ORIGINAL ARTICLE

NAD Deficiency, Congenital Malformations, and Niacin Supplementation

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ABSTRACT

BACKGROUND

Congenital malformations can be manifested as combinations of phenotypes that co-occur more often than expected by chance. In many such cases, it has proved difficult to identify a genetic cause. We sought the genetic cause of cardiac, vertebral, and renal defects, among others, in unrelated patients.

METHODS

We used genomic sequencing to identify potentially pathogenic gene variants in families in which a person had multiple congenital malformations. We tested the function of the variant by using assays of in vitro enzyme activity and by quantifying metabolites in patient plasma. We engineered mouse models with similar variants using the CRISPR (clustered regularly interspaced short palindromic repeats)–Cas9 system.

RESULTS

Variants were identified in two genes that encode enzymes of the kynurenine pathway, 3-hydroxyanthranilic acid 3,4-dioxygenase (*HAAO*) and kynureninase (*KYNU*). Three patients carried homozygous variants predicting loss-of-function changes in the HAAO or KYNU proteins (HAAO p.D162*, HAAO p.W186*, or KYNU p.V57Efs*21). Another patient carried heterozygous KYNU variants (p.Y156* and p.F349Kfs*4). The mutant enzymes had greatly reduced activity in vitro. Nicotinamide adenine dinucleotide (NAD) is synthesized de novo from tryptophan through the kynurenine pathway. The patients had reduced levels of circulating NAD. Defects similar to those in the patients developed in the embryos of *Haao*-null or *Kynu*-null mice owing to NAD deficiency. In null mice, the prevention of NAD deficiency during gestation averted defects.

CONCLUSIONS

Disruption of NAD synthesis caused a deficiency of NAD and congenital malformations in humans and mice. Niacin supplementation during gestation prevented the malformations in mice. (Funded by the National Health and Medical Research Council of Australia and others.)

Major congenital malformations occur in at least 2% of human births.[1] There are relatively few identified causes. The origins of most malformations have been difficult to determine and probably involve genetic and environmental factors or gene–environment interaction. Although malformations frequently occur in isolation, they also occur in combination.[2] Vertebral defects, anal atresia, cardiac defects, tracheoesophageal fistula, renal anomalies, and limb abnormalities (VACTERL) together represent a nonrandom combination of congenital defects without a known cause.[3] Persons are considered to have the VACTERL association if they have any three of the above defects in the absence of a genetic cause. Our understanding of the genetic basis of congenital malformations is increasing, and many causes of isolated organ defects have been identified. Isolated heart defects are many and varied and are caused by pathogenic variation in approximately 60 genes.[4] Vertebral defects also show variability and are caused by mutations in genes associated with Notch signal transduction and somitogenesis.[5-11] However, the genetic causes of isolated cardiac or vertebral defects appear to have little relevance when these defects occur in combination.[12]

To identify the genetic basis of multiple congenital malformations in individuals, we used genomic sequencing to identify potential disease-causing mutations. We functionally assessed these mutations in vitro and defined their role in mouse embryogenesis.

Methods

Study Participants

Patients with congenital vertebral and heart malformations from Families A, B, and C were identified by clinical geneticists at the Children's Hospital at Westmead and Liverpool Hospital (Australia). Family D underwent diagnostic sequencing at GeneDx (United States) and was subsequently identified through GeneMatcher[13] (Fig. 1). Patients A, B, and C were part of a series of 13 families (3 consanguineous) with multiple congenital malformations, including vertebral and heart defects. Persons from all 13 families underwent sequencing.

Genomic and Genetic Analyses

All the families underwent whole-exome sequencing except for Family B, which underwent whole-genome sequencing. Methods of library

