Supplemental Data

Analysis of copy number variation in men with non-obstructive azoospermia

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Study cohort

We included data from our previous study[1] that was analysed with the same laboratory workup and a comparable resolution of the arrays.

Control men derived from Tüttelmann *et al*., 2011[1]:

M370, M385, M419, M420, M422, M424, M443, M447, M449, M484, M485, M492, M494, M495, M590, M599, M622, M670, M671, M672, M676

Men with Sertoli cell-only phenotype derived from Tüttelmann *et al*., 2011[1]:

M12, M15, M30, M94, M126, M159, M173, M177, M185, M187, M188, M226, M245, M248, M263, M267, M291, M301, M310, M319, M324, M352, M354, M364, M371, M374, M396, M432, M433, M464, M490, M499, M550, M567, M578, M609, M666

SNP-array

SNP-array was conducted to confirm deletions which were primarily detected via CNV calling from exome sequencing data. The Infinium CytoSNP-850k-Bead Array (Illumina, San Diego, California, USA) with a median probe spacing of 1.8 kb was used. 200 ng of DNA from each proband was amplified and fragmented. Next, DNA fragments were hybridized against oligonucleotides on the BeadChip (Illumina, San Diego, California, USA), distributed over the whole human genome. Subsequently single-base extension was carried out and staining of DNA was performed. All steps were carried out according to the manusfacturer's protocol. Scanning of the BeadChips was done with a NextSeq 550 (Illumina, San Diego, California, USA). The BlueFuse Multi software (Illumina, San Diego, California, USA) was used for analyses. Signals larger than 10 adjacent probes were considered to be a genomic CNV.

Infertility-associated genes (Reviewed in Houston *et al.*, 2021[2])

ABCA1, ADCY10, ADGRG2, AK7, AKAP4, ALX4, AMH, AMHR2, ANOS1, APOA1, AR, ARL2BP, ARMC2, AURKC, BCORL1, BMP4, BMP7, BNC2, BRAF, BRDT, BSCL2, C2CD6, C7orf61, CATIP, CATSPER1, CATSPER2, CATSPERE, CCDC103, CCDC141, CCDC39, CCDC40, CCDC62, CCDC9, CCIN, CDC14A, CEP112, CEP135, CEP290, CEP78, CFAP251, CFAP300, CFAP43, CFAP44, CFAP65, CFAP69, CFAP70, CFAP91, CFTR, CHD7, CLDN2, CYP11A1, CYP11B1, CYP17A1, CYP19A1, CYP21A2, DCC, DHH, DHX37, DMC1, DMRT1, DNAAF2, DNAAF4, DNAAF5, DNAAF6, DNAH1, DNAH11, DNAH17, DNAH2, DNAH6, DNAH9, DNAI1, DNAI2, DNAJB13, DNMT1, DPY19L2, DRC1, DUSP6, DZIP1, E2F1, EIF4G1, ERBB4, ESR1, FAM47C, FANCA, FANCM, FBXO43, FEZF1, FGF17, FGF8, FGFR1,

FLNA, FSHB, FSHR, FSIP2, GALNTL5, GAS8, GATA4, GFPT2, GGN, GH1, GLI3, GNRH1, GNRHR, H2BW1, HAUS7, HESX1, HOXD13, HS6ST1, HSD17B3, HSD3B2, HSF2, HYDIN, IFT140, IGSF10, IL17RD, INSL3, KASH5, KDM3A, KISS1R, KLHL10, LHB, LHCGR, LHX3, LRRC6, M1AP, MAGEB4, MAMLD1, MAP2K2, MAP3K1, MAP7D3, MC4R, MCM9, MEI1, MEIOB, MLH3, MNS1, MTOR, MYRF, NANOS1, NANOS2, NDNF, NLRP3, NNT, NOS1, NOTCH1, NR0B1, NR5A1, NRAS, NSMF, OFD1, PANK2, PDHA2, PIWIL2, PKD1, PLCZ1, PLK4, PLXNA1, PMFBP1, POU1F1, PPP2R3C, PROK2, PROP1, PSMC3IP, QRICH2, RAF1, RBMXL2, RELN, RNF212, RNF220, RPL10L, RSPH1, RSPH3, RSPH9, RSPO1, RXFP2, SCAPER, SECISBP2, SEMA3A, SEPTIN12, SLC26A3, SOS1, SOX10, SOX2, SOX3, SOX8, SOX9, SPAG17, SPAG6, SPATA16, SPEF2, SPINK2, SPO11, SRA1, SRD5A2, SRY, STAG3, STAR, STK36, STX2, SUN5, SYCE1, SYCP2, SYCP3, TAC3, TACR3, TAF4B, TDRD6, TDRD7, TDRD9, TEX11, TEX14, TEX15, TRIM37, TSGA10, TTC12, TTC21A, TTC29, TTLL5, UBE2B, UBR2, USP26, VAMP7, WDR11, WDR19, WNK3, WT1, XRCC2, ZFX, ZMYND15, ZPBP

