

Parent community day 9 September 2022

**ADNP as the causative gene for the
Helsmoortel – Van der Aa syndrome**

Frank Kooy

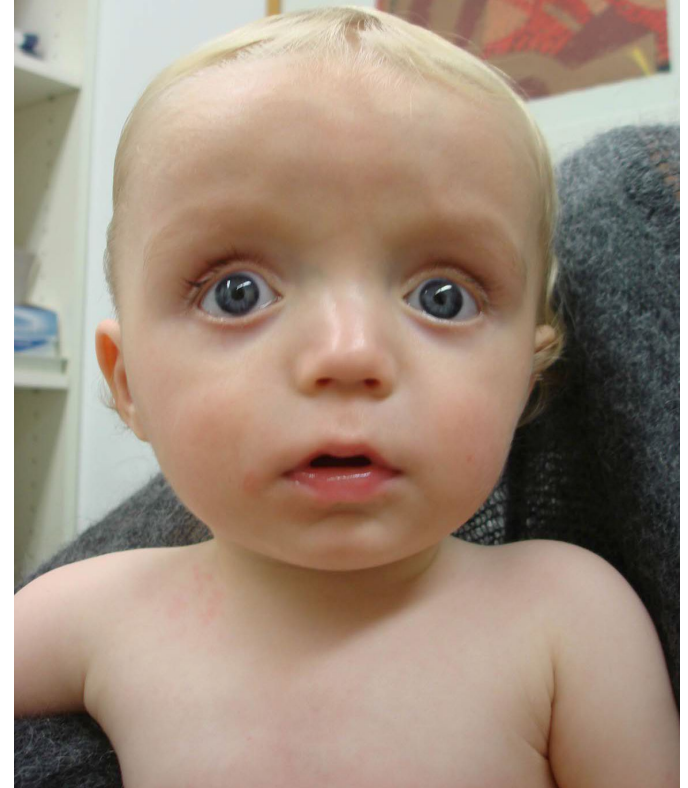


Cognitive Genetics
Centre of Medical Genetics
University of Antwerp



Young boy

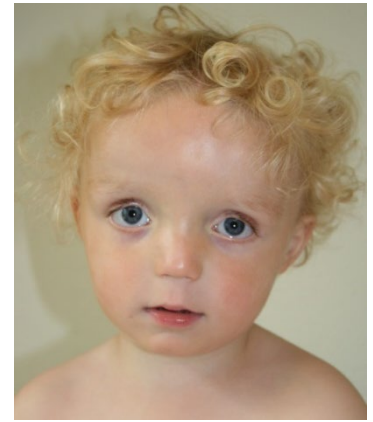
- Global developmental delay
- Failure to thrive
- Autism Spectrum Disorder
- Cardiac: atrial septal defect
- White matter lesions
- Facial Dysmorphism





