**Supplementary Data**

**Table e-1:** *NR4A2* mutations

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Nucleotide Change | Amino Acid Change | Type of mutation | Clinical picture | References |
| c.-469delG | - | Small deletion | Schizophrenia | (1) |
| c.-309C>T | - | Regulatory | Parkinson disease | (2) |
| c.-291delT | - | Small deletion | Parkinson disease | (3) |
| c.289A>G | p.M97V | Missense | Schizophrenia | (4) |
| c.308A>G | p.H103R | Missense | Bipolar disorder | (4) |
| c.326dupA | p.S110Vfs\*2 | Small insertion | a Epilepsy, language impairment & intellectual deficiencyb Mild intellectual disability & dystonia-parkinsonism | a (5)b (6) |
| c.364\_366delTAC | p.Y122del | Small deletion | Schizophrenia | (4) |
| c.374C>G | p.S125C | Missense | Parkinson disease | (7) |
| c.881dupA | p.N294Kfs\*10 | Small insertion | Mild intellectual disability & dystonia-parkinsonism | (6) |
| c.920T>G | p.V307G | Missense | Pediatric, psychomotor retardation | (8) |
| c.956G>A | p.R319Q | Missense | Mild intellectual disability, dystonia-parkinsonism & motor tics | Present paper |
| Nucleotide Alteration | Deletion size | Type of mutation | Clinical picture | References |
| - | ~89 kb | Gross deletion | Neurodevelopmental disorder with language impairment | (9) |
| c.-27827\_\*87408del121845 | 122 kb | Gross deletion | Neurodevelopmental disorder including language impairment, developmental delay, intellectual disability and/or autism spectrum disorder | (10) |
| c.-126883\_\*40976del174469 | 174 kb including exon 1 of *GPD2* | Gross deletion | Neurodevelopmental disorder including language impairment, developmental delay, intellectual disability and/or autism spectrum disorder | (10) |
| c.-27827\_\*40976del75413 | 75 kb | Gross deletion | Neurodevelopmental disorder including language impairment, developmental delay, intellectual disability and/or autism spectrum disorder | (10) |

**Table e-2:** Ancillary tests developed during the diagnosis work-out.

|  |  |
| --- | --- |
| Laboratory test | Result |
| Plasma copper and ceruloplasmin 24 hours urine copper Urine organic acidsHemogram Tiroid profileLipids aProteinogramB12 and D vitamins aFolic acid aIron profileBlood smearNH4Chitotriosidasi enzime activity a | NormalNormalNormalNormalNormalTriglycerides 240 mg/dl (0-170)Normal265 pg/ml (211-946), 66.5 nmol/l (50-250)6.5 ng/ml (2.9-16.9)NormalNormal, no acantocytosNormal40.8 mmol/ml/h (4-76) |

a The normal ranges according to the local laboratory are listed in parentheses.

**Table e-3:** Novel candidate causative genes detected in heterozygosis in the proband by whole exome sequencing

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Gene | \*MIM | Position | NucleotideChange | Amino AcidChange | Type ofMutation | Clinical Picture [Reference or MIM#] |
| *NR4A2* | 601828 | 2:157184954 | c.956G>A | p.R319Q | missense | Schizophrenia, bipolar disorder (1, 4)Parkinson Disease (2, 3, 7)Epilepsy, language impairment and intellectual deficiency (5)Mild intellectual disability & dystonia-parkinsonism (6)Neurodevelopmental disorder with language impairment (9)Autism spectrum disorder & intellectual disability (10)Pediatric, psychomotor retardation (8) |
| *CEP170* | 613023 | 1:243328887 | c.2375C>A | p.S792\* | stop gained | Schizophrenia (11)Chiari malformation (12)Intellectual disability, gross motor delay, seizures, scoliosis, hearing & sight issues (13) |
| *KCNQ2* | 602235 | 20:62044939 | c.1807-5A>T | --- | splicing variant | Epileptic encephalopathy [MIM# 613720]Myokimia, seizures [MIM# 121200] |

