BRIEF COMMUNICATION

Missense NAA20 variants impairing the NatB protein N-terminal acetyltransferase cause autosomal recessive developmental delay, intellectual disability, and microcephaly

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PURPOSE: N-terminal acetyltransferases modify proteins by adding an acetyl moiety to the first amino acid and are vital for protein and cell function. The NatB complex acetylates 20% of the human proteome and is composed of the catalytic subunit NAA20 and the auxiliary subunit NAA25. In five individuals with overlapping phenotypes, we identified recessive homozygous missense variants in NAA20.

METHODS: Two different NAA20 variants were identified in affected individuals in two consanguineous families by exome and genome sequencing. Biochemical studies were employed to assess the impact of the NAA20 variants on NatB complex formation and catalytic activity.

RESULTS: Two homozygous variants, NAA20 p.Met54Val and p.Ala80Val (GenBank: NM_016100.4, c.160A>G and c.239C>T), segregated with affected individuals in two unrelated families presenting with developmental delay, intellectual disability, and microcephaly. Both NAA20-M54V and NAA20-A80V were impaired in their capacity to form a NatB complex with NAA25, and in vitro acetylation assays revealed reduced catalytic activities toward different NatB substrates. Thus, both NAA20 variants are impaired in their ability to perform cellular NatB-mediated N-terminal acetylation.

CONCLUSION: We present here a report of pathogenic NAA20 variants causing human disease and data supporting an essential role for NatB-mediated N-terminal acetylation in human development and physiology.

INTRODUCTION

N-terminal acetylation is a common protein modification in eukaryotes, and approximately 80% of all human proteins carry this modification [1, 2]. Although not fully understood, N-terminal acetylation may have a range of functional consequences for the modified proteins including stability/degradation, subcellular targeting, and complex formation [1]. NatB is one of the major eukaryotic N-terminal acetyltransferases (NATs) acetylating around 20% of the human proteome in a cotranslational manner. Proteins harboring Met-Glu-, Met-Asp-, Met-Gln-, and Met-Asn-N-termini are substrates of NatB [3]. The catalytic subunit NAA20 forms a stable heterodimer with the large ribosomal anchor subunit NAA25 [4, 5]. NatB activity has been linked to cancer cell survival and progression [4, 6, 7] as well as shutoff activity of influenza A virus and viral polymerase activity [8] and NAD+/NADH metabolism [9]. However, no genetic disease has so far been linked to pathogenic variants of the NAA20 or NAA25 genes.

We report here five affected individuals of two unrelated families presenting with developmental delay (DD), intellectual disability (ID), and microcephaly. Homozygous NAA20 variants (MIM 610833) segregated with the phenotypes. Protein studies revealed impaired functionality of both identified variants supporting that reduced cellular N-terminal acetylation is causal for disease.

MATERIALS AND METHODS

NAA20 variants were discovered through exome or genome sequencing after clinical evaluation. Contact between clinicians and researchers was mediated by GeneDx/GeneMatcher [10]. For further experimental details, see Supplemental Materials and Methods.

RESULTS

Genetic findings

The index case in family 1, a 13-year-old female (F1:V.2) (Fig. 1a) of Saudi origin, was referred for neuropsychological evaluation for baseline cognitive assessment because of her global DD and significant ID. She is the eldest of three siblings, with a healthy sister and a brother similarly suffering from DD and ID (F1:V.4). Parents are both healthy and are paternal cousins. Exome sequencing performed on DNA from the two affected siblings uncovered a homozygous missense variant of uncertain significance in NAA20 (NM_016100.5): c.160A>G (p.Met54Val) (GenBank: NM_016100.4). We employed both positional mapping to highlight candidate common regions within the genomes of the

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Fig. 1 Rare NAA20 variants segregating with developmental phenotypes in two families. (a) Pedigrees of family 1 and family 2 with affected members indicated as filled circles (females) or squares (males). Triangle: pregnancy not carried to term. Double horizontal lines indicate consanguinity. Wt and mut indicate absence or presence of the NAA20 variants, respectively. (b) Three-dimensional structure model of NAA20 and NAA25 visualizing the positions of the variant sites in the wildtype NAA20 structure. Gray, NAA25; blue, NAA20; green, Ac-CoA; yellow, substrate peptide.
types of NatB substrates, Met-Glu-, Met-Asp-, Met-Gln-, and Met-Asn-. While NAA20-M54V exhibited a reduced NatB activity toward all four substrate classes, NAA20-A80V displayed alterations in a substrate-specific manner. NAA20-A80V was not reduced in its capacity to acetylate a Met-Asp substrate, but it revealed a significant loss in its capacity to acetylate Met-Glu, Met-Asn, and Met-Gln substrates (Fig. 2c). Since the catalytic subunit NAA20 depends on complex formation with NAA25 to form the active NatB complex on the ribosome, impaired binding between NAA20 and NAA25 will result in less active NatB complexes capable of modifying nascent polypeptides, including those starting with Met-Asp. In addition, the decreased intrinsic activities of NAA20-M54V and NAA20-A80V will further reduce the cellular N-terminal acetylation of many NatB substrates. In sum, both NAA20 variants
are less competent than NAA20-WT in performing cellular NatB-mediated N-terminal acetylation of Met-Glu, Met-Asp, Met-Gln, and Met-Asn-N-termini.

