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RESEARCH REPORT

Protective inherited mutations in activity-dependent neuroprotective protein (ADNP): the good, the bad, and the ugly

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Activity-dependent neuroprotective protein (ADNP), essential for brain formation/function, reveals multiple cytoplasmic and chromatin interacting sites. Computational modeling, alongside the Vineland Adaptive Behavior Scales, a leading instrument supporting the diagnosis of intellectual/developmental disabilities, now revealed a protective frame shift/stop mutation in ADNP. Thus, a woman with inherited mutation, ADNP_Glu931Glyfs*12 (VB), showed above average Vineland performance. Bioinformatics/*in silico* protein modeling indicated that while ADNP contains four 14-3-3 protein interaction sites (instrumental for ADNP nuclear/cytoplasmic shuttling), ADNP_Glu931Glyfs*12 contains an additional fifth 14-3-3 interaction site, implicating stronger associations. Furthermore, the endogenous neuroprotective (investigational drug, davunetide) NAPVSIPQ (NAP) site was involved in the ADNP and ADNP_Glu931Glyfs*12-14-3-3 interactions. In this respect, the mutation also enhanced ADNP-SH3 associations (another NAPVISP interaction site 354-361 aa on ADNP, critical for cytoskeletal/cellular signaling). HB, the 8-year-old VB's son, while inheriting the mother's ADNP mutation, further presented a heterozygous pathogenic *de novo* mutation ADNP, p.Arg730Thrfs*5. However, in comparison to carriers of a similar p.Arg730* mutation (part of the autistic/intellectual disability ADNP syndrome), HB exhibited overall better Vineland 3 standard score of 70–80 for all measures, compared to the nominal score of 20 in a 27-year-old ADNP, p.Arg730* subject and the 100 ± 15 norm, corroborating ADNP_Glu931Glyfs*12 protection.

Keywords: Activity-Dependent Neuroprotective Protein (ADNP), ADNP Syndrome (Helsmoortel Van Der Aa Syndrome), Davunetide (NAP), In Silico Modeling, Vineland Adaptive Behavior Scales.

Introduction

Discovered in our laboratory, activity-dependent neuroprotective protein (ADNP) (1, 2) is essential for brain formation (3). As such, aberrations in ADNP are associated with neurodevelopment (4), neuropsychiatry (5), and neurodegeneration (6, 7). The ADNP syndrome (also known as Helsmoortel Van Der Aa syndrome) is caused by *de novo* mutations in ADNP (8–10). Over the last years, we have established cellular (6, 11–14) and animal models (4, 15, 16) to characterize ADNP function and the potential protection by the ADNP-derived investigational drug, davunetide (also known as NAP, AL-108, CP201). Our haplo-insufficient *Adnp*$^{+/-}$ mouse model originally showed ADNP's/davunetide crucial function in cognitive protection through microtubule fortification, enhancement of axonal transport (17) and synapse formation (16), as well as inhibition of tauopathy (3). We have further shown a gain of toxic function with ADNP pathological mutation like the p.Tyr719* (p.Tyr718* in mice) associated with the ADNP nuclear localization signal (4). Thus, mice modeling this heterozygous truncation mutation exhibited more extensive motor (gait) impediments as well as early Tau deposition compared to the *Adnp*$^{+/-}$ mouse model. Davunetide provided significant protection in both cases (4). Mechanistically, through direct interaction with microtubule end binding proteins (EB1 and EB3) SH3 binding and association with Wnt signaling through the armadillo domain of beta catenin, davunetide enhances Tau binding to the microtubules, protecting the cytoskeleton while inhibiting tauopathy. This cytoskeletal fortification is further required to maintain nuclear envelope integrity protecting against accelerated aging/death (which is additionally accentuated with the p.Tyr718* mutation in mouse cell cultures) (13, 14). The correction by davunetide has been further extended to a human mutation, a novel davunetide (NAPVSIPQQ to NAPVSIPQE) point mutation in ADNP causing a mild developmental syndrome in a young child (9). Most importantly, davunetide has recently shown efficacy coupled with neuroprotection in women suffering from the neurodegenerative disorder, progressive supranuclear palsy, a late-onset tauopathy (18) as well as sex-dependent memory boost in elderly individuals presenting amnestic mild cognitive impairments, preceding Alzheimer's disease (19) .