Table S1: Descriptive statistics of clinical data of all 252 men analysed by aCGH.

	All (252)	MeiA (37)	SCO (194)	Controls (21)
Age [y]	33 (26 - 43)	33 (23 - 44)	33 (26 - 42)	35 (28 - 49)
Bi-testicular volume [mL]	23 (9 - 54)	18 (9 - 53)	22 (9 - 43)	52 (23 - 94)
Serum FSH [U/L]	19.2 (2.6 - 39.7)	19.4 (2.0 – 36.0)	20.6 (6.6 - 41.4)	3.1 (1.4 - 9.3)
Serum LH [U/L]	6.4 (2.4 - 13.4)	7 (2.1 - 12.6)	6.7 (2.6 - 14.2)	3.1 (1.6 - 5.8)
Serum testosterone [nmol/L]	13.6 (6.3 - 27)	13.1 (7.7 - 29.9)	13.2 (5.9 - 27)	15.9 (8.6 - 37.7)
Origin	198 men of European origin	29 men of European origin	152 men of European origin	20 men of European origin

Data shown are the median values with the respective 95% confidence intervals.

Abbreviations: y: years, d: days, FSH: follicle stimulating hormone, LH: luteinising hormone, TESE: testicular sperm extraction. Data were not normally distributed and compared between the full cohort and subgroups by Kruskal-Wallis test with Dunn's post-hoc multiple comparison. Grey shaded parameters differed significantly comparing the control group with the other groups. Origin was determined based on self-reporting. All parameters were obtained at their first visit at the Centre of Reproductive Medicine and Andrology.

Reference values: Bi-testicular volume >24 mL, semen volume: 1.5 mL, semen pH: 7.2-8.0, semen fructose >13 µmol/sample, serum FSH 1-7 U/L, serum LH 2-10 U/L, serum testosterone >12 nmol/L.

Table S2. Primersequences used for qPCR validation of deletions of interest.

Genomic region	Located genes	Primer sequence (5' -> 3')
10q26.3	*SYCE1, CYP2E1, SCART1*	AGAGACACATGGTGCCATCT CTGGCTGCCTCTCTTCAACA
10q26.3	*SYCE1, CYP2E1, SCART1*	CATGGTGGCGGTAGTTGTCT AGGACAACCCTGAAGCTTCA
14q24.3	*MLH3, EIF2B2*	CCCTAGAGAGCCAAGGTCGA TCACTTGGACCGCAATGACA
14q24.3	*MLH3, EIF2B2*	TGTAAATCTGCTCCTAAGATTACAACA AGGCATGGCAGACTAGAGTA
16p13.3	*SLX4, CLUAP1, NLRC3*	GCAGTCCAGTTCACCTTGGA CCCAGGTCACCAGAGTTTCC
16p13.3	*SLX4, CLUAP1, NLRC3*	GGAGGGGAATGGGATGTGAC AGACTTGTCCAACCACCACC
16p13.13	*TEKT5*	TGCCCATGCACTTCTCCTTG TTTGTCACCTGGGCTGGAG
16p13.13	*TEKT5*	CCATGGTAATGAGAACTGCAGTGAAAGCAGGGCTATGTCTGGG
19p13.3	*CLPP*	CCTGAATCTTGGCGAGGGTT CTCAAGTTCTCCGGTCTGGG
19p13.3	*CLPP*	ACTTTTAATTTGCAGGGGTGCC GTGCAGAAGGGAGGGTGTC