**DISCUSSION**

Based on our functional studies, it is highly likely that the individuals homozygous for the NAA20 c.160A>G (p.Met54Val) or NAA20 c.239C>T (p.Ala80Val) variants suffer from impaired NatB-mediated N-terminal acetylation of numerous cellular substrates. Because there are several thousand different NatB substrates in human cells [3] and because NatB steers many cellular pathways [1], pathogenic NAA20 variants are likely to have pleiotropic effects. This fits well with the overall findings of DD and ID in all individuals. However, NAA20-M54V and NAA20-A80V displayed differences in their substrate specificities, with NAA20-M54V relatively more impaired in its ability to acetylate Met-Asp substrates while NAA20-A80V was comparatively less active toward the other substrate types (Fig. 2c). This might suggest that there are also certain cellular NatB substrates that are specifically impacted for each of these two NAA20 variants. Thus, unique clinical findings for affected individuals harboring a specific NAA20 variant may relate to disrupted signaling via specific NatB substrates only impaired for a specific variant (Fig. 2d). For example, only affected family 2 individuals, not affected family 1 individuals, presented with cardiac anomalies (Table S2). However, more individuals need to be identified to properly define the genotype–phenotype relationship, and differences in genetic background between individuals may significantly contribute to observed phenotypic differences.

In humans, seven distinct NAT enzymes (NatA–NatF and NatH) have been identified [1]. Each NAT is composed of unique subunits and catalyzes N-terminal acetylation of a unique set of substrates. NatA–NatE perform cotranslational N-terminal acetylation. While NatA, NatB, and NatC perform bulk acetylation of large substrate pools, NatD and NatE have more specialized roles toward a few substrates. In contrast, NatF and NatH act posttranslationally toward transmembrane proteins and actins, respectively [1].

Until now, pathogenic variants were only identified for genes encoding the catalytic NAA10 and auxiliary NAA15 subunits of the NatA complex. In 2011, the lethal X-linked Ogden syndrome was presented. Eight boys harboring a NAA10 missense variant displayed an aged appearance, craniofacial anomalies, hypotonia, global DD, cryptorchidism, and cardiac arrhythmias [11]. Investigations in budding yeast and patient cells suggested that a reduced NatA-mediated N-terminal acetylation was involved in disease etiology [12–14]. In the last decade, a number of additional pathogenic NAA10 variants were identified in boys and girls presenting with ID, DD, and cardiac abnormalities [15–17]. Distinct phenotypes such as Lenz microphthalmia syndrome (MIM 309800) were also correlated to specific effects of some variants. The potential multifunctionality of NAA10 as a monomeric NAT and KAT in addition to its role as a catalytic subunit of the NatA complex (together with NAA15) makes it very challenging to define disease mechanisms [1], although some variants are more impaired in NatA function while others are more impaired in monomeric NAA10 function. More recently, patients harboring pathogenic NAA15 variants also presented with phenotypes partially overlapping with those observed for NAA10 variants, including cohorts of patients with congenital heart disease and autism spectrum disorder [17–20]. Thus, it is likely that impaired NatA-mediated N-terminal acetylation is at least in part causative for disease seen in these individuals. Despite the fact that NatA and NatB acylate unique subsets of cellular substrates, at present, it is difficult to distinguish between NatA and NatB-mediated impairment of N-terminal acetylation at the level of human pathophysiology. This is due to the pleiotropic nature of overlapping phenotypes as well as extensive phenotype variability among individuals with pathogenic NAA10, NAA15, and NAA20 variants. Microcephaly is potentially a distinguishing parameter that is only found in some NAA10 and NAA15 variant cases [17], but was found in this study among all affected individuals with NAA20 variants (Table S2). Unlike NAA10 and NAA15, NAA20 appears to be more tolerant to haploinsufficiency (probability of loss of function intolerance [pLI] = 0.01) and less constrained for missense variation (Z = 0.31). These characteristics are consistent with the strictly recessive inheritance of the variants we report in this study in NAA20 in contrast to the monoallelic disease-causing variants reported previously in NAA10 and NAA15.

In conclusion, we present here pathogenic NAA20 variants that disrupt NAA20 function and support an essential role for NatB-mediated N-terminal acetylation in human development and physiology. All affected individuals display DD, ID, and microcephaly. We propose to use the term NAA20-related syndrome to describe this novel disorder caused by pathogenic NAA20 variants.

**DATA AVAILABILITY**

The NAA20 variants with accession numbers are available at LDV: https://databases.l dov.nl/shared/variants/000076362000014229 and https://databases.ldov.nl/shared/variants/000076361900014229.

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REFERENCES

1. Aksnes H, Ree R, Arnesen T. Co-translational, post-translational, and non-catalytic roles of N-Terminal acetyltransferases. Mol Cell. 2019;73:1097–1114.

