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The Transcriptional Regulator *ADNP* Links the BAF (SWI/SNF) Complexes With Autism

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Mutations in *ADNP* were recently identified as a frequent cause of syndromic autism, characterized by deficits in social communication and interaction and restricted, repetitive behavioral patterns. Based on its functional domains, *ADNP* is a presumed transcription factor. The gene interacts closely with the SWI/SNF complex by direct and experimentally verified binding of its C-terminus to three of its core components. A detailed and systematic clinical assessment of the symptoms observed in our patients allows a detailed comparison with the symptoms observed in other SWI/SNF disorders. While the mutational mechanism of the first 10 patients identified suggested a gain of function mechanism, an 11th patient reported here is predicted haploinsufficient. The latter

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observation may raise hope for therapy, as addition of NAP, a neuroprotective octapeptide named after the first three amino acids of the sequence NAPVSPIQ, has been reported by others to ameliorate some of the cognitive abnormalities observed in a knockout mouse model. It is concluded that detailed clinical and molecular studies on larger cohorts of patients are necessary to establish a better insight in the genotype phenotype correlation and in the mutational mechanism. © 2014 Wiley Periodicals, Inc.

KEY WORDS: autism; SWI/SNF; BAF complexes; ADNP

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INTRODUCTION

Autism is a neurodevelopmental disorder characterized by limitations in social interaction and communication in combination with stereotypic, repetitive behavior and restricted interest [APA, 2013]. The symptoms usually emerge in early childhood, before the age of three. The prevalence of the disorder appears to be on the rise over the last decades, with as many as 1 in 68 individuals affected according to the most recent estimates [Surveillance, 2014]. Consistent among all prevalence estimates, more boys than girls are affected. All population studies indicate a significant contribution of genetic components underlying the disorder. While the heritability—the proportion of phenotypic variation explained by genetic factors—was once estimated to be as high as 90% in the first reported twin studies [Folstein and Rutter, 1977; Steffenburg et al., 1989], there is now a consensus that these initial studies may have overestimated the genetic contribution of the disorder. The largest population based study so far reports a heritability of 50% with an increased risk of recurrence of about 10-fold for a first degree relative and of about 2-fold for cousins [Sandin et al., 2014].

While the genetic causes of non-syndromic autism remain elusive, searches for a genetic cause have been successful to some extent in syndromic forms of autism. Syndromic autism is defined as autism in combination with additional clinical features. Co-morbidities often observed include intellectual disability, epilepsy, and psychiatric disorders. Frequent monogenic causes of syndromic autism include the fragile X syndrome and Rett syndrome [Amir et al., 1999; Rooms and Kooy, 2011]. Specific genomic disorders—submicroscopic chromosomal deletions or duplications at fixed positions in the genome—presenting with autism, include the duplication of the Prader–Willi/Angelman region at 15q11-13 and recurrent copy number variants (CNV) at 16p11.2 [Sanders et al., 2011]. A microscopically visible chromosomal abnormality associated with autism is an additional supernumerary isodicentric chromosome 15 [Mendelsohn and Schaefer, 2008]. Apart from recurrent genomic disorders, an excess of non-recurrent de novo CNVs as compared to healthy siblings and control subjects is also evident in patients with Autistic Spectrum Disorder (ASD) [Pinto et al., 2010].

Most recently, whole exome—consisting of all protein coding regions in the genome—sequencing (WES) has been applied to identify the genes involved in autism. This approach was inspired by the successful introduction of WES to identify genetic causes of neurodevelopmental disorders [Veltman and Brunner, 2012]. After a proof of principle study comparing the exomes of a pilot cohort of 10 ID patients with those of the parents, likely causative mutations were identified in six cases [Vissers et al., 2010]. Subsequent studies using the same so called trio approach in larger cohorts of up to 250 patients showed a diagnostic yield in the range of up to 50% [de Ligt et al., 2012; Rauch et al., 2012; Yang et al., 2013]. In autism, it was discovered that de novo potentially deleterious (e.g., amino acid changing) SNPs were significantly more prevalent in patients than in unaffected relatives or controls. Based on the trio and quartet approach, consisting of patient, parents, and unaffected sibling, the most frequently mutated genes identified so far include *CDH8*, *SCN2A*, *DYRK1A*, and *CTNNB1* [O'Roak et al., 2012a; Krumm et al., 2014]. Many genes implicated in autism were previously identified as causative for ID, epilepsy, schizophrenia or bipolar disorder, suggesting an overlap in the underlying etiology of neurodevelopmental disorders.