Table S3. Infertility-associated genes (reported recently in independent studies)

ADAD2	Krausz *et al*., 2020[3]
GCNA	Hardy *et al*., 2021[4]
MAJIN	Salas-Huetos *et al*., 2021[5]
M1AP	Wyrwoll *et al*., 2020[6]
MSH4	Krausz *et al*., 2020[3]
MSH5	Wyrwoll *et al*., 2021[7]
RAD21L1	Krausz *et al*., 2020[3]
RNF212	Riera-Escamilla *et al*., 2019[8]
SHOC1	Krausz *et al*., 2020[3]
STAG3	Riera-Escamilla *et al*., 2019; van der Bijl *et al*. 2019[8,9]
SYCP2	Schilit *et al*., 2019[10]
TERB1	Krausz *et al*., 2020; Salas-Huetos *et al.*, 2021[3,5]
TERB2	Salas-Huetos *et al.,* 2021[5]
TRIM71	Torres-Fernández *et al.,* 2021[11]

Table S4: Results of literature search for candidate genes.

Gene	Expression acc. to Gtex[12]	Described in context of male infertility	Described in context of female infertility	Interaction with a protein involved in spermatogenesis acc. to String database[13]	Animal model matching human disease
SYCE1	Highest expression in testis	Pashaei *et al.*, 2020[14], Maor-Sagie *et al.*, 2015[15], Krausz *et al.*, 2020[3]	Hernández-López *et al.*, 2020[16]	*REC8, SMC1B, SYCP2*	Bolcun-Filas *et al.*, 2007&2009[17,18]
TEKT5	Only expressed in testis	No	No	No	Cao *et al.*, 2011[19], Aoki and Matsui, 2019[20]
MLH3	High expression in any tissue	Xu *et al.*, 2010[21], Markandona *et al.*, 2015[22], Zhang *et al.*, 2015, Chen *et al.*, 2020[23], Zhao *et al.*, 2019[25], Ji *et al.*, 2012[26], Ferrás *et al.*, 2007[27]	Pashaiefar *et al.*, 2013[28]	*MLH1, MSH4, BRCA2*	Lipkin *et al.*, 2002[29]
SLX4	Highest expression in testis and brain	No	No	*FANCM*	Holloway *et al.*, 2011[30]
CLPP	High expression in any tissue	Demain *et al.*, 2017[31]	Tiosano *et al.*, 2019[32]	No	Gispert *et al.*, 2013[33]

Table S5. Genetic and clinical data of azoospermic individuals carrying deletions in the prioritized genes.

Indi-vidual	Age, origin	Gene symbols; prioritized genes in bold	Fertility parameters	Gonadal phenotype, TESE outcome
M1369	30 y, Syria	***SYCE1***, *CYP2E1, SCART1*	FSH: 4.2 LH: 5.3 T: 11.5 TV: 17/21 Azoospermia	Meiotic arrest, No sperm retrieved
M3187	32 y, Yemen	***SYCE1,*** *CYP2E1, SCART1*	FSH: NA LH: NA T: NA TV: 12/12 Azoospermia	Meiotic arrest, No sperm retrieved
M2681	37 y, Germany	***SYCE1,*** *CYP2E1, SCART1*	FSH: NA LH: NA T: NA TV: NA Azoospermia	SCO, No sperm retrieved
M663	33 y, Germany	***SYCE1***, *CYP2E1, SCART1*	FSH: 21.1 LH: 10.4 T: 13.8 TV: 13/10 Azoospermia	SCO, No sperm retrieved
M921	41 y, Iraq	***SYCE1***, *CYP2E1, SCART1*	FSH: 9.8 LH: 12.5 T: 9.0 TV: 3/3 Azoospermia	Meiotic Arrest, No sperm retrieved
M226	36 y, Syria	***MLH3***, *EIF2B2*	FSH: 28.4 LH: 7.5 T: 12.7 TV: 6/7 Azoospermia	SCO, No sperm retrieved
M1635	27 y, Germany	***SLX4***, *CLUAP1, NLRC3*	FSH: 19.0 LH: 6.2 T: 16.3 TV: 11/10 Azoospermia	SCO, No sperm retrieved
M877	31 y, Poland	***TEKT5***	FSH: 21.2 LH: 8.1 T: 23.2 TV: 17/20 Azoospermia	SCO, No sperm retrieved