Therefore, from a translational medicine point of view, we have been characterizing ADNP syndrome individuals utilizing the Vineland Adaptive Behavior Scales, a leading instrument for supporting the diagnosis of intellectual and developmental disabilities (20–22). Given potential genotype-phenotype differences and age-dependency, we have recently assessed a cohort of 15 individuals (1- to 27-year-old), using 1–3 longitudinal parent (caretaker) interview/s (Vineland 3 questionnaire) over several years (21). Our results indicated developmental delays, and potential developmental arrests. We have positively correlated symptom severity (e.g., communicative problems) with the heterozygous pathogenic ADNP allele protein size as well as with age (with all individuals seem to acquire an age equivalent of 1–6 years). Additionally, correlations were discovered between the two previously described mouse phenotypes alluded to above (4), which in humans translated to two epigenetic signatures in ADNP emphasizing aberrant acquisition of motor behaviors, with truncating mutations around the nuclear localization signal being mostly affected (21).

Further detailed analysis of ADNP mutations in transfected cell cultures revealed that the *de novo* addition of protein sequences in ADNP frame shift mutations may provide protection, such as the addition of SH3 binding site (12). Here, by *in silico* modeling coupled with Vineland 3 questionnaire, we investigated a rare case of inherited (mother, VB) and *de novo* plus inherited mutation in a son (HB), with only the son exhibiting ADNP syndrome characteristics.

The current paper reviews, cites, and focuses on our work on ADNP. Our original findings on ADNP stemmed from structure analysis of protective proteins stimulated by vasoactive intestinal peptide to provide protection and enhance synapse formation by neuroglial interaction (23–25). We have initially identified activity-dependent neurotrophic factor (23) and by molecular cloning, structure, and function (neuroprotection) analysis coupled to a reductionist approach of small active peptides identified ADNP and its investigational drug, davunetide (NAP) (1). By further structural analysis, we have identified ADNP2, which partly mimics ADNP, but does not contain the NAP (davunetide) motif (2). Together, this work connects structure predictions (currently awarded the Nobel prize

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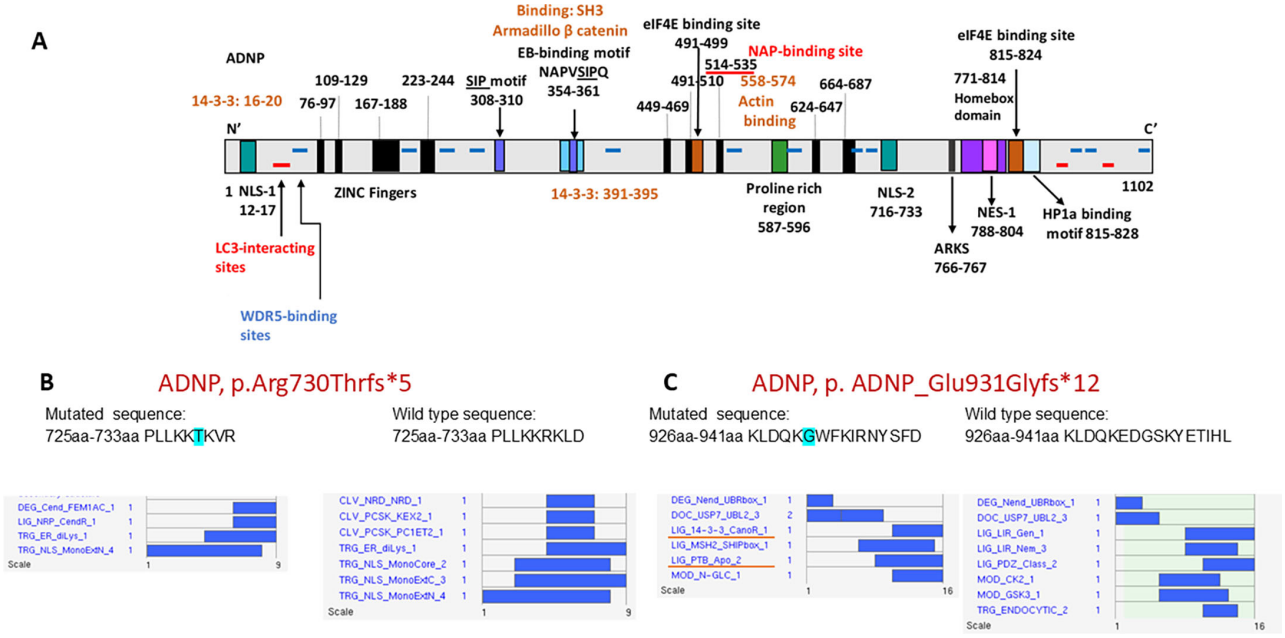