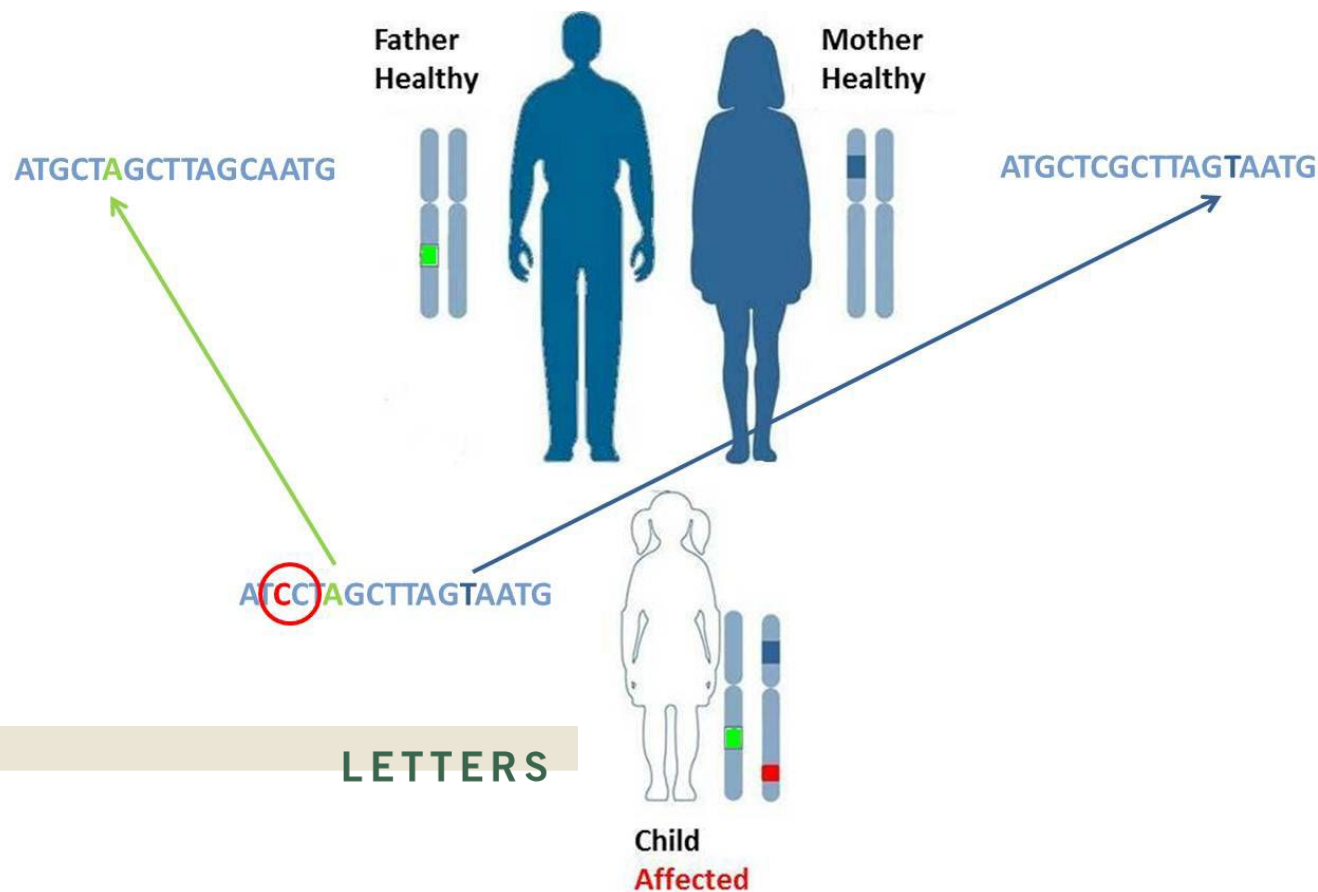
Diagnostic work up

- Karyotype: normal 46, XY
- SNP array: no abnormalities detected
- Differential diagnosis: Noonan spectrum disease, but targeted screening of relevant *PTPN11*, *SOS1*, *RAF1*, *KRAS* genes revealed no mutations