Collectively, the genetic abnormalities identified in autism to date explain maximally 15% of cases [Mendelsohn and Schaefer, 2008; Carter and Scherer, 2013]. While no single gene is mutated in more than 1% of patients, autism genes functionally converge to commonly affected cellular pathways and protein-protein interaction networks [O'Roak et al., 2012b; Krumm et al., 2014; Pinto et al., 2014]. The most commonly affected networks are the developmental wnt signaling pathway, the pathway involving synaptic function, and the chromatin remodeling pathway.

The Identification of *ADNP* Mutations in Autistic Patients

In an attempt to identify novel genes responsible for autism, a first de novo p.Lys408Valfs*31 mutation in the activity-dependent neuroprotective protein (ADNP) gene was identified in a large

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cohort of autistic patients [O'Roak et al., 2012b]. As sequencing of the 209 families in this cohort did not reveal a second mutation in *ADNP* nor hardly in any other candidate gene, a large scale resequencing study of the most promising candidates, including *ADNP*, was initiated using the molecular inversion probe (MIP) sequencing technology [O'Roak et al., 2012a]. In this study of 2,446 probands, an additional p. Tyr719*de novo *ADNP* mutation was identified. However, a single p.Q361* mutation in a patient of the NHLBI GO Exome Sequencing Project (ESP) cohort, consisting of patients with non-neurological disorders affecting heart, lung, and blood was present, and it was concluded that statistical evidence was not sufficient to prove causality of the mutations [ESP]. Confusingly, a p. Gly1094Profs*5 mutation, inherited from an unaffected parent, was reported in this same large scale targeted resequencing study. While this finding seemed to argue against causality at first sight, this mutation is located close to the C-terminus of the encoded protein, beyond the last known functional domain. Typically variations that close to the end of a protein are unlikely to affect protein function.

In a trial of optimizing the diagnostic workflow for the introduction of WES in a diagnostic setting, we discovered another *ADNP* mutation in a small cohort of ID/autism patients [Helsmoortel et al., 2014a]. Targeted screening of a cohort of 148 autistic patients revealed yet another mutation. By combining the data from WES and targeted resequencing studies initiated in multiple centers, we identified a total of 10 patients with mutations in *ADNP*, including the cases described above [Helsmoortel et al., 2014b]. We calculated that 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. In addition to the case-control analysis, we calculated a locus specific enrichment for truncating variation using a probabilistic model as described [O'Roak et al., 2012a]. The probability of detecting eight or more de novo truncating events in *ADNP* within our cohort by chance was estimated to be $P = 2.65\text{e-}18$ (binomial test) under a de novo rate of 1.2 non-synonymous coding variants per individual. To further delineate the clinical characteristics of this novel disorder, an online portal was set up to collect phenotypic information of additional patients in a collaborative effort (Fig. 1). One additional patient with a de novo c.118C > T (p.Q40*) mutation was already submitted to the system.

The *ADNP* gene

The *ADNP* gene was first identified in murine P19 carcinoma cells as a vasoactive intestinal peptide (VIP) responsive gene showing increased expression after VIP treatment [Bassan et al., 1999; Pinhasov et al., 2003]. VIP is a neuroprotective peptide that is active during embryonic development, especially during the time of neuronal tube closure,

The ADNP gene was first identified in murine P19 carcinoma cells as a vasoactive intestinal peptide (VIP) responsive gene showing increased expression after VIP treatment. VIP is a neuroprotective peptide that is active during embryonic development, especially during the time of neuronal tube closure, and protects damaged nerve cells from cell death by inducing glia-derived, survival promoting substances.