Figure 1. ADNP linear structure highlighting multiple protein interaction sites and presumptive additional domains of two different frameshift mutations. (A) ADNP linear structure, highlighting protein interaction domains. The structure was assembled based on our previous publications (1–7, 14, 16, 17, 28–33). (B) On the left, the potential additional domains added for ADNP with the mutation p.Arg730Thrfs*5 in the sequence 725-733 aa are displayed. On the right, the domains present in the compatible wild-type ADNP sequence 725-733 aa can be found. (C) On the left, the potential additional domains added for ADNP with the mutation ADNP, p. ADNP_Glu931Glyfs*12 in the sequence 926-941 aa are displayed. On the right, the domains present in the compatible wild-type ADNP sequence 926-941 aa can be found.

in chemistry (26, 27)) to functional behavior impacting our daily lives and illuminating the beauty of genomic psychiatry.

Results

The ADNP p.Arg730Thrfs*5 Frame Shift *de Novo* Mutation Does not Duplicate a Known ADNP Motif

Figure 1A shows selected molecular interaction motifs on ADNP indicating multiple partners and involvement in key cellular pathways. A most prevalent pathogenic mutation in the ADNP syndrome is p.Arg730* (34, 35). Here, a child presenting developmental delays was subjected to whole exome sequencing discovering a unique ADNP mutation p.Arg730Thrfs*5 (Figure 1B). The additional amino acids in this case are associated with proteasomal turnover (DEG_Cend_FEM1AC_1) as well as neuropilin (LIG_NRP_CendR_1), which is linked to protection against viral infection (Figure 1B). The potential additional domains, while not directly found in the native ADNP sequence, are linked to ADNP's function, with ADNP syndrome children being more susceptible to infections with slower recuperation time (34) and with ADNP regulated genes linked with the proteasomal system (35).

Potentially Accelerated Mutated Protein Degradation (http://elm.eu.org/). More specifically, regarding the additional DEG_Cend_FEM1AC_1 motif: "C-degrons play vital functions in targeting receptors of several cullin-RING E3 ligase complexes (CRLs) to initiate protein degradation. FEM1 proteins, including FEM1A/B/C, act as the receptors to specifically recognize C degrons ending with arginine (Arg (R)/C-degron) to enable CRL2-mediated proteasomal turnover. Cul2 ligase complexes are responsible for targeting substrates with arginine as their C-terminal residue (36) with some of the known substrates having a native C-termini ending in Arg (R)." Indeed, in HB case, a C-terminal R has been added because of the frameshift mutation.

Potential Protection Against Viral Infection (http://elm.eu.org/). Regarding the LIG_NRP_CendR_1 – "CendR Motif Binding to Neuropilin Receptors, neuropilins (NRPs) are vital multifunctional cell surface receptors playing important roles in various cellular signaling pathways that include VEGF-dependent vascular permeability, semaphorin-dependent axon guidance, angiogenesis, immunity, cell survival, migration, and invasion. NRPs specifically recognize a C-terminal motif, sometimes at a polybasic Furin cleavage site, known as the CendR motif. Physiological ligands such as VEGF-165 and semaphorin 3A interact with the b1 domain of NRP1 and promote cellular internalization. Several viruses such as EBV, HTLV-1, and Lujo also use NRP1 for cellular entry. Recently NRP1 has been identified as an entry point of SARS-CoV-2 via a Furin-generated CendR motif present in the viral S1 protein. Loss of NRP function results in significant cardiovascular and neuronal phenotypes and is also associated with embryonic lethality. Thus, NRPs play critical roles in both physiological and pathological contexts and are potential therapeutic targets for viral infection." With peripheral and circulating (37) ADNP directly linked to spleen protein expression (4) and immune response (38), these findings are of further interest.

ADNP Glu931Glyfs*12 Inherited Mutation Contains Additional 14-3-3 Binding Site (Nuclear Cytoplasm Shuttle) and a Phosphotyrosine Binding Domain – TRAIL (TNF-Related Apoptosis Inducing Ligand)

Four identified protein motifs added to ADNP because of the Glu931Glyfs*12 frame-shift-STOP mutation (Figure 1C) are of interest, as follows. LIG_MSH2_SHIPbox_1, mismatch repair contributing to the overall fidelity of DNA replication; N-linked glycosylation (MOD_N-GLC_1) is a co-translational process involving the transfer of a oligosaccharide chain to asparagine residue in the protein; 14-3-3 protein association (LIG_14_3_3_CanoR_1), with 14-3-3 formerly found to be involved in ADNP nuclear-cytoplasmic shuttling (28); and a PTB_Apo_2 Phosphotyrosine binding (LIG_PTB_Apo_2) domains recognizing short peptides with a core Asn-X-X-Tyr. The classical phosphotyrosine binding (PTB) domains bind the motif when it is phosphorylated on the Tyr residue (Figure 1C). Importantly, the last two added motifs are additional to similar internal ADNP motifs (Figures 2 and 3).

