Not causative'.

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399 **Table 2: Rare potentially pathogenic missense mutations in exome data from various cohorts of infertile men and fertile control cohorts.**

Gene	Missense Z-score	NIJ/NCL Cohort of Patient-Parent Trios (n=185)	NIJ/NCL Cohort of Infertile Men (Singleton) (n=145)	MERGE Cohort of Infertile Men (n=887)	GEMINI Cohort of NOA Men (n=926)	Geisinger-Regeneron DiscovEHR Cohort of Infertile Men (n=88)	Italian Cohort of NOA Men (n=48)	Total Infertile Men (n=2,279)	Fertile Dutch Men (n=5,784)	Fertile Dutch Women (n=5,803)	Burden test Infertile vs Fertile Men (Bonf)	Burden test Fertile Men vs Women (Bonf)
ABLIM1	1.62	1	1	1	1	1	0	5	1	1	0.15	1
ATP1A1	6.22	0	0	0	1	0	0	1	0	1	1	1
CDC5L	2.78	1	1	1	3	0	0	6	2	4	0.15	1
CDK5RAP2	-0.37	1	0	1	1	0	0	3	5	5	1	1
HUWE1	8.87	1	0	2	0	0	0	3	0	0	0.41	1
INO80	3.53	1	0	1	0	0	0	2	3	3	1	1
MAP3K3	2.04	1	0	2	0	0	0	3	1	2	1	1
MCM6	1.07	1	1	1	3	0	0	6	4	8	0.64	1
PPP1R7	1.86	0	0	0	1	0	0	1	1	1	1	1
QSER1	1.34	0	1	1	0	0	0	2	8	1	1	0.38
RASAL2	1.40	0	1	1	2	1	0	5	25	13	1	0.94
RBM5	4.17	1	2	2	0	1	0	6	0	2	0.009	1
RPA1	1.22	1	0	0	1	0	0	2	3	3	1	1
SDF4	0.53	1	0	0	0	0	1	2	1	1	1	1
SOGA1	2.27	1	0	1	1	0	0	3	15	5	1	0.47
STARD10	1.34	1	0	2	0	0	0	3	4	5	1	1
TENM2	3.30	1	0	2	2	0	2	7	16	16	1	1
ZFHX4	1.01	0	0	3	3	0	0	6	14	8	1	1

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400 The genes included in this analysis were among the strongest candidate genes affected by a DNM (either missense or LoF mutation). The missense Z-score is included here to indicate a
401 relative (in)tolerance to missense mutation[22]. For the original NIJ/NCL discovery cohort, only the missense DNMs are included in this Table (7 of these genes were affected by a LoF DNM). A
402 burden test was done to compare the total number of predicted pathogenic missense mutations observed in the infertile vs. fertile men, as well as between fertile men and fertile women
403 (Fisher's Exact test, adjusted for multiple testing following Bonferroni correction).

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