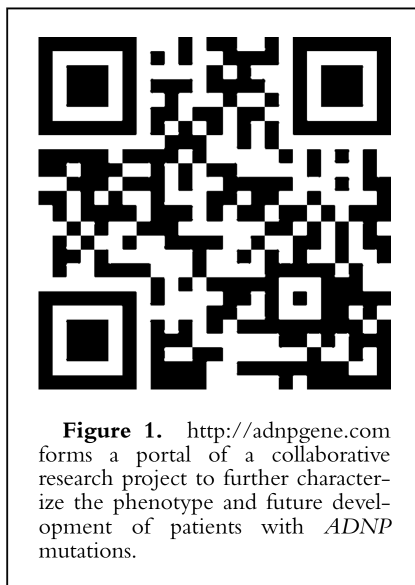
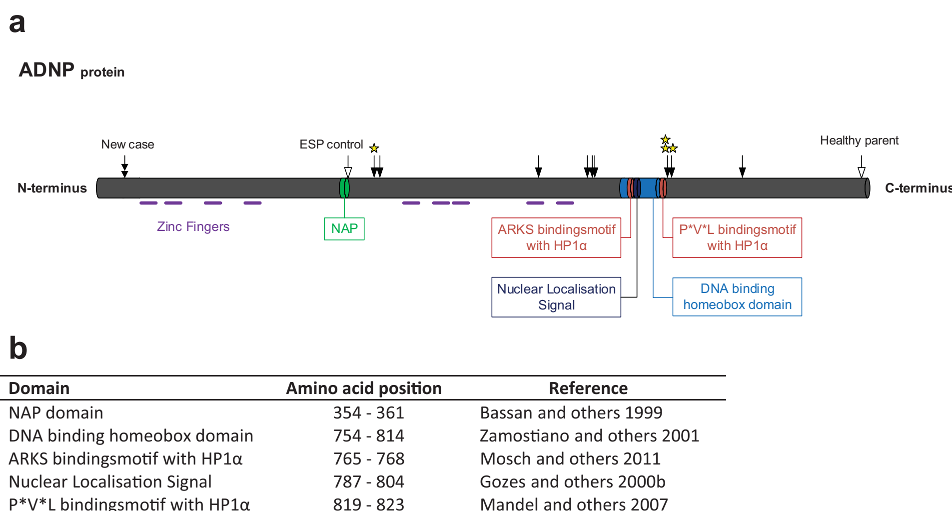


Figure 1. http://adnpgene.com forms a portal of a collaborative research project to further characterize the phenotype and future development of patients with *ADNP* mutations.

and protects damaged nerve cells from cell death by inducing glia-derived, survival promoting substances [Said, 1996]. The human orthologue, *ADNP*, spans about 40 kb of genomic DNA and consists of five exons and four introns with alternative splicing of an untranslated second intron [Zamostiano et al., 2001]. Human and murine mRNA are 90% identical and the region is highly conserved between vertebrates. The encoded protein contains nine zinc fingers and a homeobox domain with a strong homology to that found in hox genes, suggesting a firm role in embryonic development (Fig. 2). Bioinformatic analysis also identified PxVxL as a potential heterochromatin protein 1α (HP1α) binding motif, together with an ARKS motif in the homeobox domain [Mosch et al., 2011]. Indeed, HP1α is found in co-immunoprecipitates from P19 nuclear protein extracts with



Domain	Amino acid position	Reference
NAP domain	354 - 361	Bassan and others 1999
DNA binding homeobox domain	754 - 814	Zamostiano and others 2001
ARKS bindingsmotif with HP1α	765 - 768	Mosch and others 2011
Nuclear Localisation Signal	787 - 804	Gozes and others 2000b
P*V*L bindingsmotif with HP1α	819 - 823	Mandel and others 2007

Figure 2. **a**: Schematic representation of the ADNP protein. Symbols: ↓ previous cases, ↓ new case, ↓ controls and ☆ cases for which expression analysis was performed. **b**: Amino acid positions of the different domains.

ADNP antibodies and vice versa. Additional proteins in the precipitates with ADNP antibodies were BRG1, BAF250A, and BAF170, all members of the mating-type switching/sucrose non-fermenting (SWI/SNF) remodeling complex. Despite the presence of a bipartite nuclear localization signal (NLS), the protein is predominantly, but not exclusively, cytoplasmatic in neuronal cells, though in non-neural cells it is mostly located in the nucleus [Gennet et al., 2008; Mandel et al., 2008]. The protein also contains signals involved in cellular secretion and uptake and ADNP has been found in the extracellular space of VIP stimulated astrocytes [Furman et al., 2004]. Finally, ADNP exhibits a strong neuroprotective function that can be attributed in its entirety to an octapeptide Asn-Ala-Pro-Val-Ser-Ile-Pro-Alaor NAPVSPIQ domain called NAP [Bassan et al., 1999; Magen and Gozes, 2014]. The mechanism of action is believed to involve P53, a key regulator of cellular apoptosis, as silencing of *ADNP* results in an increase of p53 [Zamostiano et al., 2001]. Subsequently, it was shown that addition of NAP to PC12 cells subjected to oxidative stress protected the cells against elevated p53 levels, normally caused by this treatment [Gozes et al., 2004]. ADNP2 is a sister protein to ADNP which is 33% identical at the protein level, shares the zinc finger and homeobox domains, but lacks the NAP motif [Zamostiano et al., 2001]. Expression levels of *ADNP* and *ADNP2* appear to be correlated [Dresner et al., 2011; Helsmoortel et al., 2014b].