Concentrating on LIG_14_3_3_CanoR_1 Associating with the 14-3-3 Proteins, a Family of Conserved Regulatory Molecules That are Involved in Diverse Cellular Processes Through the Interaction with Hundreds of Different Proteins (http://elm.eu.org/). "In mammals, seven isoforms are present. 14-3-3 proteins form either homo- or heterodimers that

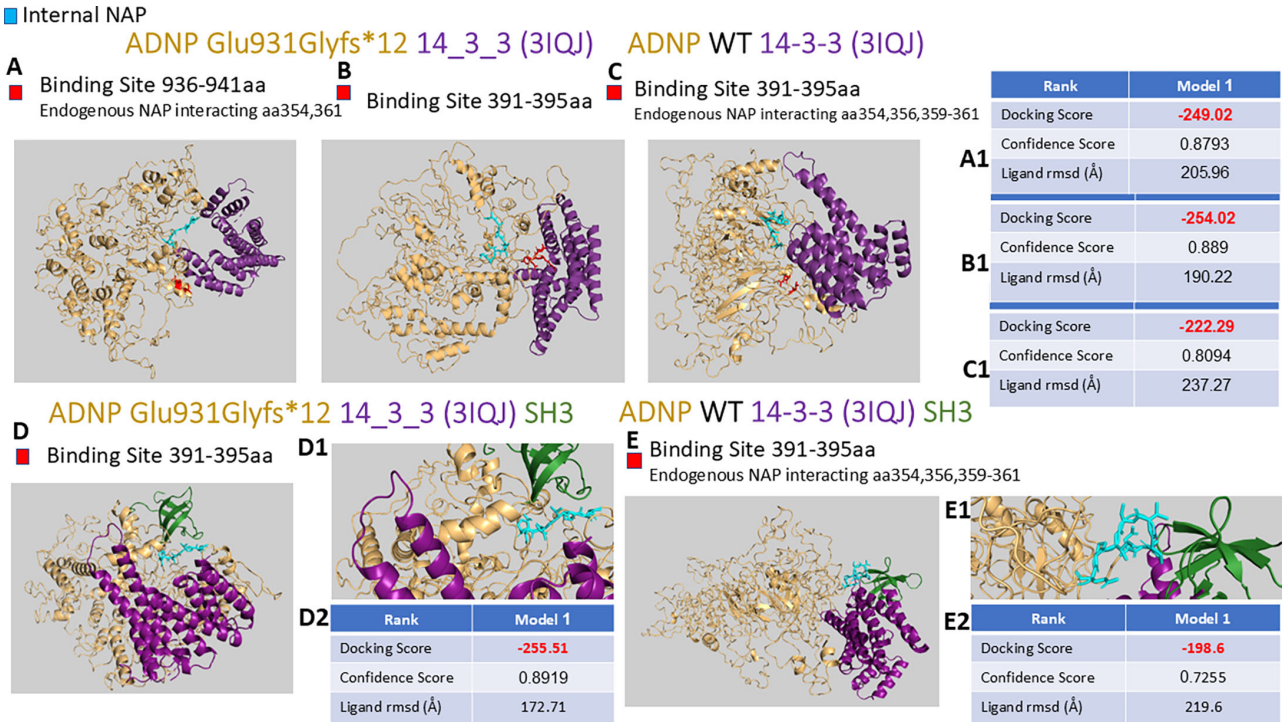

Figure 2. ADNP Glu931Glyfs*12 interaction with 14_3_3 and with an SH3 domain. (A) The results of docking ADNP_Glu931Glyfs12 (light orange) to the 14-3-3 protein (PDB code 3IQJ, dark purple) in the 936-941 amino acid region (red)—one of the 14-3-3 binding sites within ADNP_Glu931Glyfs12—presented here. The docking results indicate that the internal NAP (cyan) interacts with the 14-3-3 protein through residues 354 and 361. (A1) To evaluate the accuracy of the docking results, three measures were used. The docking score is a shape-based pairwise scoring function, where a lower value indicates better shape complementarity between the two docking elements. The confidence score reflects the reliability of the predicted binding mode. This score typically ranges from 0 to 1, with higher values indicating greater confidence in the predicted interaction. Finally, the Ligand RMSD measures the deviation of the protein's predicted position from other models. A smaller RMSD value is associated with greater confidence, as it suggests that the current model is like other docking models, thereby supporting its validity. (B) The results of docking ADNP_Glu931Glyfs12 (light orange) to the 14-3-3 protein (PDB code 3IQJ, dark purple) in the 391-395 amino acid region (red)—one of the 14-3-3 binding sites within ADNP_Glu931Glyfs12—presented here. The docking results indicate that the internal NAP (cyan) does not interact with the 14-3-3 protein. (B1) Three measures were used to evaluate the docking in Figure 2B: the docking score, the confidence score, and the ligand RMSD, as explained in A1. (C) The results of docking ADNP wild type (light orange) to the 14-3-3 protein (PDB code 3IQJ, dark purple) in the 391-395 amino acid region (red)—one of the 14-3-3 binding sites within ADNP wild type—presented here. The docking results indicate that the internal NAP (cyan) interacts with 14-3-3 through residues 354,356 and 359-361. (C1) Three measures were used as explained (A1). (2D) The results of docking ADNP_Glu931Glyfs12 (light orange) to the 14-3-3 protein (PDB code 3IQJ, dark purple) in the 391-395 amino acid region (red)—one of the 14-3-3 binding sites within ADNP_Glu931Glyfs12—presented here. The docking results indicate that the internal NAP (cyan) does not interact with the 14-3-3 protein. In addition, SH3 domain (dark green is docked to ADNP). (D1) A