M1281	31 y, Cosovo	***CLPP***	FSH: 23.8 LH: 19.0 T: 6.3 TV: 2/2 Azoospermia	SCO, No sperm retrieved

Abbreviations: FSH: follicle stimulating hormone (IU/L), LH: luteinizing hormone (IU/L), T: testosterone (nmol/L), TV: testicular volume right/left (mL), y: years, SCO: Sertoli cell-only phenotype, NA: not available. Reference values: FSH 1-7 IU/L, LH 2-10 IU/L, T >12 nmol/L, TV >12 mL per testis.

Table S6. Deletions detected in individuals M921, M1369, M663, M226, M1635, M877, M1281, M2681 and M3187 based on the exome sequencing data.

Individual	Chromosome	CNV Start	CNV End	CNV Type	Gene Name	Transcript	Zygosity
M921	10	135267485	135381888	DEL	*SYCE1*	NM_001143764	heterozygous
M3187	10	135267252	135379283	DEL	*SYCE1*	NM_001143764	homozygous
M2681	10	135267252	135379283	DEL	*SYCE1*	NM_001143764	heterozygous
M1369	10	135267485	135381888	DEL	*SYCE1*	NM_001143764	homozygous
M663	10	135340900	135379033	DEL	*SYCE1*	NM_001143764	heterozygous
M226	14	75469978	75489721	DEL	*MLH3*	NM_001040108	heterozygous
M1635	16	3586122	3658965	DEL	*SLX4*	NM_032444	heterozygous
M877	16	10721440	10788730	DEL	*TEKT5*	NM_144674	heterozygous
M1281	19	6333470	6374387	DEL	*CLPP*	NM_006012	heterozygous

Table S7. Genetic and clinical data of infertile men carrying two single nucleotide variants in the genes *TEKT5* and *SLX4*.

Indi-vidual	Age, origin	Genotype	Fertility parameters	Gonadal phenotype, TESE outcome
M1767	38 y, Iraq	*TEKT5*: c.[263G>A(;)1022C>T] p.(Arg88His)(;)(Ala341Val)	FSH: 36.5 LH: 5.6 T: 9.6 TV: 6/5 Azoospermia	Round spermatid arrest, No sperm retrieved
PIT10	21 y, European	*TEKT5:* c.[531G>C(;)938C>G] p.(Glu177Asp)(;)(Ser313Cys)	FSH: NA LH: NA T: NA TV: NA Azoospermia	Early meiotic arrest, No sperm retrieved
M1626	25 y, Serbia	*SLX4*: c.[2359G>A(;)5468G>A] p.(Glu787Lys)(;)(Arg1823Gln)	FSH: 2.2 LH: 2.7 T: 18.7 TV: 28/25 Azoospermia	NA
M2023	32 y, Syria	*SLX4*: c.[2359G>A];[2359G>A] p.[Glu787Lys];[Glu787Lys]	FSH: 10.9 LH: 6.2 T: 12.2 TV: 10/7 Azoospermia	NA

Abbreviations: FSH: follicle stimulating hormone (IU/L), LH: luteinizing hormone (IU/L), NA: not available/applicable, T: testosterone (nmol/L), TV: testicular volume right/left (mL), y: years, TESE: testicular sperm extraction, SCO: Sertoli cell-only phenotype.

Reference values: FSH 1-7 IU/L, LH 2-10 IU/L, T >12 nmol/L, TV >12 mL per testis.

Table S8: ACMG-AMP criteria for *TEKT5* and *SLX*4 variants (excel file).

Figure S1: SNP-array results demontrating deletions affecting *SYCE1*.

M2681 is affected by a heterozygous deletion (**A**) as well as a missense variant in *SYCE1* on the second allele. M3187 is affected by a homozygous deletion covering *SYCE1* (**B**).