ADNP Knockout Mouse Model

A knockout mouse model has been generated by targeted replacement of the protein coding exons III–V with a neomycin cassette [Pinhasov et al., 2003]. Homozygous mice are not viable and die prenatally at E8.5–9 due to a failure of cranial neural tube closure. Heterozygous mice develop normally, be it with a slight developmental delays. A detailed differential expression analysis of E9 full knockout, heterozygous and control mice in parallel with the same analysis in P19 cells showed a significant upregulation of genes involved in lipid transport, lytic vacuoles, and coagulation. Downregulated genes clustered in pathways involving regulation of transcription, organogenesis, and neurogenesis [Pinhasov et al., 2003; Mandel et al., 2007]. Typical examples in the first category include the apolipoproteins A1 and E, metalotionine 1 and neurogenin with overexpression in the range of 5–30 fold. For the second category, examples include myosin light chain 2 and neurogenin 1 with a 12-fold and 5-fold underexpression, respectively. The heterozygous knockout mouse model shows tauopathy and cognitive abnormalities as demonstrated in the passive avoidance and Morris water maze tests [Vulih-Shultzman et al., 2007; Gozes et al., 2014]. Interestingly, treatment with NAP or its derivative isoNAP was able to, at least partially, ameliorate the abnormalities in the behavioral tests.

CLINICAL PRESENTATION

All patients had autism, co-morbid in each case with mild to severe intellectual disability. Dysmorphic features as described in the first cohort of 10 patients included a prominent forehead, high hairline, eversion or notch of the eyelid, broad nasal bridge, and thin upper lip [Helsmoortel et al., 2014b]. In the meantime an 11th patient has been identified. His phenotype shares many of the characteristics of the first 10 patients (Tables I and II).

Family history was unremarkable in each case. At the time of birth, the parental ages were in the normal range. None of the couples were consanguineous and no affected siblings have been observed. All children were born at term with birth weight, length, and

TABLE I. Comparison of Clinical Features of Patients Caused by Mutations in ADNP or Coffin-Siris genes (Growth, Craniofacial Features, Skeletal Features, Complications, Neurology, Development and Behavior)

Genes	*ADNP*	Coffin-siris genes caused by mutations in *SMARCB1*, *SMARCA4*, *SMARCE1*, *ARID1A*
Growth and feeding		
Prenatal growth		
Birth weight	-0,17 (n = 8)	-1.3 (n = 31)
Birth length	+0,34 (n = 7)	-1.7 (n = 17)
Birth OFC	+0,7 (n = 4)	-0.9 (n = 15)
Postnatal growth at last observation		
Weight (mean SD score)	+0,5 (n = 10)	-2.3 (n = 28)
Height (mean SD score)	-1,0 (n = 10)	-3.3 (n = 33)
OFC (mean SD score)	+0,2 (n = 9)	-2.6 (n = 28)
Sucking/feeding difficulty	64% (7/11)	99% (32/33)
Tube feeding	0% (0/9)	95% (19/20)
Weaned off tube feeding	0% (0/9)	38% (6/16)
Craniofacial features		
Sparse scalp hair	0% (0/8)	65% (20/31)
Hypertrichosis	0% (0/7)	91% (29/32)
Thick eyebrows	0% (0/8)	82% (28/34)
Long eyelashes	0% (0/8)	90% (28/31)
Ptosis	33% (3/9)	58% (19/33)
Nasal bridge		
Wide	66% (6/9)	33% (10/30)
Flat	11% (1/9)	27% (8/30)
Normal	33% (3/9)	27% (8/30)
Narrow	0% (0/9)	13% (4/30)
Philtrum		
Long	13% (1/8)	30% (9/30)
Short	13% (1/8)	27% (8/30)
Normal	75% (6/8)	17% (5/30)
Broad/long	13% (1/8)	17% (5/30)
Broad	0% (0/8)	7% (2/30)
Broad/short	0% (0/8)	3% (1/30)
Upper lip vermilion		
Thin	90% (9/10)	53% (16/30)
Normal	10% (1/10)	30% (9/30)
Everted	10% (1/10)	13% (4/30)
Thick	0% (0/10)	3% (1/30)
Lower lip vermilion		
Thick	25% (2/8)	82% (27/33)
Normal	75% (6/8)	18% (6/33)
Palatal abnormalities		
Cleft palate	0% (0/9)	30% (9/30)
Skeletal-limb features		
Hypoplastic 5th fingers or toes	14%(1/7)	88% (29/33)
Hypoplastic 5th fingernails or toenails	0% (0/7)	97% (33/34)
Hypoplastic other fingernails and toenails	14%(1/7)	65% (20/31)
Prominent interphalangeal joints	14%(1/7)	33% (9/27)
Prominent distal phalanges	14%(1/7)	50% (13/26)
Scoliosis	33% (3/9)	39% (11/28)
Internal complications		
Cardiovascular	27% (3/11)	15/34 (44%)
Gastrointestinal	66% (6/9)	65% (20/31)
Genitourinary	11% (1/9)	32% (10/31)
Hernia	0% (0/7)	56% (14/25)