closer look at the docking (D). (D2) Three measures were used to evaluate the docking as A1. (E) The results of docking ADNP wild type (light orange) to 14-3-3 (PDB code 3IQJ, dark purple) in the 391-395 amino acid region (red)—one of the 14-3-3 binding sites within ADNP wild type—are presented here. The docking results indicate that the internal NAP (cyan) interacts with the 14-3-3 protein through residues 354,356 and 359-361. In addition, SH3 domain (dark green is docked to ADNP). (E1) A closer look at the docking is provided for E. (E2) Three measures were used to evaluate the docking in Figure 2E as above (A1).

target certain phosphoserine/threonine-containing motifs with a low micromolar affinity. Binding to a small set of unmodified proteins has also been reported. Phosphorylation-dependent and -independent binding occurs via the same deep ligand-binding groove. There are canonical arginine-containing motifs and a noncanonical motif group that are difficult to classify but utilize additional hydrophobic interactions. The canonical Arg-containing 14-3-3 binding peptides are phosphorylated by members of basophilic kinases."

Specifically concentrating on ADNP – 14-3-3 protein association (Figure 2A–C) we showed that the additional ADNP mutated site (aa936-941) interacted *in silico* with further association of aa354,361 in NAPVSIPQ (spanning aa354-361 in ADNP, Figure 2C). In the Glu931Glyfs*12 mutated ADNP, the internal aa391-395/14-3-3 interaction, did not show further interaction with NAP aa (Figure 2B), contrasting the control/nonmutated ADNP showing extensive interaction with NAP aa354,356,359-361. The most favorable docking score was with the internal site on the mutated ADNP (−254.02) (Figure 2A1), with second best being on the additional site in the mutated ADNP (Figure 2A2), and the least favorable on the native ADNP (Figure 2A3). With SH3 domains interacting with the NAP sequence, docking SH3 together with 14-3-3 resulted in a much favorable interaction with the mutated protein internal interaction site (−255.51) (Figure 2D, D1, D2) versus (−198.6) for the native protein, including a more extensive NAP involvement (Figure 2E, E1, E2).

Focusing on LIG_PTB_Apo_2, PTB Domains Recognizing Short Peptides with a Core Asn-X-X-Tyr Motif Preceded by a Short Peptide Segment That Docks by Beta Augmentation (http://elm.eu.org/). "The classical PTB domains bind the motif when it is phosphorylated on the Tyr residue. However other PTBs recognize essentially the same motif when unmodified."

Further focusing on PTB and ADNP also resulted in a much-preferred mutated protein interaction with PTB (Figure 3, −264.85 vs. −133.06). Similarly, the additional site showed preferred interaction −268.88.