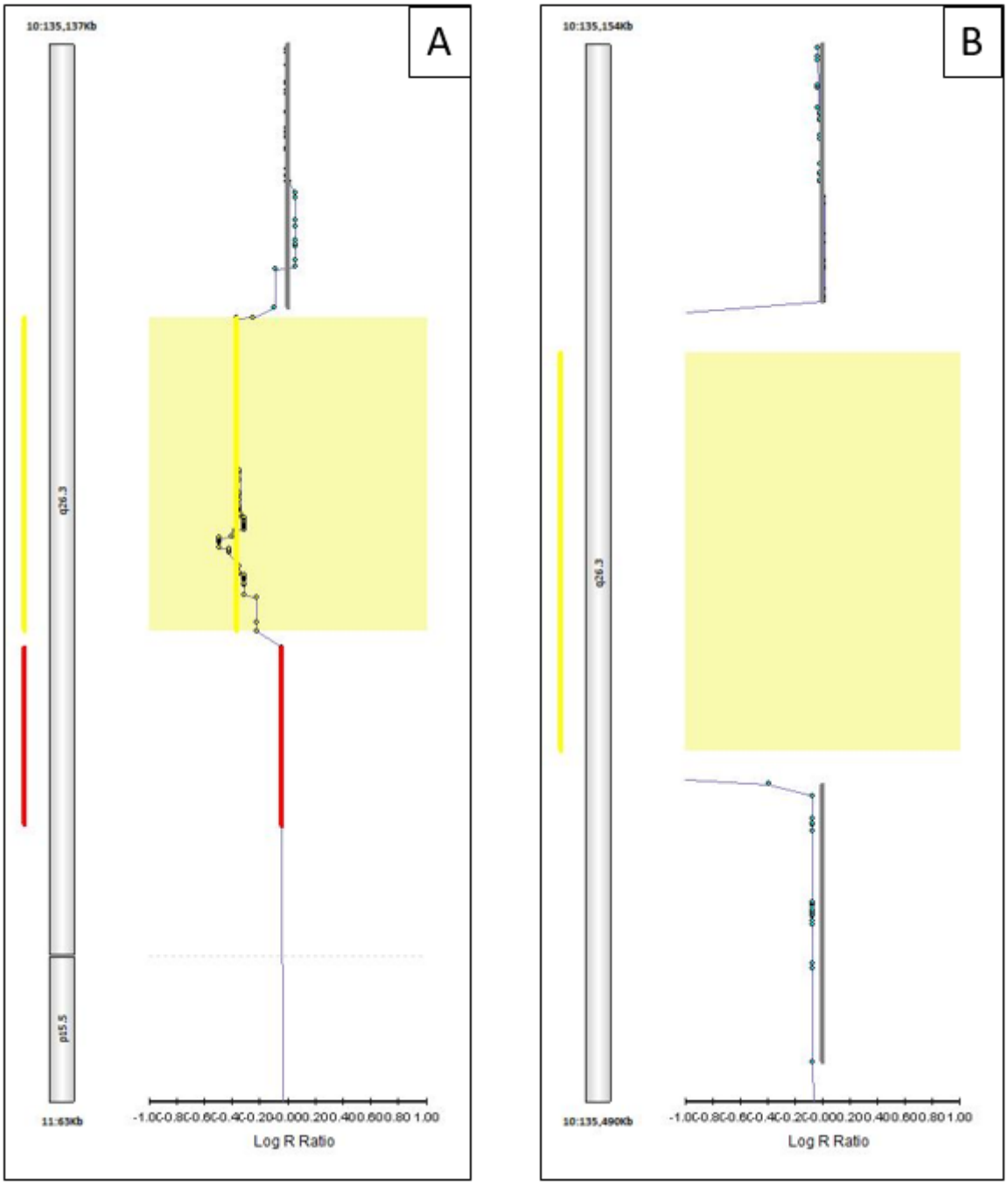


Figure S2: Electropherograms of the *TEKT5* variants in M1767 and his mother.

M1767 was identfied with two heterozygous *TEKT5* variants: c.263G>A and c.1022C>T. The mother carries only the variant c.263G>A while the other variant was not present, suggesting compound-heterozygosity of both variants in M1767. DNA from the father of M1767 was not available.