(*Continued*)

TABLE I. (*Continued*)

Genes	*ADNP*	Coffin-siris genes caused by mutations in *SMARCB1, SMARCA4, SMARCE1, ARID1A*
Hearing and vision		
Hearing impairment	22% (2/9)	46% (13/28)
Visual impairment	73% (8/11)	56% (14/25)
Immunology		
Frequent infection	64% (7/11)	72% (21/29)
Neurology		
Hypotonia	73% (8/11)	73% (24/33)
Seizures	18% (2/11)	44% (14/32)
Structural CNS abnormalities	50% (5/10)	92% (24/26)
Development and intelligence		
Developmental delay and ID		
Severe	55% (6/11)	56% (19/32)
Moderate to severe	0% (0/11)	6% (2/32)
Moderate	18% (2/11)	22% (7/32)
Mild	27% (3/11)	13% (4/32)
Speech impairment		
No words	22% (2/9)	63% (19/30)
Several words	66% (6/9)	10% (3/30)
Sentences	11% (1/9)	27% (8/30)
Behavior		
Behavioral abnormalities	78% (7/9)	65% (15/23)

SD, standard deviation; CNS, central nervous system; ID, intellectual disability.

occipitofrontal circumference in the normal range. Developmental milestones were delayed in all patients. They could sit between 7.5 months and 12 months and walked independently between 19 months and 4.5 years old. All of them have speech problems including one patient that has never developed speech and is now 8.5 years old. Seven of 11 patients have had feeding difficulties, including decreased sucking or chewing and abnormally increased appetite. Bladder training was delayed in five patients. In infancy, eight children have had hypotonia and two have had seizures, but without abnormalities on EEG. More than half of the children have eye defects, mostly hypermetropia or strabismus, but these abnormalities did not always result in visual impairment. A high anterior hairline is seen in five of eight patients of whom sufficiently detailed clinical information is available and one patient has a low hairline (Fig. 3). Seven of 10 patients have a prominent forehead. Other craniofacial features include ptosis, abnormal slant of palpebral fissures, wide nasal bridge, upturned nasal tip, and a thin upper lip. Six of nine patients have ear abnormalities, including small low-set ears, protruding cup shaped ears, and bilateral helical indentation.

TABLE II. Additional Features of ADNP Patients

Clinical features	Percentage affected
Craniofacial features	
High hairline	63% (5/8)
Prominent forehead	70% (7/10)
Malformed ears	66% (6/9)
Obesity	57% (4/7)
Visual impairment	
Strabismus	27% (3/11)
Hypermetropia	55% (6/11)
Cardiovascular complications	
Atrial Septal Defect	18% (2/11)
Bladder training delay	100% (5/5)
Sleep problems	45% (5/11)
Autism	100% (11/11)
ADHD	18% (2/11)

Joint hyperlaxity is noted in six of nine children. Almost all children have hand abnormalities, including clinodactyly, polydactyly, small fifth fingers, fetal finger pads, prominent interphalangeal joints, and distal phalanges. Three of eleven patients have cardiac defects, namely atrial septal defect and mitral valve prolapse. Six of nine patients