Glu931Glyfs*12 is a Protective Mutation and p.Arg730Thrfs*5 May be Less Deleterious than p.Arg730*

Vineland 3 analysis indicated that VB (aged 43 years) Vineland 3 standard scores (SS) were above average in terms of performance, with SS of communication domain of 106, daily living skills of 107, socialization

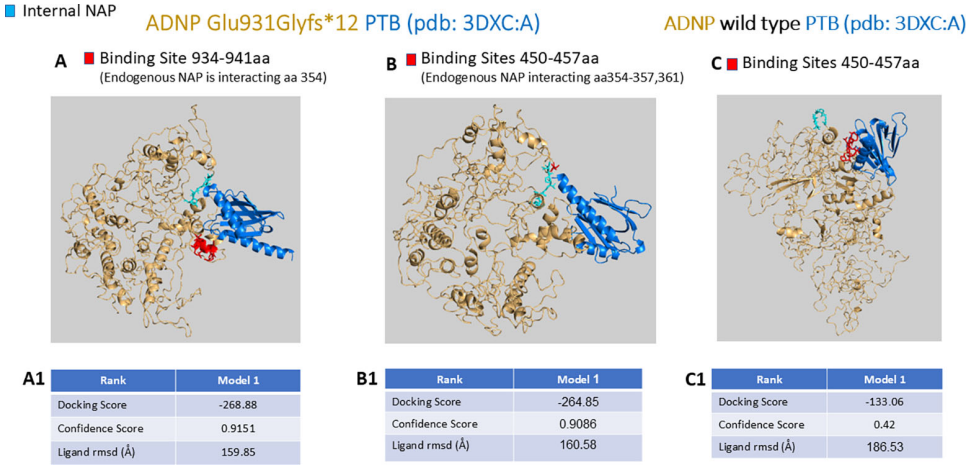

Figure 3. Docking of ADNP_Glu931Glyfs12 to the PTB protein. (A) The results of docking ADNP_Glu931Glyfs12 (light orange) to the PTB protein (PDB code 3DXC, navy blue) in the 934-941 amino acid region (red)—one of the PTB binding sites within ADNP_Glu931Glyfs12—are presented here. The docking results indicate that the internal NAP (cyan) interacts with the PTB protein through residue 354. (A1) Three measures were used to evaluate the docking as explained in Figure 2A1. (B) The results of docking ADNP_Glu931Glyfs12 (light orange) to the PTB protein (PDB code 3DXC, navy blue) in the 450-457 amino acid region (red)—one of the PTB binding sites within ADNP_Glu931Glyfs12—are presented here. The docking results indicate that the internal NAP (cyan) interacts with the PTB protein through residues 354-357 and 361. (B1) Three measures were used to evaluate the docking as in Figure 2A1. (C) Docking ADNP wild type (light orange) to the PTB protein (PDB code 3DXC, navy blue) in the 450-457 amino acid region (red)—one of the PTB binding sites within ADNP wild type—are presented. The docking results indicate that the internal NAP (cyan) does not interact with the PTB protein. (C1) As above, three measures were used to evaluate the docking in C as explained in Figure 2A1.

domain of 107, and the Adaptive Behavior Composite (ABC) of 108. Figure 4 shows HB results (aged 8 years), with SS averaging 70–80, much higher than scores observed before for ADNP syndrome (21), (averaging 35–53 for 10 ADNP syndrome randomized individuals with some tested longitudinally). Regardless, when using the Random Forest algorithm (with Weka 3.8.6) to classify the two instances of HB and VB, based on the Vineland 3 data presented above (21) and control set of an equal number of instances with values randomly generated between 85 and 115, the results clearly classified the mother (VB) as normal, and the child (HB) as having ADNP mutation deficits, albeit within the higher performing ADNP syndrome individuals.

Discussion

We discovered here correlations between ADNP structure and function on the adaptive behavior scale looking at yet undescribed ADNP mutations. Our paper underscores the importance of diving into precise details. As such, VB's inherited mutation, ADNP p.Glu931Glyfs*12, shows a Vineland 3 SS slightly above average results of 100, while our previous study (21) showed 50–60 SS results for a child with an ADNP mutation at p.955Argfs*36. Thus, the inherited ADNP p.Glu931Glyfs*12 is apparently protective against the potential p.Arg730Thrfs*5 effects, possibly through the acquired interaction with 14-3-3, important for ADNP cytoplasmic localization (28).