Next Generation Sequencing Trio approach



nature
genetics

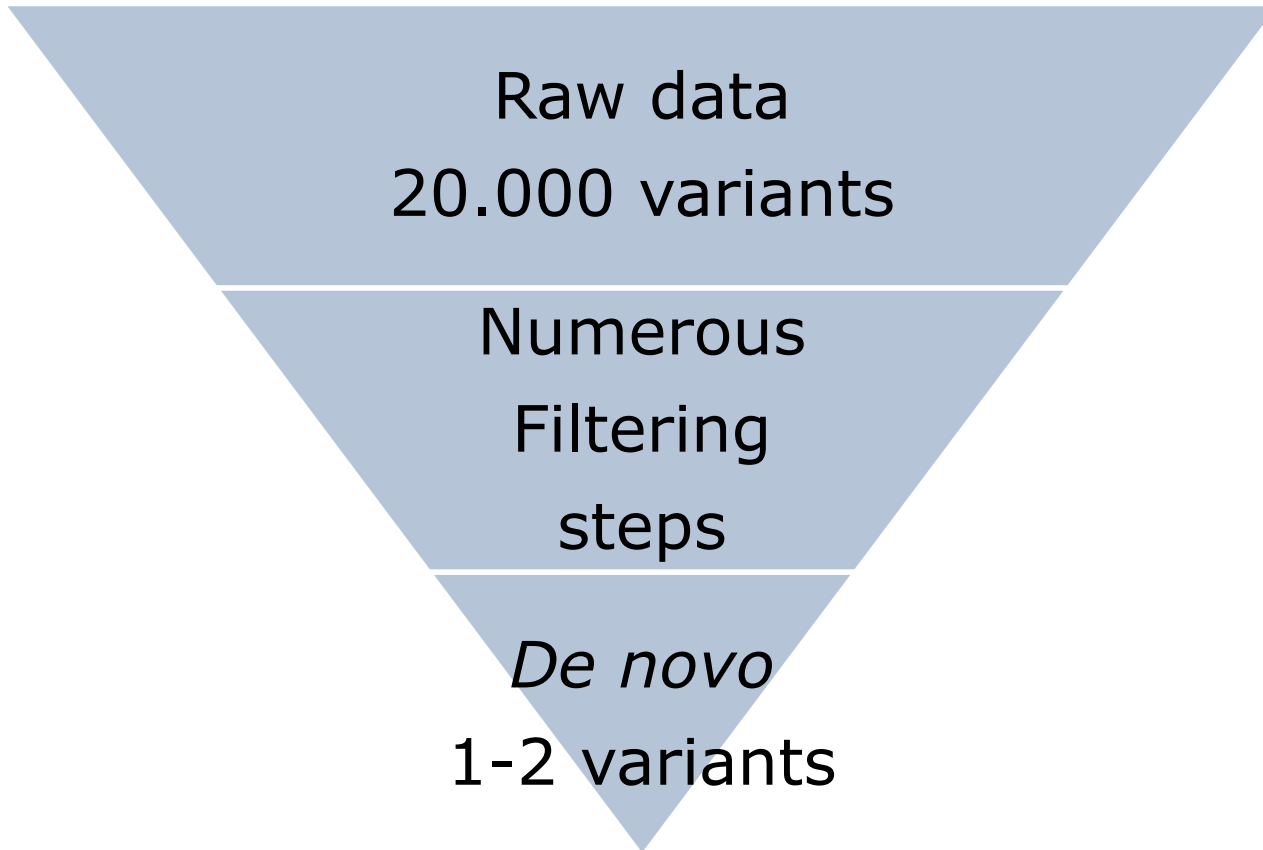
A *de novo* paradigm for mental retardation

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Trio approach

Variant calling





VariantDB Web-based interface

VCF to VariantDB (version 0.1.2)

VCF file:
 5: Unified Genotyper on data 68 and data 30 (VCF) ▾
 Unified Genotyper VCF File

Store VCF and BAM Files:
 Yes ▾
 This option allows you to send the BAM and VCF files to our storage server for dynamic loading into IGV. If you store them there, please delete them here.

BAM File:
 4: Final_BAM_In_Linkage ▾

Provide a Sample Name ::
 Type the sample name ▾
 If no name is specified, a new sample will be created, and you will be notified of the name

Sample Name:
 DemonstrationSample

Sample Gender :
 Female ▾
 This can be set from the database frontend as well.

VariantDB-Server :
 Main Server @ University of Antwerp ▾
 Specify the VariantDB server you wish to send the data to. You MUST have a valid account on the target server, identical to your account here.

Execute

What it does

This tool sends the results from the GATK unified genotyper to a VariantDB server. From there, variants can be compared between samples, filtered on various annotations etc. To add servers, specify them in the tool configuration XML file.

Input file

VCF file from the GATK Unified Genotyper.

Outputs

Text file with some results from the vcf-parser.