**Table e-4:** Twenty rare variants detected in the proband and inherited in an autosomal dominant fashion from the patient’s father or mother (shaded files).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Genotype | Chr:position | NucleotideChange | Amino AcidProtein | Gene | dbSNP | Allele Count/ Allele Number(gnomAD)a |
| ProbandNBIA-277 | FatherNBIA-387 | MotherNBIA-388 |
| 0/1 | 0/1 | 0/0 | 8:39646218 | c.612A>C | p.K204N | *ADAM2* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 13:39266417 | c.4936A>T | p.I1646F | *FREM2* | rs765560306 | 1/251068 |
| 0/1 | 0/1 | 0/0 | 17:66890387 | c.2843T>C | p.I948T | *ABCA8* | rs201434277 | 3/250908 |
| 0/1 | 0/1 | 0/0 | 18:8783996 | c.886G>A | p.E296K | *SOGA2* | rs1193304665 | 2/250386 |
| 0/1 | 0/1 | 0/0 | 7:25218815 | c.113G>A | p.R38H | *C7orf31* | rs531648664 | 8/279760 |
| 0/1 | 0/1 | 0/0 | 12:53170945 | c.131G>A | p.C44Y | *KRT76* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 19:41762491 | c.2171G>A | p.R724H | *AXL* | rs1441087022 | 1/251468 |
| 0/1 | 0/1 | 0/0 | 14:57938225 | c.739C>T | p.L247F | *C14orf105* | rs1374191015 | 1/251250 |
| 0/1 | 0/1 | 0/0 | 1:227333410 | c.923C>T | p.T308I | *CDC42BPA* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 15:40259830 | c.1303G>A | p.A435T | *EIF2AK4* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 4:100349278 | c.373C>A | p.R125S | *ADH7* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 2:74044007 | c.2657C>T | p.A886V | *C2orf78* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 7:105658301 | c.1436G>T | p.R479L | *CDHR3* | rs1302095045 | 3/228838 |
| 0/1 | 0/1 | 0/0 | 17:9497556 | c.454C>T | p.P152S | *WDR16* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 8:105360764 | c.-12-5T>C | NA | *DCSTAMP* | [rs1228062399](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=rs1228062399" \t "_blank) | 2/232824 |
| 0/1 | 0/1 | 0/0 | 22:20709738 | c.1470C>A | p.V490V | *FAM230A* | [rs1394317999](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=rs1394317999" \t "_blank) | 1/29046 |
| 0/1 | 0/1 | 0/0 | 19:35608266 | c.57G>A | p.L19L | *FXYD3* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 4:106156084 | C.985T>C | p.F329L | *TET2* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 11:4936814 | c.80G>C | p.R27P | *OR51G2* | NA | Novel |
| 0/1 | 0/1 | 0/0 | 20:1471949 | c.57G>C | p.L19L | *SIRPB2* | NA | Novel |
| 0/1 | 0/0 | 0/1 | 2:242573083 | c.489C>T | p.S163S | *THAP4* | NA | Novel |
| 0/1 | 0/0 | 0/1 | 19:1054061 | c.3529C>G | p.R1177G | *ABCA7* | rs141885771 | 1/250896 |
| 0/1 | 0/0 | 0/1 | 15:83499561 | c.1852G>A | p.V618I | *WHAMM* | rs1460546047 | 1/242040 |
| 0/1 | 0/0 | 0/1 | 20:60884471 | c.11009T>C | p.V3670A | *LAMA5* | rs368953531 | 2/202630 |
| 0/1 | 0/0 | 0/1 | 19:14262418 | c.3692T>C | p.L1231S | *LPHN1* | rs1296491013 | 1/231548 |
| 0/1 | 0/0 | 0/1 | 9:116279851 | c.1705C>T | p.L569F | *RGS3* | NA | Novel |
| 0/1 | 0/0 | 0/1 | 19:9072490 | c.14956C>A | p.Q4986K | *MUC16* | rs1377856289 | 3/248620 |
| 0/1 | 0/0 | 0/1 | 11:47354779 | c.3296G>A | p.G1099E | *MYBPC3* | rs1168771733 | 1/214272 |
| 0/1 | 0/0 | 0/1 | 7:144094488 | c.1921C>G | p.P641A | *NOBOX* | rs1458557917 | 2/172252 |
| 0/1 | 0/0 | 0/1 | 1:110019519 | c.376T>A | p.F126I | *SYPL2* | rs1359589422 | 1/249588 |
| 0/1 | 0/0 | 0/1 | 11:61727479 | c.884G>C | p.R295P | *BEST1* | NA | Novel |
| 0/1 | 0/0 | 0/1 | 3:58849553 | c.949C>A | p.L317I | *C3orf67* | NA | Novel |
| 0/1 | 0/0 | 0/1 | 13:111286942 | c.546G>A | p.A182A | *CARKD* | rs768098549 | 2/282826 |
| 0/1 | 0/0 | 0/1 | 14:105349779 | c.985G>A | p.V329I | *CEP170B* | NA | Novel |
| 0/1 | 0/0 | 0/1 | 11:105795230 | c.1582A>C | p.K528Q | *GRIA4* | NA | Novel |
| 0/1 | 0/0 | 0/1 | 10:20534350 | c.1389G>T | p.L463L | *PLXDC2* | NA | Novel |
| 0/1 | 0/0 | 0/1 | 8:125499130 | c.1240T>C | p.Y414H | *RNF139* | [rs1443907767](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=rs1443907767" \t "_blank) | 3/251342 |
| 0/1 | 0/0 | 0/1 | 2:55071240 | c.904G>T | p.D302Y | *EML6* | [rs1456438351](https://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=rs1456438351" \t "_blank) | 1/156552 |
| 0/1 | 0/0 | 0/1 | 1:6639323 | c.2205T>C | p.N735N | *TAS1R1* | NA | Novel |
| 0/1 | 0/0 | 0/1 | 22:42418293 | c.447C>T | p.F149F | *WBP2NL* | NA | Novel |