Indeed, HB mutation analysis identified the pathological mutation, p.Arg730Thrfs*5. Interestingly, another variation of the ADNP syndrome prevalent p.Arg730* mutation, was also found in postmortem Alzheimer's disease brains, namely, p.Arg730Thrfs*4 with this somatic mutation frequency correlated to Braak stage (tauopathy) and aging (6). Interestingly those mutations present a lost caspase binding site on (ADNP aa733-738) (39). However, as elaborated in the *Results*, the p.Arg730Thrfs*5 contains a C-terminal Arg (R) serving as a protein degradation signal (36). Indeed, the acquired C-terminal R could contribute to the rapid elimination of the mutated protein. With further ADNP autocrine regulation of its expression (29), this could result in increased ADNP p.Glu931Glyfs*12, encompassing the apparent better clinical outcomes.

To further put findings into context, we have previously reviewed the ADNP literature discussing genotype/phenotype correlations (19, 40), expanding on skin-related abnormalities (40). Here, summarizing new

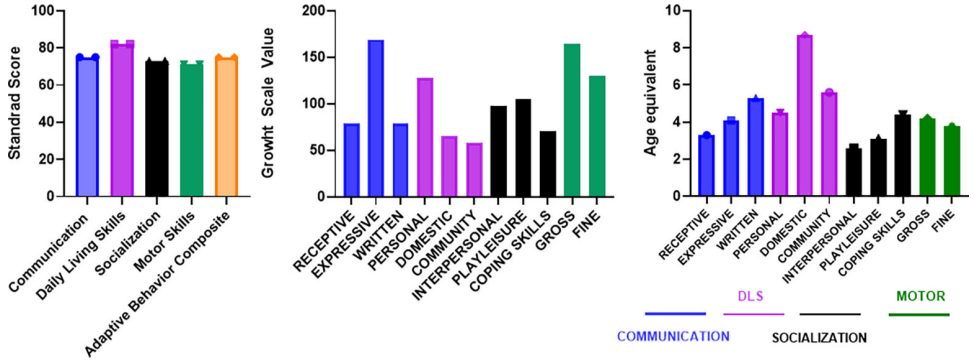

Figure 4. Vinland adaptive behavior questionnaire. This figure presents the results of the comprehensive version of the Vineland Adaptive Behavior questionnaire for HB (8-year-old) including that of the AE scores, the GSV scores both for the different subdomains of this questionnaire as well as the SS for the different domains of the questionnaire along with the SS for the ABC. Confidence interval was taken at the level of 90%. Notice that all standard score values are above 70 while the age equivalent in the domestic subdomain is even above that of a child of 8 years.

discoveries in 2024, we would like to highlight a phenotype of narrow eye openings, droopy eyelids, and folds of skin on the inner part of the eyes going from the bottom to the upper corner called blepharophimosis and associated with pathological protein truncating mutations around the nuclear localization signal of ADNP, compared to other truncating mutations (41). Another structure and function mutation in ADNP, namely, (Gln423Serf*17) exhibits renal abnormalities and polycystic ovarian disease, which may be specifically associated with this variant (42). Indeed, the additional amino acid sequence here is SVQFQTCCSCHRPSPR, which includes the sequence SVQF, interacting with caspases associated with tissue well-being (ELME000285), for example, ref. 39.

Moreover, the genetic background as well as environmental effects are instrumental in the manifestation of the ADNP mutation phenotype. For example, models of zebrafish of different strains (43), may show variable phenotypic outcomes (44, 45), as well as differential susceptibility to environmental stress (45), further capitulated in mouse models of ADNP deficiency (4, 46).

Importantly, additional study limitations include interventions methods, which may accelerate development and contribute to the apparently improved outcome in the Vineland questionnaire results. In the case of HB, given the fact that the ADNP syndrome genotype is associated with microbiome alteration (4, 47), a father son fecal transfer was performed, which resulted in an apparent improvement (VB, personal communication). Regardless, it should be kept in mind that this is a case study of unique mutations with work focusing on structure and function relations in the clinical scenario. Future studies should aim at biochemical, cellular, and animal modeling, gaining further insights and better understanding.

Regardless, the current study, and similar structural/function studies (e.g., refs. 9, 12, 21) are of importance, also in terms of therapeutic development, with intellectual disability being a major impediment in ADNP children and adults and with davunetide (NAP) showing memory boosting and protection of functional daily activities in mice and humans with the same underlying pathology that is regulated by ADNP (16, 18, 19, 48, 49). Specifically interesting in this case, davunetide (NAP) has shown corrective effects on altered ADNP syndrome-like gut microbiota composition in two independent ADNP mouse models (4, 47), correlating with behavioral improvement, which are of direct translational efficiency in HB's case, enjoying the benefits of fecal transfer, and much beyond. For more information, from a personal perspective, please see an overview of ADNP and davunetide discovery and development (50).