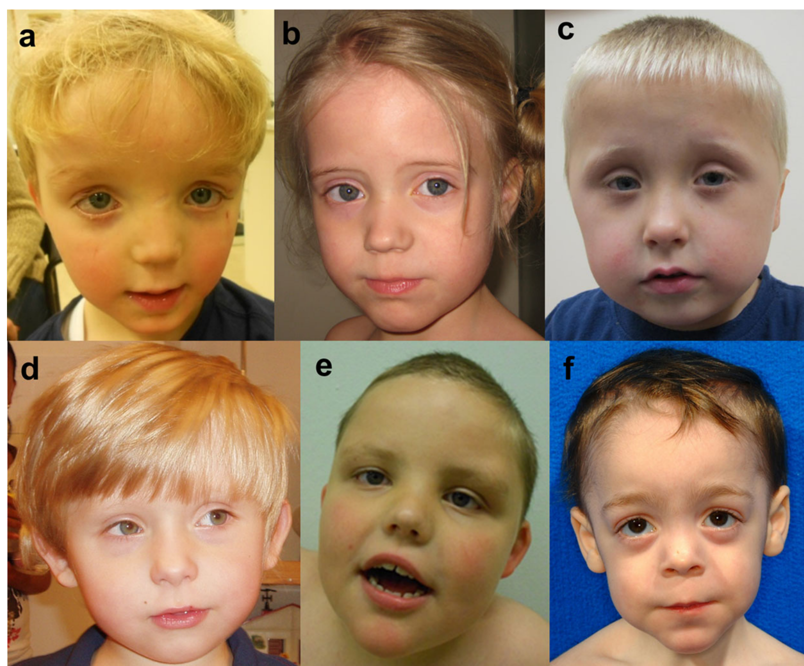


Figure 3. **a–f**: Patients 1 (a), 2 (b), 4 (c), 5 (d), 6 (e) and 8 (f) at young ages. Note the clinical similarities, including a prominent forehead, a thin upper lip and a broad nasal bridge. Reproduced with permission from Helsmoortel et al., 2004.

have had gastro-intestinal problems, including esophageal reflux disease, frequent vomiting, and constipation. Most of the children have recurrent infections, including upper respiratory tract and urinary tract infections. Sleep problems are present in five of eleven patients, with good response on melatonin. Several patients are obese. Eight patients have had brain imaging by MRI. Five of them showed abnormalities, including atypical white matter lesions, wide ventricles, and choroid cysts. Behavioral problems are common. Two children were reported to have anxiety problems, three have an obsessive compulsive disorder and two have affectional problems. One of them shows aggressive behavior.

ADNP mutation patients share several clinical features with Coffin-Siris patients, namely feeding difficulties, gastrointestinal problems, visual impairment, frequent infections, hypotonia, structural central nervous system abnormalities, speech impairment, intellectual disability, developmental delay, and behavioral problems (Table I). The

***ADNP* mutation patients share several clinical features with Coffin-Siris patients, namely feeding difficulties, gastrointestinal problems, visual impairment, frequent infections, hypotonia, structural central nervous system abnormalities, speech impairment, intellectual disability, developmental delay and behavioral problems (Table I).**

ADNP patients identified so far do not have a coarse face with sparse scalp hair, hypertrichosis, thick eyebrows or long eyelashes as observed in patients with Coffin-Siris syndrome, nor have intra-uterine or postnatal growth retardation been reported (Tables I and II).

ADNP IN nBAF (mSWI/SNF) COMPLEXES

In vertebrates, ATP-dependent chromatin remodeling is mediated by BAF complexes, which are the functional equivalents of the SWI/SNF complexes in yeast. The human BAF complex consists of 15 subunits, where one of both homologue ATPase core subunits, SMARCA4 or SMARCA2, is always present [Ronan et al., 2013]. Additional components vary with developmental stage and tissue, with theoretically several hundred possible configurations. The exact subunit composition of the complex determines the final functional characteristics and tissue specificity. With regard to neuronal development, a switch of three subunits in the complex composition predisposes the neuronal progenitor cells to a post-mitotic state and initiates activity-dependent dendritic outgrowth and axonal development. This transition occurs in all neurons, and illustrates the fundamental role of BAF complexes in development [Lessard et al., 2007]. Defects hampering the global function of the complex can result in multiple defects in an organism [Ho and Crabtree, 2010]. Illustrative for these global effects of deregulating BAF-mediated chromatin remodeling is that mutations in six components of the neuronal BAF (nBAF) complex (SMARCB1, SMARCA4, SMARCA2, SMARCE1, ARID1A, and ARID1B) have been reported to cause distinct, albeit overlapping, syndromic ID disorders [Santen et al., 2013]. These conditions are now commonly referred to as the "SWI/SNF-related ID syndromes" [Kosho et al., 2013].

Ample evidence exists that ADNP is of key importance for proper functioning of the nBAF complex. This functional relation between ADNP and the nBAF complex is mediated by direct protein-protein interaction of ADNP with several of the BAF subunits [Mandel and Gozes, 2007]. ADNP directly binds to SMARCA2, SMARCA4, and SMARCC2, through its C-terminal end (Fig. 4). Furthermore, reciprocal interactions have been demonstrated between ADNP, the

Ample evidence exists that ADNP is of key importance for proper functioning of the nBAF complex. This functional relation between ADNP and the nBAF complex is mediated by direct protein–protein interaction of ADNP with several of the BAF subunits. ADNP directly binds to SMARCA2, SMARCA4 and SMARCC2, through its C-terminal end.

heterochromatin-enriched protein HP1α, and BAF complexes. ADNP is recruited to the pericentromeric heterochromatin through binding of the P*V*L and ARKs motif to HP1α. It can be hypothesized that ADNP by its zinc fingers and homeobox domain functions as an anchoring protein that binds to specific positions in the DNA to guide the protein remodeling complex to its target regions. This way, dysfunction of ADNP results in deregulation of BAF-mediated chromatin remodeling.