adbSNP and gnomAD: last accessed March 3, 2020.

**Figure e-1.** Non-Motor Symptoms Scale for Parkinson’s Disease,

Non-Motor Symptoms Scale for Parkinson’s Disease, with a major affectation on gastro-intestinal (13/36) and sleep/fatigue (12/48) domains.

**Figure e-2**. The 39-item Parkinson's Disease Questionnaire (PDQ-39).

The score was 87/156 with maximum score for mobility (40/40) and communication (12/12) domains. Abbreviation: ADL: activities of daily living.

**Figure e-3:** Family’s pedigree.



The proband (marked with an arrow) is the only carrier of the novel *NR4A2* c.956G>A (p.R319Q) mutation in heterozygosis.

**Figure e-4:** Distribution of reported disease-causing mutations in *NR4A2*.

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The p.R319Q mutation (in red) detected in the proband is located in the Zf-C4/DBD (DNA binding domain). Changes in bold are associated with the dystonia-parkinsonism phenotype. Missense variants are marked with a green circle; stop mutations are marked with a black circle; and deletions are marked with a brown circle.

**Supplementary material:**

**Genetic analysis**

The proband was analyzed using a custom gene panel MovDisord-498 and finally, whole exome sequencing (WES) of the proband and healthy parents (trio). Filtering data from the gene panel MovDisord-498, previously reported (14, correa Vela) and from WES, as well as the analysis of the novelty of the candidate variants was carried out as previously reported (14). 15 Conservation of the residues was investigated using Clustal Omega tool. Sanger sequencing on an ABI Prism 3130XL analyzer (Applied Biosystems, Foster City, CA, USA) was performed for validation and segregation analysis.