2. Arnesen T, Van Damme P, Polevoda B, Helsens K, Ejvendh R, Colaert N, et al. Proteomics analyses reveal the evolutionary conservation and divergence of N-terminal acetyltransferases from yeast and humans. Proc Natl Acad Sci U S A. 2009;106:8157–8162.

3. Van Damme P, Lasa M, Polevoda B, Gazquez C, Elsosegui-Artola A, Kim DS, et al. N-terminal acetylome analyses and functional insights of the N-terminal acetyltransferase NatB. Proc Natl Acad Sci U S A. 2012;109:12449–12454.

4. Starheim KK, Arnesen T, Gromyko D, Ryningen A, Varhaug JE, Lillehaug JR. Identification of the human N(alpha)-acetyltransferase complex b (hNatB): a complex important for cell-cycle progression. Biochem J. 2008;415:325–331.

5. Polevoda B, Cardillo TS, Doyle TC, Bedi GS, Sherman F. Nat3p and Mdm20p are required for function of yeast NatB Nalpha-terminal acetyltransferase and of actin and tropomyosin. J Biol Chem. 2003;278:30686–30697.

6. Neri L, Lasa M, Elsosegui-Artola A, D’Avola D, Carte B, Gazquez C, et al. NatB-mediated protein N-alpha-terminal acetylation is a potential therapeutic target in hepatocellular carcinoma. Oncotarget. 2017;8:40967–40981.

7. Aksnes H, Ree R, Arnesen T. Co-translational, post-translational, and non-catalytic roles of N-Terminal acetyltransferases. Mol Cell. 2019;73:1097–1114.

8. Oishi K, Yamayoshi S, Kozuka-Hata H, Oyama M, Kawaoka Y. N-Terminal acetylation by NatB is required for the shutoff activity of influenza A virus PA-X. Cell Rep. 2016;24:851–860.

9. Croft T, Venkatakrishnan P, Raj CJT, et al. N-terminal protein acetylation by NatB modulates the levels of Nmnats, the NAD(+)-biosynthetic enzymes in Saccharomyces cerevisiae. J Biol Chem. 2020;295:7362–7375.

10. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2008;27:7296–7306.

11. Oishi K, Yamayoshi S, Kozuka-Hata H, Oyama M, Kawaoka Y. N-Terminal acetylation by NatB is required for the shutoff activity of influenza A virus PA-X. Cell Rep. 2016;24:851–860.

12. Myklebust LM, Van Damme P, Stave SI, Dörfl M, Abboud A, Kalvik TV, et al. Biochemical and cellular analysis of Ogden syndrome reveals downstream N-acetylation defects. Hum Mol Genet. 2015;24:1956–1976.

13. Van Damme P, Stove SI, Glomnes N, Arnesen T. A Saccharomyces cerevisiae model reveals in vivo functional impairment of the Ogden syndrome N-terminal acetyltransferase NAA10 Ser37Pro mutant. Mol Cell Proteomics. 2014;13:2031–2041.

14. Dorfl MJ, Fang H, Crain J, Klingener M, Weiser J, Lyon GJ. Proteomic and genomic characterization of a yeast model for Ogden syndrome. Yeast. 2017;34:19–37.

15. Esmaipour T, Riazifar H, Liu L, Donkervoort S, Huang VH, Madaan S, et al. A splice donor mutation in NAA10 results in the dysregulation of the retinoic acid signalling pathway and causes Lenz microphthalmia syndrome. J Med Genet. 2014;51:185–196.

16. Saunier C, Stave SI, Popp B, Gérard B, Blenski M. Expanding the phenotype associated with NAA10 related N-terminal acetylation deficiency. Hum Mutat. 2016;37:755–764.

17. Cheng H, Gottlieb A, Marchi E, Kleyner R, Bhadjwaj P, Rope AF, et al. Phenotypic and biochemical analysis of an international cohort of individuals with variants in NAA10 and NAA15. Hum Mol Genet. 2019;28:2900–2919.

18. Cheng H, Dharmadhikari AV, Varland S, Ma N, Domingo D, Kleyner R, et al. Truncating variants in NAA15 are associated with variable levels of intellectual disability, autism spectrum disorder, and congenital anomalies. Am J Hum Genet. 2018;102:985–994.

19. Ward T, Tai W, Morton S, Impens F, Van Damme P, Van Haver D, et al. Mechanisms of congenital heart disease caused by NAA15 haploinsufficiency. Circ Res. 2021;128:1156–1169.

20. Ritter A, Berger JH, Deardorff M, Izumi K, Lin KY, Medne L, et al. Variants in NAA15 cause pediatric hypertrophic cardiomyopathy. Am J Med Genet A. 2021;185:228–233.

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ETHICS DECLARATION
The study was conducted in accordance with the principles of the Declaration of Helsinki and written informed consent was obtained from adult participants and legal guardians of child participants. The study was approved by the institutional review board (IRBs) at the Icahn School of Medicine at Mount Sinai (13-00495) and the King Faisal Specialist Hospital and Research Center (RAC 212053).

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
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