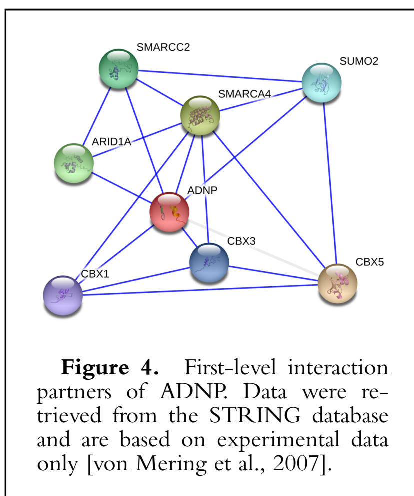


Figure 4. First-level interaction partners of ADNP. Data were retrieved from the STRING database and are based on experimental data only [von Mering et al., 2007].

Mutational Mechanism

The first reported patients all had stop mutations in the fifth and last exon of *ADNP*. Consistent with predicted escape from nonsense mediated decay for mRNA mutations in the last exon, *ADNP* mRNA levels were not downregulated in the four available cell lines of patients with stop mutations [Kervestin and Jacobson, 2012; Helsmoortel et al., 2014b]. Unexpectedly, instead of downregulation, overexpression of the *ADNP* transcripts was observed in these cell lines. Because three of the mutations clustered within basepairs of each other, we were able to discriminate between expression of the mutant and wild type mRNA. Despite the presence of only one copy of the ADNP gene in the genome, this analysis showed unaltered mRNA levels of the wild-type transcript and the excess level of mRNA in these patients corresponds to the amount of mutated mRNA. It would be interesting to see whether the mutated RNA is translated into protein, but Western blots have so far not been reported for these patients. Recently, the expression of the *ADNP* gene was reported to be auto-regulated by a negative feedback loop mechanism [Oz et al., 2012]. Hence, a plausible explanation for the observed overexpression in the cell lines of the patients is the inability of the mutant protein to bind to its own promoter region, resulting in a homeostatic correction by up regulation of transcription.

These observations are compatible with a dominant negative mutational mechanism. A dominant negative model is also in line with a discordance in over- or underexpression in our patients versus the results in mice/P19 cells for several genes, but p53 and ADNP2 [Vulih-Shultzman et al., 2007; Helsmoortel et al., 2014b]. However, since the initial report on 10 patients, an 11th patient has been identified with a stop mutation in the fourth exon, which is unlikely to escape nonsense mediated decay. Since the clinical presentation of this patient is not different from that of patients with a mutation in exon five, it challenges the dominant negative hypothesis as the

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only mutational mechanism. It would be interesting to investigate whether the homeostatic correction of *ADNP* mRNA levels observed in the initial patients with mutations in exon five, escaping nonsense mediated decay, is present in this patient, where mRNA degradation is predicted. Full homeostatic correction in the presence of NMD would favor the dominant-negative model, but leaves the clinical presentation of Patient 11 unexplained. A reduced *ADNP* expression in haploinsufficient samples would indicate that the mutant protein actively deregulates the feedback loop. Deregulation might be a consequence of the inability to harvest additional cofactors to the promoter region, while at the same time preventing wild-type protein to bind to the promoter binding site. Similarly, mutant *ADNP* might occupy alternative target sequences, while being unable to bind to HP1α or BAF. As such, despite the presence of functional BAF complexes, chromatin remodeling is hampered, leading to downstream alterations in gene expression patterns. To test this hypothesis, ChIP-seq and RNA-seq analysis of cell lines of the available patients could be useful.

Therapeutic Potential of NAP

The octapeptide NAP has femtomolar activity and is able to restore some of the anomalies caused by haploinsufficiency

of the entire ADNP protein [Bassan et al., 1999; Zamostiano et al., 2001]. In cellular models, it is able to protect the cells from chemical, electrical or stress induced damage [reviewed in Gozes et al., 2005]. The presumed mode of action is NAPs ability to bind to tubulin, facilitating microtubule assembly and increasing cellular survival. Davunetide, the drug name for NAP, is a candidate for the treatment of multiple selected neurological disorders [Gozes, 2011]. Intranasal and intravenous formulations of the drug exist and both have been shown to cross the blood-brain barrier [Gozes et al., 2000a; Leker et al., 2002]. The drug has been in Phase II and even in Phase III clinical trials and appears to be well tolerated without significant side effects [Magen and Gozes, 2014]. As stated above, specific cognitive abnormalities were also ameliorated by NAP in an *ADNP* heterozygous knockout mouse model. While these observations may raise hope for treatment in patients with *ADNP* mutations, it has to be noted that the mouse model has not been evaluated for autistic traits so far. Consequently, it is not known whether davunetide is able to interfere with the autistic traits. A second point of interest is that, generally speaking, drug testing in a mouse model needs independent confirmation because of the intrinsic variance associated with this type of experiments and because environmental conditions might interfere with the test result [Crabbe et al., 1999; Wahlsten et al., 2003].