Additionally, we confirmed maternity and paternity by the segregation analysis of 40 selected rare variants distributed throughout the genome from WES data (Supplementary Table 4).

**List of genes included in the gene panel MovDisord-498.**

*AARS2,ABCB7,ABCD1,ABHD12,ACAT1,ACO2,ACTB,ADAR,ADCK3,ADCY5,AFG3L2,AHI1,AIFM1,AIMP1,ALDH18A1,ALDH3A2,ALG6,ALS2,AMACR,AMPD2,AMT,ANO10,ANO3,AP4B1,AP4E1,AP4M1,AP4S1,AP5Z1,APTX,ARG1,ARL13B,ARL6IP1,ARSA,ARSI,ARX,ASPA,ASS1,ATCAY,ATL1,ATM,ATN1,ATP13A2,ATP1A2,ATP1A3,ATP2B3,ATP6AP2,ATP7A,ATP7B,ATP8A2,ATR,ATXN1,ATXN10,ATXN2,ATXN3,ATXN7,B4GALNT1,BCAP31,BCKDHA,BCKDHB,BCS1L,BEAN1,BICD2,BSCL2,BTD,C10ORF2,C12ORF65,C19ORF12,C5ORF42,C9ORF72,CA8,CACNA1A,CACNA1B,CACNA1G,CACNB4,CAMTA1,CASK,CC2D2A,CCDC88C,CCT5,CEP290,CEP41,CHCHD2,CHMP1A,CIZ1,CLCN2,CLN5,CLN6,CLP1,COASY,COL18A1,COL6A3,COQ2,COQ4,COQ7,COQ9,COX10,COX15,COX20,COX8A,CP,CPT1C,CSTB,CTDP1,CTSD,CUL4B,CWF19L1,CYP27A1,CYP2U1,CYP7B1,DARS2,DBT,DCAF17,DCLRE1B,DCTN1,DDB2,DDC,DDHD1,DDHD2,DKC1,DLAT,DLD,DNAJC13,DNAJC19,DNAJC6,DNMT1,DYRK1A,EARS2,ECHS1,EEF2,EIF2B1,EIF2B2,EIF2B3,EIF2B4,EIF2B5,EIF4G1,ELOVL4,ELOVL5,EMC1,ENTPD1,EPM2A,ERCC2,ERCC3,ERCC5,ERCC6,ERCC8,ERLIN1,ERLIN2,ETFA,ETFB,ETFDH,ETHE1,EXOSC3,EXOSC8,FA2H,FAAH2,FAM126A,FAM134B,FARS2,FBXL4,FBXO7,FGF14,FLRT1,FLVCR1,FMR1,FOLR1,FOXC1,FOXG1,FOXRED1,FTH1,FTL,FUCA1,FUS,FXN,GAD1,GALC,GAMT,GAN,GATM,GBA,GBA2,GCDH,GCH1,GFAP,GFM1,GFM2,GJB1,GJC2,GLB1,GLDC,GLRX5,GNAL,GNAO1,GOSR2,GPR56,GRID2,GRM1,GTPBP2,GTPBP3,HACE1,HEXA,HEXB,HIBCH,HPCA,HPRT1,HSD17B4,HSPD1,HTRA1,HTRA2,HTT,IARS2,IBA57,IFIH1,IFRD1,INPP5E,ISG15,ITM2B,ITPR1,JPH3,KCNA1,KCNA2,KCNC3,KCND3,KCNJ10,KCTD17,KCTD7,KDM6A,KIAA0196,KIAA0226,KIF1A,KIF1C,KIF5A,