| Location | Ref Allele | Alt Allele | Ref.Depth | Alt.Depth | Allelic Balance | Genotype |
|-------------------------|------------------------------|----------------------------------|------------------------|--------------------------------|---|----------------------------------|
| chr1:45973938 | C | T | 18 | 13 | 0.4194 | Heterozygous |
| | Base_Quality_Rank_Sum = -0.5 | Fisher_Strand_Bias 0 | | Mapping_Quality 67 | | Mapping_Quality_Rank_Sum = 0.781 |
| | Phred_Genotype 93 | Phred_Polymorphism 144.19 | | Read_Depth 11.1 | | Read_Position_Rank_Sum = -1.021 |
| | Strand_Bias = 185.25 | | | | | |
| | SV_Status | SV_Type | Class | Class Comment | Disease | Gene |
| | overlap | copy number gain | Uncertain significance | classified by single submitter | multiple conditions | MMACHC |
| | overlap | copy number gain | Uncertain significance | classified by single submitter | Autism | CDC17 |
| | exact | single nucleotide variant | Pathogenic | classified by single submitter | Methylenic acidemia with homocystinuria | MMACHC |
| | | | | | | STOP-GAIN |
| | | | | | | |
| Panel Definition | Panel Gene | Panel Gene Comment | | | | |
| ID gene | MMACHC | OMIM:608514 | | | | |
| RefSeq_Exon | RefSeq_GeneLocation | RefSeq_Protein_Length_Difference | RefSeq_Transcript | RefSeq_VariantType | RefSeq_Polymorph | RefSeq_Polymorph |
| | exon | 5 | NM_015058 | | | |
| Amino_Acid_Change_37.66 | Color_Change_37.66 | Effect_37.66 | Effect_Invested_37.66 | Functional_Class_37.66 | Gene_Coding_37.66 | Gene_Exon_37.66 |
| | | DOWNSTREAM | MODIFIER | | CODING | PROX1 |
| | | DOWNSTREAM | MODIFIER | | CODING | PROX1 |
| | | DOWNSTREAM | MODIFIER | | CODING | PROX1 |
| | | STOP_GAINED | HIGH | NONSENSE | CODING | xxxx_1_45973984_45974038 |
| | | UPSTREAM | MODIFIER | | CODING | MMACHC |
| | | UPSTREAM | MODIFIER | | CODING | MMACHC |

Use This Sample/Region
 Type the sample name | F7_Index

Filter Settings | Annotations | Export | Statistical Charts | Sample Log

Select Annotations To show
 Hover the mouse over available annotations to get more information.

GATK Annotations Information

| | | | |
|--|--|--|--|
| <input checked="" type="checkbox"/> AllelicRatio | <input checked="" type="checkbox"/> Alt_Allele_Depth | <input checked="" type="checkbox"/> Base_Quality_Rank_Sum | <input checked="" type="checkbox"/> Fisher_Strand_Bias |
| <input checked="" type="checkbox"/> Genotype | <input checked="" type="checkbox"/> Mapping_Quality | <input checked="" type="checkbox"/> Mapping_Quality_Rank_Sum | <input checked="" type="checkbox"/> Phred_Genotype |
| <input checked="" type="checkbox"/> Phred_Polymorphism | <input checked="" type="checkbox"/> Quality_By_Depth | <input checked="" type="checkbox"/> Read_Position_Rank_Sum | <input checked="" type="checkbox"/> Ref_Allele_Depth |
| <input checked="" type="checkbox"/> Strand_Bias | <input type="checkbox"/> Tranches_Filter | | |

ANNOVAR Information

| | | | |
|--|--|---|--|
| <input type="checkbox"/> 1000g2012apr_all | <input type="checkbox"/> 1000g2012apr_all | <input type="checkbox"/> 1000g2012apr_amr | <input type="checkbox"/> 1000g2012apr_asn |
| <input type="checkbox"/> 1000g2012apr_eur | <input type="checkbox"/> CADD_phred | <input type="checkbox"/> CADD_raw | <input type="checkbox"/> Ensembl_Exon |
| <input type="checkbox"/> Ensembl_GeneID | <input type="checkbox"/> Ensembl_GeneLocation | <input type="checkbox"/> Ensembl_TranscriptID | <input type="checkbox"/> Ensembl_VariantType |
| <input type="checkbox"/> Ensembl_cPointAA | <input type="checkbox"/> Ensembl_cPointMT | <input type="checkbox"/> Genomic_SuperDups_Location | <input type="checkbox"/> Genomic_SuperDups_Score |
| <input type="checkbox"/> RefSeq_Exon | <input type="checkbox"/> RefSeq_DetectedLocation | <input type="checkbox"/> RefSeq_Protein_Length_Difference | <input type="checkbox"/> RefSeq_Symbol |
| <input type="checkbox"/> RefSeq_Transcript | <input type="checkbox"/> RefSeq_VariantType | <input type="checkbox"/> RefSeq_cPointAA | <input type="checkbox"/> RefSeq_cPointMT |
| <input type="checkbox"/> UCSC_Exon | <input type="checkbox"/> UCSC_GeneLocation | <input type="checkbox"/> UCSC_Symbol | <input type="checkbox"/> UCSC_Transcript |
| <input type="checkbox"/> UCSC_VariantType | <input type="checkbox"/> UCSC_cPointAA | <input type="checkbox"/> UCSC_cPointMT | <input type="checkbox"/> esp5400_aa |
| <input type="checkbox"/> esp5400_all | <input type="checkbox"/> esp5400_ea | <input type="checkbox"/> esp5500_aa | <input type="checkbox"/> esp5500_all |
| <input type="checkbox"/> esp6500_ea | <input type="checkbox"/> jpb_GERP | <input type="checkbox"/> jpb_LR | <input type="checkbox"/> jpb_MultiTast |
| <input type="checkbox"/> jpb_Phlyop | <input type="checkbox"/> jpb_PolyPhen2 | <input type="checkbox"/> jpb_SIR | <input type="checkbox"/> snp130_rsid |
| <input type="checkbox"/> snp135_Clinical | <input type="checkbox"/> snp135_JMAP | <input type="checkbox"/> snp135_rsid | <input type="checkbox"/> snp137_rsid |
| <input type="checkbox"/> snp137_Clinical | <input type="checkbox"/> snp137_JMAP | <input type="checkbox"/> snp137_NChr | |