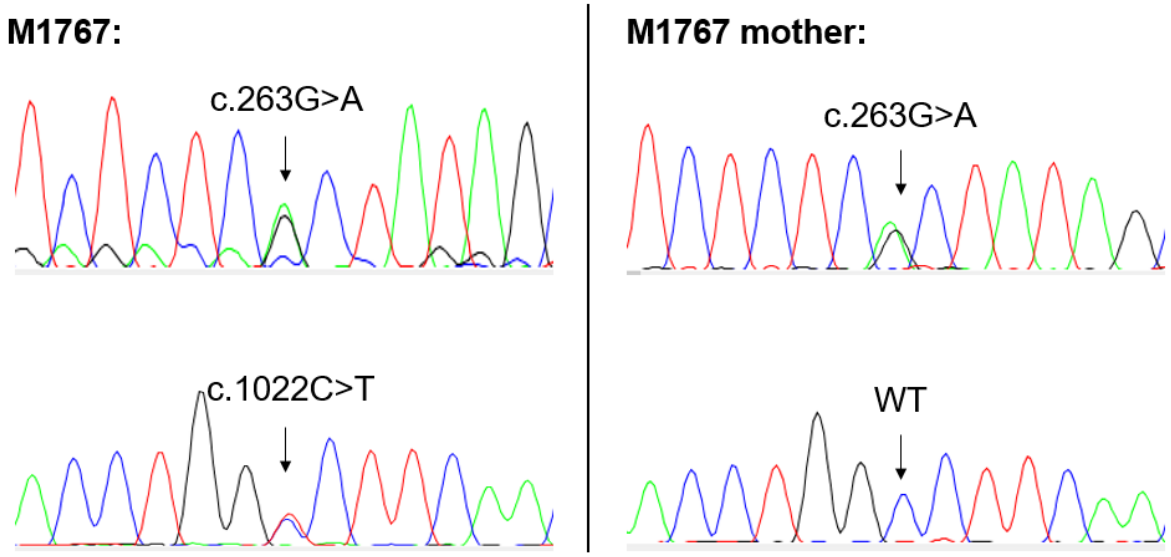


Figure S3: Prevalence of all CNVs, deletions and duplications.

The prevalence of all CNVs as well as deletions and duplications detected in 21 men from the control group was compared to the prevalence of those detected in 37 men with MeiA and 194 men with SCO. There were significantly more CNVs detected in the control group compared to men with MeiA. All other statistical tests did not reveal significant differences.