KIF7,KLC2,KMT2B,KMT2D,L1CAM,L2HGDH,LAMA1,LIAS,LIPT1,LMNB1,LMNB2,LRPPRC,LRRK2,LYST,MAG,MAN2B1,MARS,MARS2,MCEE,MECP2,MECR,MICU1,MLC1,MMACHC,MMADHC,MME,MPZ,MRE11A,MRPL10,MTFMT,MTHFR,MTPAP,MTTP,MUT,NALCN,NARS2,NDUFA1,NDUFA10,NDUFA11,NDUFA12,NDUFA2,NDUFA4,NDUFA9,NDUFAF2,NDUFAF5,NDUFAF6,NDUFS1,NDUFS2,NDUFS3,NDUFS4,NDUFS7,NDUFS8,NDUFV1,NDUFV2,NHLRC1,NIPA1,NKX21,NOL3,NOP56,NPC1,NPC2,NPHP1,NT5C2,NUP62,OFD1,OPA1,OPA3,OPHN1,PANK2,PARK2,PARK7,PARN,PAX6,PC,PCBD1,PCCA,PCCB,PCNA,PDE10A,PDE8B,PDGFB,PDGFRB,PDHA1,PDHB,PDHX,PDSS1,PDSS2,PDYN,PET100,PEX10,PEX7,PGAP1,PHYH,PIK3R5,PINK1,PITX2,PLA2G6,PLEKHG4,PLP1,PMM2,PMPCA,PNKD,PNKP,PNPLA6,PNPT1,POLG,POLR3A,POLR3B,PPCDC,PPCS,PPP2R2B,PRICKLE1,PRKCG,PRKRA,PRNP,PRPS1,PRRT2,PSEN1,PTEN,PTS,QDPR,RAB39B,RAB3GAP2,RAD1,RARS,RARS2,REEP1,REEP2,RELN,RIPPLY1,RNASEH2A,RNASEH2B,RNASEH2C,RNF170,RNF216,RPGRIP1L,RPIA,RTEL1,RTN2,SACS,SAMD9L,SAMHD1,SCARB2,SCN1A,SCN4A,SCO2,SCP2,SCYL1,SDHA,SDHAF1,SEPSECS,SERAC1,SETX,SGCE,SIL1,SLC16A2,SLC17A5,SLC19A3,SLC1A3,SLC20A2,SLC25A15,SLC25A19,SLC25A42,SLC2A1,SLC30A10,SLC33A1,SLC39A14,SLC46A1,SLC52A2,SLC52A3,SLC6A19,SLC6A3,SLC6A8,SLC9A1,SLC9A6,SMPD1,SNAP25,SNCA,SNX14,SPAST,SPG11,SPG20,SPG21,SPG7,SPR,SPTAN1,SPTBN2,SQSTM1,STUB1,SUCLA2,SUCLG1,SUOX,SURF1,SYNE1,SYNJ1,SYT14,TACO1,TAF1,TARDBP,TBCE,TBP,TCTN1,TCTN2,TCTN3,TDP1,TDP2,TECPR2,TENM4,TERT,TFG,TGFB1,TGM6,TH,THAP1,TIMM8A,TINF2,TK1,TMEM138,TMEM216,TMEM231,TMEM237,TMEM240,TMEM67,TOR1A,TPK1,TPP1,TRAPPC11,TREX1,TRMU,TRPC3,TSEN2,TSEN54,TSFM,TTBK2,TTC19,TTPA,TTR,TUBB4A,TXN2,UBA5,UCHL1,UQCRQ,USP8,VAMP1,VCP,VHL,VLDLR,VPS13A,VPS35,VPS37A,VPS53,VRK1,VWA3B,WDR45,WDR48,WDR73,WDR81,WFS1,WWOX,XK,XPA,XPC,XPR1,ZFR,ZFYVE26,ZFYVE27,ZNF592*