Presets

Presets
 ALL_GATK #
 Effect On RefSeq #
 Demo_Annotations #
 snpDE_Orch37_66 #
 all #
 GO_anno #
 WebTools #

Save Current Annotations
 Add Annotations #
 Save Current Annotations

Use This Sample/Region
 Type the sample name | F7_Index

Filter Settings | Annotations | Export | Statistical Charts | Sample Log

Build your query

| Negate | Filter On | Argument | Values |
|-------------|---|-------------------------|-------------------|
| Not Match ▾ | In Parents ▾ | F7_Filter = F7_Mother | As Any Genotype ▾ |
| Match ▾ | Rel Occ. Control Samples (Any Genotype) ▾ | Smaller Or Equal Than ▾ | 0.05 |
| Match ▾ | Quality By Depth ▾ | Bigger Or Equal Than ▾ | 4.8 |

Presets
 Quality #
 HaplPos_Quality #
 not in snp130 #
 common filters #
 Apply Filter

Save Current Filter
 Save Current Filter

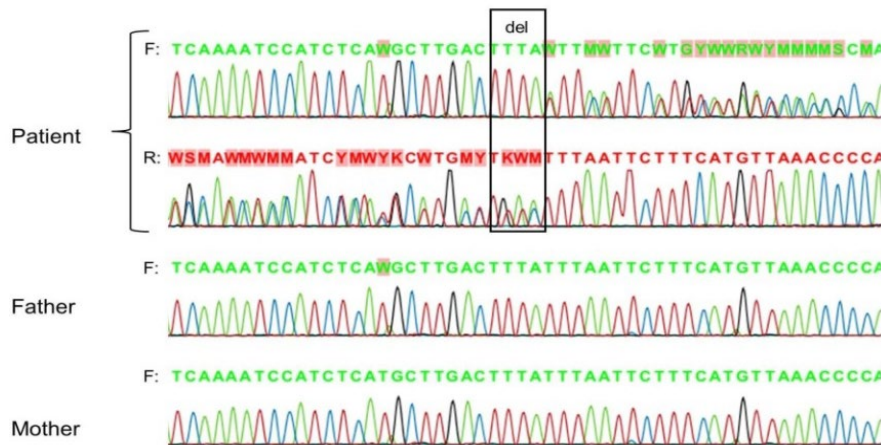


NGS analysis





NGS analysis: a single de novo mutation



- 4bp del in *ADNP*
- Causes frameshift introducing stop codon
- Mutation not control databases, e.g., ESP, 1000g, dbSNP



ADNP gene

Activity Dependent Neuroprotective Protein

- Expressed in brain
- Zinc fingers/homeobox domain: potential transcription factor
- Involved in neurogenesis
- Involved in heart development
- Homozygous KO mice are embryonically lethal
- Heterozygous KO mice have cognitive & behavioral problems