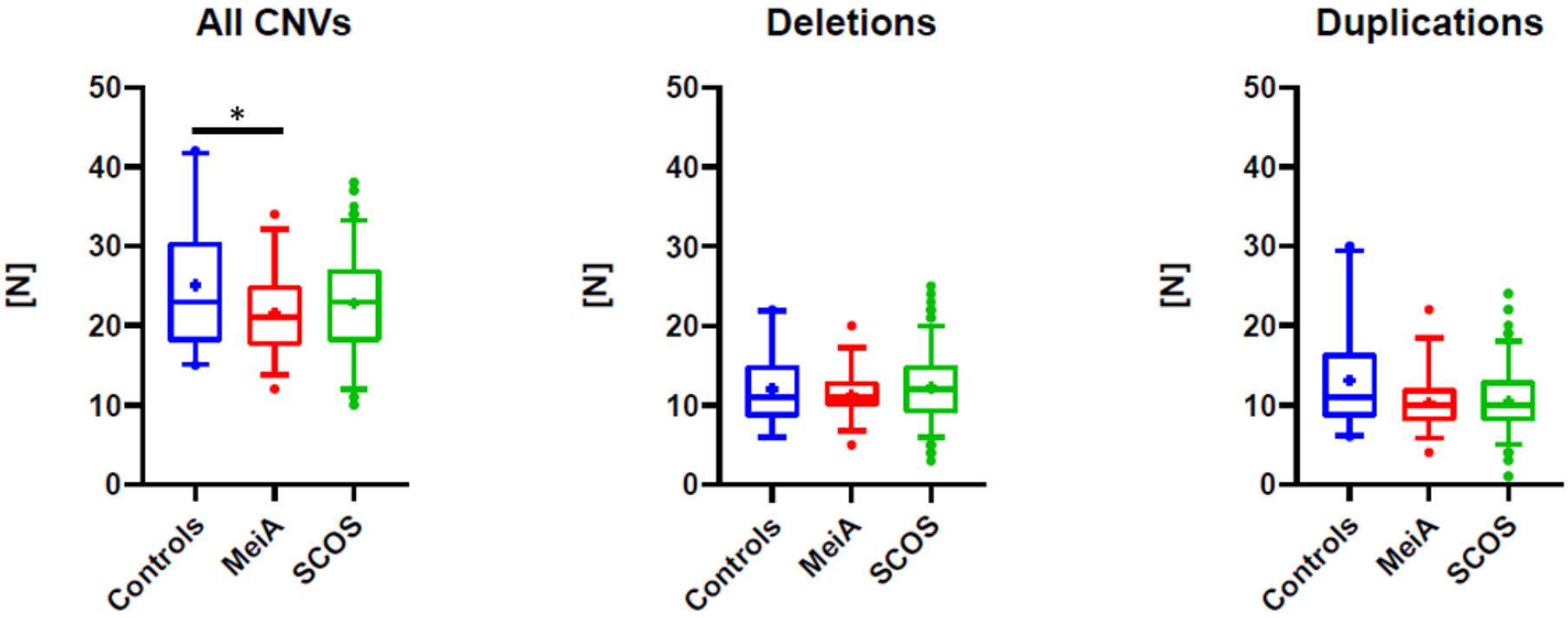


Figure S4: Chromosomal distribution of deletions and duplications.

The distribution of duplications and deletions was calculated per chromosome and normalized to 100 men for control men, men with MeiA and men with SCO. Positive values indicate duplications, while negative values indicate deletions.