EPILOGUE

The data presented show that mutations in the *ADNP* gene cause syndromic autism. These findings are in line with the observations by others that genes involved in the chromatin remodeling pathway are over represented in autism/ID. In contrast to related disorders caused by mutations in the SWI/SNF genes *SMARCB1*, *SMARCA4*, *SMARCA2*, *SMARCE1*, *ARID1A*, and *ARID1B*, *ADNP* is not part of the core nBAF complex, suggesting that a potentially much broader range of SWI/SNF related disorders might exist. All patients reported so far suffer from a combination of clinical characteristics that show some consistency. It should be realized though that all are in their childhood (5–12 years old) and we do not know the clinical presentation of older patients. At present the clinical heterogeneity appears too large to enable identification of additional patients on the basis of clinical selection criteria only. *ADNP*-related syndromic ASD seems to be more clinically heterogeneous than existing monogenic syndromes. However, this greater heterogeneity between patients with *ADNP* mutations might be caused by applying a more unbiased gene identification strategy. Until recently, patients were selected for screening a specific gene based on clinical similarity with an existing disorder. This biased selection procedure increases the chance of detecting a mutation in the target gene in two patients with convincing clinical similarity. In contrast, WES is applied on very large cohorts without phenotypic bias, except for a small set of broad inclusion criteria, such as ASD/ID. The clinical heterogeneity of these

TABLE III. Likely Pathogenic Variants in Coffin-Siris Genes, Present in the Exome Sequencing Project

Gene	Variant type	Protein change	ESP frequency	Phred (CADD)
ARID1A	Truncating	p.(M940Hfs*67)	0.00399361	33.0
	Truncating	p.(L2259Vfs*19)	0.003035144	37.0
ARID1B	Truncating	p.(V2005Wfs*16)	0.000159898	44.0
SMARCA2	Missense	p.(D82H)	0.000153775	27.6
	Missense	p.(P109A)	0.000153775	32.0
	Missense	p.(K862R)	0.000153775	26.9
	Missense	p.(V1567L)	0.001999077	26.9
	Missense	p.(D1573N)	0.002460403	28.5
SMARCA4	Missense	p.(R425Q)	0.000153775	34.0
	Missense	p.(R1119H)	0.000153775	31.0
	Missense	p.(S1209N)	0.000153775	24.0
	Missense	p.(R1463H)	0.000153846	34.0
	Missense	p.(V1501M)	0.000153775	24.5
SMARCE1	Missense	p.(G360D)	0.003229279	25.8
	Missense	p.(E336K)	0.000153775	27.5
	Missense	p.(R313H)	0.000461326	32.0
	Missense	p.(R229W)	0.000153775	23.6
	Missense	p.(R148C)	0.000153775	34.0

Genes were selected as causative for Coffin-Siris from Tsurusaki et al. [2012]. ESP frequency is combined frequency for all populations. Phred (CADD) is the Phred-scaled relative rank of CADD score for this variant [Kircher et al., 2014]. Scaled scores higher than 23 represent the 0.5% most likely pathogenic variants amongst all 8.6 billion possible genomic variants.

cohorts diminishes the chances that identical mutations are found in two or more patients with a strong clinical resemblance. If this hypothesis is correct, the phenotypic spectrum of many existing, clinically well delineated rare disorders is likely to expand in the post-exomic era [Yu et al., 2013].

It remains to be seen whether specific *ADNP* mutations may be associated with non-syndromic autism. The phenotypes of the patients reported here, range from severe autism, co-morbid with ID to less severely affected patients. It is very well possible that the phenotype in larger cohorts of patients with truncating or less damaging *ADNP* mutations will expand into a much milder range. Seemingly, support of this hypothesis comes from the presence of a likely pathogenic *ADNP* mutation in the ESP cohort. However, care should be taken in treating ESP as a healthy control cohort with regard to neurological disorders. This is illustrated by multiple likely pathogenic variants in several other genes involved in SWI/SNF related syndromes (Table III), we identified in the ESP database by in silico analysis [Tsurusaki et al., 2012; Kircher et al., 2014]. Unfortunately, detailed phenotypic information is not available on the individuals included in these control databases. The detailed molecular and phenotypical characterization of larger patient cohorts extending in all age groups is mandatory for a better understanding of the role of *ADNP* mutations in human disease.

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