Other mutations

Table 2. Recurrent disruptive mutations in ID and ASD

| Gene ^{a,b} | ID cases | ASD cases | Summary ^{c,d,e} | ESP samples | |
|---------------------|----------|-----------|-------------------------------|-------------|-----------------------|
| | | | | Variants | Frequency |
| <i>CHD8</i> | – | 9/2446 | 2 (O), 7 (O*) [+3 (N*)] | 0 | 0/6503 |
| <i>SCN2A</i> | 3/151 | 2/593 | 1 (L), 2 (R), 2 (S) [+1 (N*)] | 1 | 7/6503 |
| <i>SYNGAP1</i> | 3/151 | – | 1 (L), 2 (R) | 1 | 207/6503 ^f |
| <i>GRIN2B</i> | – | 3/2446 | 1 (O), 2 (O*) | 0 | 0/6503 |
| <i>DYRK1A</i> | – | 3/2446 | 1 (I), 1 (O), 1 (O*) | 0 | 0/6503 |
| <i>ZNF292</i> | 1/151 | 1/593 | 1 (L), 1 (N) | 1 | 2/6503 |
| <i>POGZ</i> | – | 2/593 | 1 (I), 1 (N) | 1 | 1/6503 |
| <i>KATNAL2</i> | – | 2/593 | 1 (O), 1 (S) | 1 | 1/6503 |
| <i>TBR1</i> | – | 2/2446 | 1 (O), 1 (O*) | 0 | 0/6503 |
| <i>CTNNA1</i> | 1/151 | 1/2446 | 1 (L), 1 (O*), [+1 (L*)] | 0 | 0/6503 |
| <i>SETBP1</i> | 1/151 | 1/593 | 1 (O), 1 (R) | 3 | 58/6503 ^f |
| <i>ADNP</i> | – | 2/2446 | 1 (O), 1 (O*) | 1 | 1/6500 |
| <i>LRP2</i> | 1/151 | 1/593 | 1 (I), 1 (L) | 6 | 53/6500 |
| <i>ARID1B</i> | – | 2/2446 | 1 (O), 1 (O*) | 5 | 314/6500 |

- 1 patient in exome study - O’Roak ea, Nature 2012
- 1 patient in MIPS screening - O’Roak ea, Science 2012
- 1 in ESP non-neurological control cohort



ADNP mutation





ADNP mutation





ADNP mutation





ADNP mutation





ADNP mutation



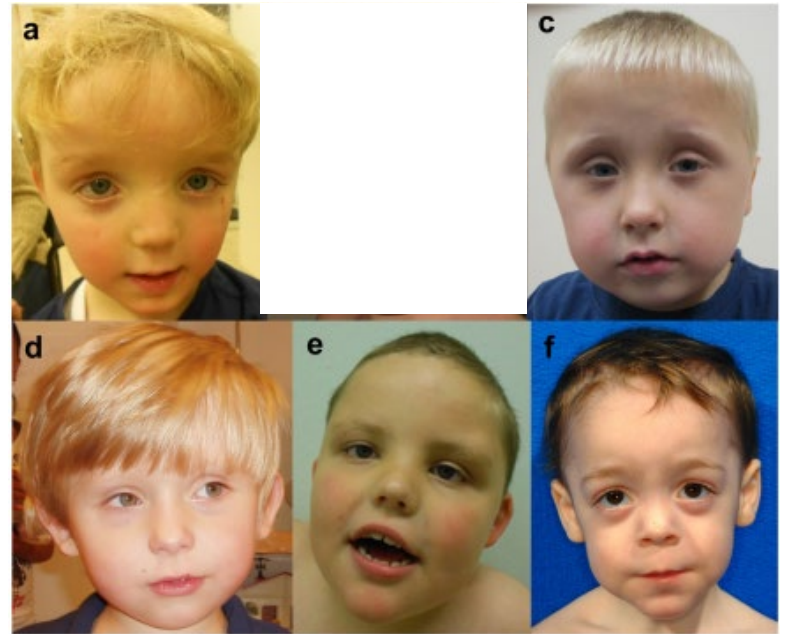
Ten patients with truncating mutations in *ADNP*