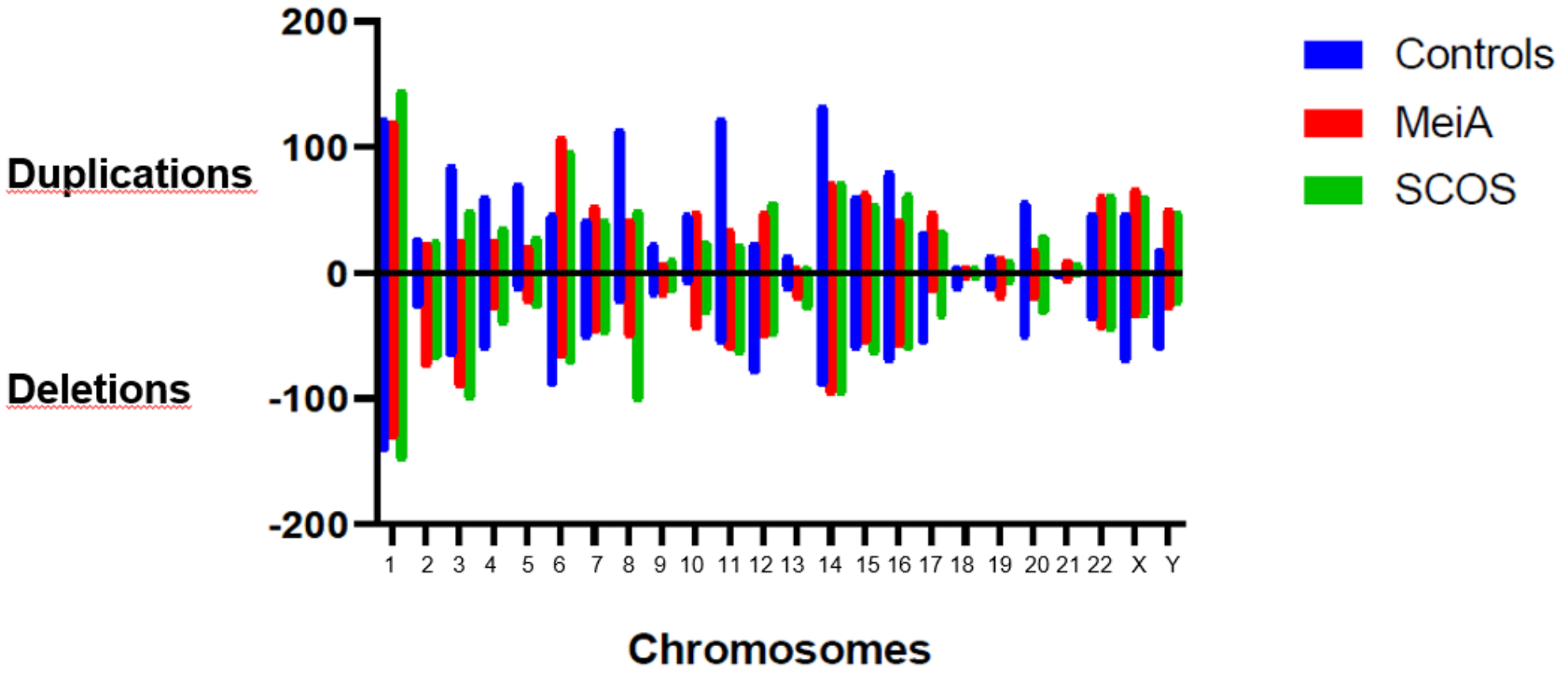
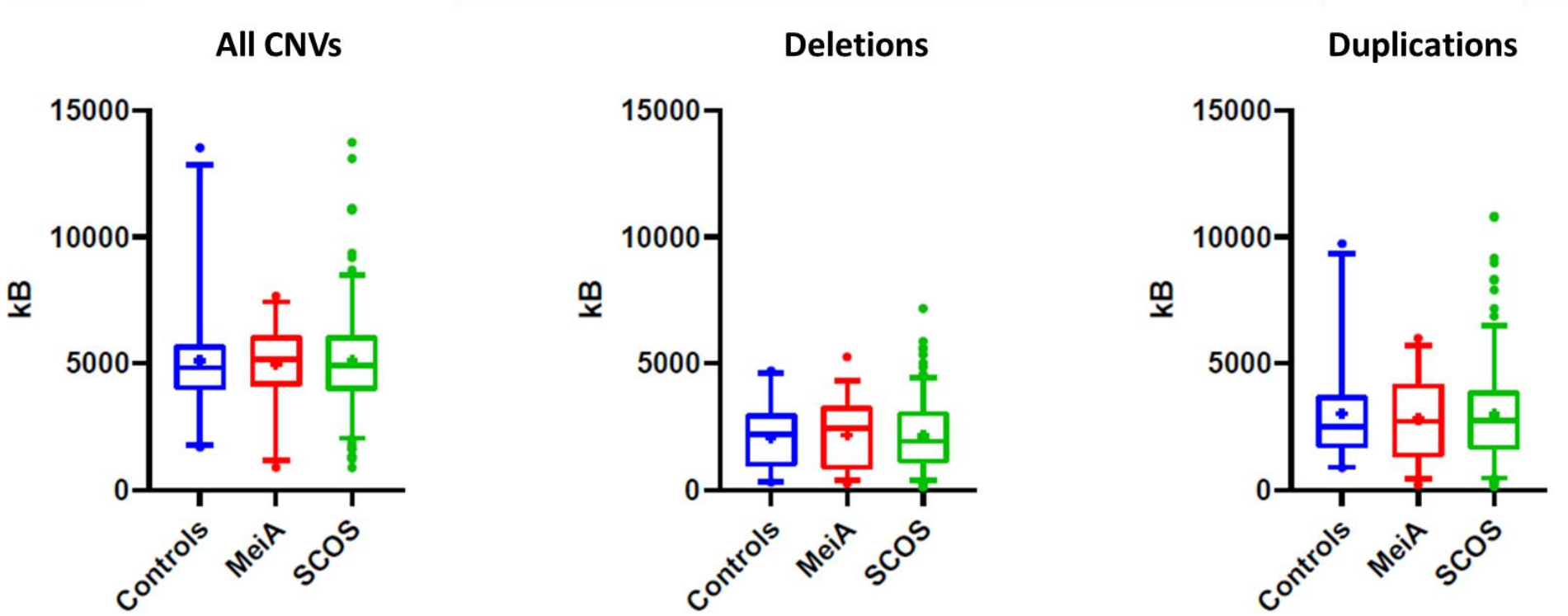


Figure S5: Size in kilo base pairs of CNVs, deletions and duplications.

The size of all CNVs as well as deletions and duplications detected in 21 men from the control group was compared to the size of those detected in 37 men with MeiA and 194 men with SCO. There were no significant differences regarding the size of all CNVs, deletions or duplications between control men, men with MeiA and men with SCO.



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