Materials and Methods

Subjects

Two subjects are discussed here, a mother (VB) with an inherited mutation ADNP p.Glu931Glyfs*12, that is, ADNP 921 SESEEKLDQKGWF KIRNYSFD* 942 (single amino acid code) versus control, 921 SESEEK LDQKEDGSKYETIH 939 and an 8-year-old son (HB) with the inherited mutation as well as a *de novo* mutation, ADNP p.Arg730Thrfs*5, that is, 721 QMEFPLLKKTKVR* 737, versus control, 721 QMEFPLLKKRKLDDDSD-SPS 740.

In Silico Modeling

We used the eukaryotic linear motif (ELM) resource for assessement of functionality (51).

I-TASSER (https://zhanggroup.org/I-TASSER) was used for protein structure modeling and HDOCK (http://hdock.phys.hust.edu.cn/) was used for *in silico* protein/protein docking.

PyMOL software was used to create figures.

Vineland 3 Questionnaire

The Vineland 3 (52) is a standardized measure of adaptive behavior—describing what subjects actually do to function in their everyday lives. A precise description of the assessment and data analysis was described previously (21, 22). In short, this questionnaire includes several domains as follows. 1) The Communication domain contains three subdomains: receptive, expressive and written. 2) The Daily Living Skills domain containing three subdomains: personal, domestic, and community. 3) The Socialization domain contains three subdomains: interpersonal relationships, play and leisure and coping skills. 4) The Motor Skills domain containing two subdomains: Gross motor and Fine motor. This domain is normed only through the age of 9 years.

The assessments of adaptive behaviors were carried out in a quiet comfortable place by interviewing the parents using the Zoom platform. Individuals were assessed at three levels (1–3, below) as per the definitions of the comprehensive version of the Vineland Adaptive Behavior Scales.

1) SSs provide a standardized measure of the individual's adaptive behavior relative to their same-aged peers. The Vineland 3 questionnaire uses a SS range of 1–160, with a mean score of 100 and a standard deviation of 15. A SS of 100 is considered average, with scores below 70 indicating significant deficits in adaptive behavior.
2) Growth Scale Values (GSV) are designed to measure change over time. Like a raw score, the GSV score is an indicator of absolute, not relative, performance.
3) Age-equivalent (AE) scores represent the age at which the individual's adaptive behavior is typically observed. It is important to note that AE scores should not be interpreted as the individual's developmental age or intellectual ability.

It is important to interpret both AEs and SSs together, as they provide different but complementary information about an individual's adaptive behavior. While AEs give a general sense of the individual's adaptive functioning in different subdomains, SSs offer a more precise measure of the individual strengths and weaknesses compared to his/her same-aged peers in the different domains as well as an ABC, which is based on the SSs for three specific adaptive behavior domains: Communication, Daily Living Skills, and Socialization.

GraphPad Prism 7.0 software included D'Agostino and Pearson test for normality coupled with log10 transformation to achieve normal distribution.

Random Forest Algorithm (with Weka 3.8.6)

Given the small sample size, Random Forest using bootstrapping, as part of its ensemble learning process, was applied. In a random forest, multiple decision trees are trained on different subsets of the training data. These subsets are created using bootstrapping, which involves randomly sampling the data with replacement. This means that some samples may be repeated in a subset, while others may be left out. This technique helps to increase the diversity among the trees, which can improve the overall performance of the model. Thus, to further compare HB to the normal (SS 85-115) versus the ADNP syndrome population, a randomly selected previous data were used (21) alongside with a control set of an equal number of instances with SS values randomly generated between 85 and 115.

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Author Contributions

I.G. initiated and led the study. E.G. mapped the frame shift additional sequences. S.S. provided the molecular models in consultation with I.G., Y.L. implemented the Vineland questionnaire. I.G. wrote the paper with editorial inputs from all authors.

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Author Disclosures

I.G. serves as VP Drug Development at Exonavis Therapeutics Ltd. Developing davunetide for the ADNP syndrome (under patent protection, I.G. inventor). Other contributors have confirmed that no conflict of interest exists. The manuscript has been read and approved by all authors.

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