| Patient | Patient ID | Origin | Screening method | Cohort composition | Cohort size | mutation in genomic DNA (chr20) | mutation in cDNA (NM_015339.2) | Protein | Mutation Type | Inheritance |
|---------|------------|-----------|---------------------|---|-------------------|--------------------------------------|--------------------------------|------------------|---------------|-----------------------|
| 1 | 111294 | Antwerp | WES | Moderate to severe ID and/or autism + dysmorphic features | 10 | g.49508752_49508755 delTTTA | c.2496_2499delTAAA | p.Asp832Lysfs*80 | Frameshift | <i>de novo</i> |
| 2 | 11-08612 | Nijmegen | WES | Non-syndromic severe ID | 100 | g.49510040G>T | c.1211C>A | p.Ser404* | Nonsense | <i>de novo</i> |
| 3 | 12130.p1 | Seattle | WES ^{2,16} | ASD from the Simon Simplex Collection | 189 | g.49510028_49510029 delTT | c.1222_1223delAA | p.Lys408Valfs*31 | Frameshift | <i>de novo</i> |
| 4 | 1050237 | Westmead | WES | Non-syndromic severe ID | 95 | g.49509086_49509098 delATTTGCTCGTAAG | c.2153_2165delCTTAC GAGCAAAT | p.Thr718Glyfs*12 | Frameshift | <i>de novo</i> |
| 5 | 3061-08D | Stockholm | WES | Moderate to severe ID and/or autism + dysmorphic features | 45 | g.49509094G>C | c.2157C>G | p.Tyr719* | Nonsense | <i>de novo</i> |
| 6 | 122793 | Antwerp | HRM | Autism | 148 | g.49508757_49508760 delTTAA | c.2491_2494delTTAA | p.Lys831Ilefs*81 | Frameshift | <i>de novo</i> |
| 7 | 07-06960 | Nijmegen | MIPS | ID and/or autism | 2743 | g.49508443delG | c.2808delC | P.Tyr936* | Frameshift | <i>de novo</i> |
| 8 | 2376 | Troina | MIPS | ID and/or autism | Idem as patient 7 | g.49508757_49508760 delTTAA | c.2491_2494delTTAA | p.Lys831Ilefs*81 | Frameshift | <i>de novo</i> |
| 9 | 2533 | Troina | MIPS | ID and/or autism | Idem as patient 7 | g.49509321G>A | c.1930C>T | p.644Arg* | Nonsense | parents not available |
| 10 | 13545.p1 | Seattle | MIPS ¹⁶ | ASD from the Simon Simplex Collection | 2446 | g.49509094_49509095 insT | c.2156_2157insA | p.Tyr719* | Frameshift | <i>de novo</i> |



Dysmorphic features

| Phenotype | Frequency |
|-----------------------|-----------|
| Prominent forehead | 5/8 |
| High hairline | 7/8 |
| Eversion/notch eyelid | 3/7 |
| Hypertelorism | 1/8 |
| Broad nasal bridge | 6/8 |
| Short nose | 2/8 |
| Thin upper lip | 6/7 |





Clinical characteristics

| Phenotype | Frequency |
|---------------------------------------|--------------|
| Autism Spectrum Disorder (ASD) | 10/10 |
| Intellectual Disability (ID) | 10/10 |
| Developmental delay (motor) | 9/10 |
| Developmental delay (speech) | 8/9 |
| ADHD | 2/9 |
| Hypotonia | 7/9 |
| Growth retardation / Short stature | 5/8 |
| Feeding problems | 5/8 |
| Recurrent infections | 5/8 |
| Congenital heart defect | 3/8 |
| Hyperlaxity | 6/8 |
| Obesity | 4/7 |
| Hypermetropia | 6/6 |
| Seizures | 2/7 |
| Behavior | 5/7 |
| Insensitivity to pain | 2/5 |
| MRI brain abnormality | 5/9 |
| Hand abnormalities | 6/8 |
| Constipation | 2/6 |



Statistics

- The frequency of truncating *de novo* mutations in *ADNP* is significantly higher (p : 0.001852, odds ratio 13.24668, one-sided Fisher's exact test) in patients compared to the ESP cohort and additional controls from the Simons Siblings.
- The probability of detecting 8 or more *de novo* truncating events in *ADNP* within our cohort by chance was estimated to be $p = 2.65e^{-18}$ (binomial test) under a *de novo* rate of 1.2 non-synonymous coding variants per individual according to a probabilistic model of a locus specific enrichment for truncating variation [O'Roak et al., 2012].





De novo mutations in *ADNP* cause new autism syndrome

Autism spectrum disorder
Intellectual Disability
Facial dysmorphism
Congenital malformations

may explain etiology of
0.17% of ASD patients

LETTERS

nature
genetics

A SWI/SNF-related autism syndrome caused by *de novo* mutations in *ADNP*

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Acknowledgements

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Meredith Wilson
Madhura Bakshi



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