**Video legend**

At examination a marked craniocervical dystonia is shown. There is a severe retrocollis with right laterocollis and jaw opening dystonia that improved with a postural trick by touching the face. In the pull test there is not clear instability. No cerebellar signs were present. The sagittal view demonstrated an important axial involvement with a forward posture. At walking, the patient showed dragging steps, being mainly affected the left side of the body.

**e-References**

1. Chen Y-H, Tsai M-T, Shaw C-K, Chen C-H. Mutation analysis of the human NR4A2 gene, an essential gene for midbrain dopaminergic neurogenesis, in schizophrenic patients. American Journal of Medical Genetics (Neuropsychiatric Genetics). 2001;105:753-7.

2. Sleiman PMA, Healy DG, Muqit MMK, Yang YX, van der Brug M, Holton JL, et al. Characterisation of a novel NR4A2 mutation in Parkinson's disease brain. Neuroscience Letter. 2009;457:75-9.

3. Le WD, Xu P, Jankovic J, Jiang H, Appel SH, Smith RG, et al. Mutations in NR4A2 associated with familial Parkinson Disease. Nature Genetics 2003;33:85-9.

4. Buervenich S, Carmine A, Arvidsson M, Xiang F, Zhang Z, Sydow O, et al. NURR1 mutations in cases of schizophrenia and manic-depressive disorder. American Journal of Medical Genetics (Neuropsychiatric Genetics). 2000;96:808-13.

5. Ramos LLP, Monteiro FP, Sampaio LPB, Costa LA, Ribeiro MDO, Freitas EL, et al. Heterozygous loss of function of NR4A2 is associated with intellectual deficiency, rolandic epilepsy, and language impairment. Clinical Case Reports. 2019;7:1582-4.

6. Wirth T, Mariani LL, Bergant G, Baulac M, Habert MO, Drouot N, et al. Loss-of-function mutations in NR4A2 cause dopa-responsive dystonia Parkinsonism. Mov Disord. 2020.

7. Grimes DA, Han F, Panisset M, Racacho L, Xiao F, Zou R, et al. Translated mutation in the Nurr1 gene as a cause for Parkinson's disease. Movement Disorders. 2006;21:906-9.

8. Vissers LELM, Kirsten JM, van Nimwegen KJM, Schieving JH, Kamsteeg E-J, Kleefstra T, et al. A clinical study of exome sequencing versus conventional genetic testing in pediatric neurology. Genetics in Medicine. 2017;19:1055-63.

9. Reuter MS, Krumbiegel M, Schluter G, Ekici AB, Reis A, Zweier C. Haploinsufficiency of NR4A2 is associated with a neurodevelopmental phenotype with prominent language impairment. Am J Med Genet A. 2017;173(8):2231-4.

10. Levy J, Grotto S, Mignot C, Maruani A, Delahaye-Duriez A, Benzacken B, et al. NR4A2 haploinsufficiency is associated with intellectual disability and autism spectrum disorder. Clin Genet. 2018;94(2):264-8.

11. Wang Q, Li M, Yang Z, Hu X, Wu HM, Ni P, et al. Increased co-expression of genes harboring the damaging de novo mutations in Chinese schizophrenic patients during prenatal development. Sci Rep. 2015;5:18209.

12. Musolf AM, Ho WSC, Long KA, Zhuang Z, Argersinger DP, Sun H, et al. Small posterior fossa in Chiari I malformation affected families is significantly linked to 1q43-44 and 12q23-24.11 using whole exome sequencing. Eur J Hum Genet. 2019;27(10):1599-610.

13. Zahir FR, Mwenifumbo JC, Chun HE, Lim EL, Van Karnebeek CDM, Couse M, et al. Comprehensive whole genome sequence analyses yields novel genetic and structural insights for Intellectual Disability. BMC Genomics. 2017;18(1):403.

14. Sanchez-Monteagudo A, Alvarez-Sauco M, Sastre I, Martinez-Torres I, Lupo V, Berenguer M, et al. Genetics of Wilson disease and Wilson-like phenotype in a clinical series from eastern Spain. Clin Genet. 2020;10.1111/cge.13719.

Correa-Vela M, Lupo V, Montpeyo M, et al. Impaired proteasome activity and neurodegeneration with brain iron accumulation in FBXO7 defect. Ann Clin Transl Neurol 2020;7:1436-1442.