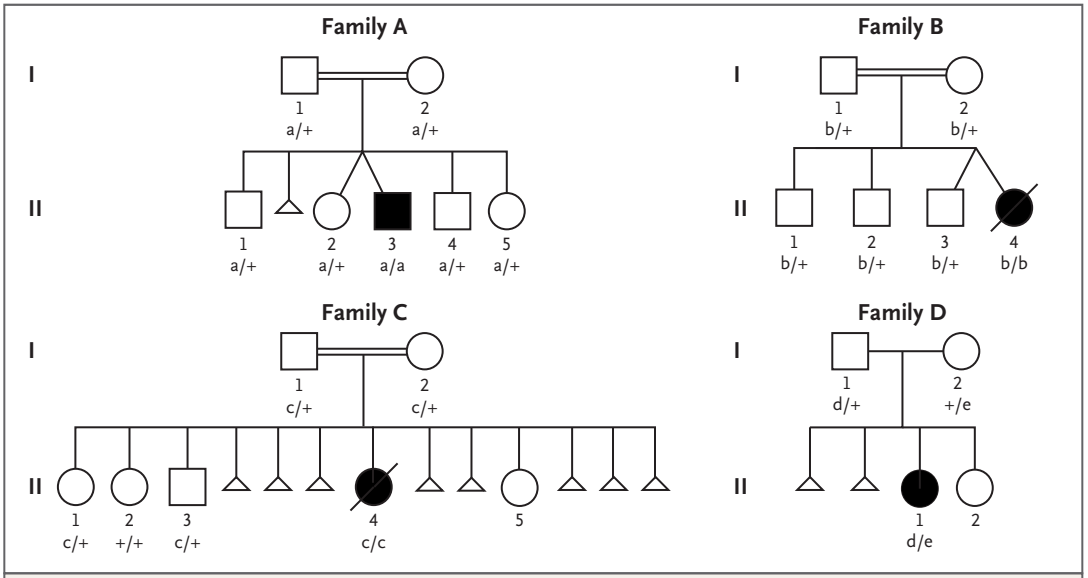

Figure 1. Pedigrees of Families with Congenital Malformations and *HAAO* or *KYNU* Variants.

Squares indicate male persons, circles female persons, triangles first-trimester deaths, solid symbols affected persons (patients), slashes deceased persons, and double horizontal lines consanguineous marriages. All persons in Families A, C, and D were evaluated by means of exome sequencing. All persons in Family B were evaluated by means of genome sequencing. In family D, two distinct variants in the *KYNU* gene were identified. Mutated alleles are depicted as "a" or "b" for *HAAO* and as "c," "d," or "e" for *KYNU*. The reference allele is depicted by a plus sign.

preparation, sequencing, variant detection, and resequencing are described in the Supplementary Appendix (available with the full text of this article at NEJM.org). Details of the enzyme assays, the quantification of metabolites in the kynurenine pathway, the generation of null alleles in mice, and analysis of mice embryos are also available in the Supplementary Appendix.

RESULTS

CLINICAL FEATURES OF AFFECTED FAMILIES

The clinical features of the study participants are summarized in Table 1; details are provided in the Results section and Table S1 in the Supplementary Appendix. The four families include one consanguineous family from Iraq (Family A), two consanguineous families from Lebanon (Families B and C), and a family from the United States without a history of consanguinity (Family D) (Fig. 1). There is no other history of congenital anomalies or intellectual disability in these families. The mother in Family B had insulin-requiring gestational diabetes. The mother in Family C had prepregnancy insulin-requiring diabetes, hypercholesterolemia, and a body-mass index (BMI, the weight in kilograms divided by the square of the height in meters) of 32.8. The mother in Family D had a BMI of 29.3. Patient A and Patient B were each one of dizygotic twins.

All the affected persons were born with vertebral defects predominantly affecting the thoracolumbar spine. Patients A and B had a spinal lipoma, which was associated with sacral agenesis in Patient A and with spinal dysraphism in Patient B. All the patients had cardiac defects: patent ductus arteriosus in Patient C, an atrial septal defect in Patient A, and hypoplastic left heart in Patients B and D. Patients A, B, and C had hypoplastic kidneys, and Patient D had a solitary left kidney with moderate chronic kidney disease. Patient C had rhizomelia, and Patient D had shortened long bones. Patients A and C had talipes. Patients A and B had sensorineural hearing loss. Patient A had a submucous cleft palate, a bifid uvula, and a laryngeal web with persistent laryngeal tracheomalacia. Patient B had palsy in the left vocal cord that was possibly iatrogenic. None of the patients had a tracheoesophageal defect. Patient C had an anterior anus. Patient C died at 4 months of age from restrictive respiratory disease due to spondylocostal defects and Patient B at 11 months of age from complications of hypoplastic left heart.

In addition to congenital malformations, postnatal growth and cognitive defects were evident. Patients A, B, and C had microcephaly. Patients A and D have extreme short stature. Patient A has moderate intellectual disability and behavioral issues at 12 years of age, and Patient D has speech delay at 3 years of age.

PATHOGENIC VARIANTS IN *HAAO* AND *KYNU*

In the consanguineous families, variants were filtered according to a recessive inheritance model and a compound heterozygous inheritance model and on the assumption that a de novo mutation in the patient was a possibility. Additional filtering was used to select variants that were nonsynonymous, rare, and predicted to be damaging, and these variants were assessed for further evidence of disease causation (Tables S2 through S7 in the Supplementary Appendix). Of these, predicted loss-of-function variants in two genes (*HAAO*, encoding 3-hydroxyanthranilic acid 3,4-dioxygenase, and *KYNU*, encoding kynureninase) were identified in three consanguineous families and were prioritized for further analysis. HAAO and KYNU are enzymes of the kynurenine pathway and are involved in the synthesis of nicotinamide adenine dinucleotide (NAD) (Fig. 2). Pathogenic variants in genes that are associated with NAD synthesis were not identified in the remaining 10 families. Neither *HAAO* nor *KYNU* has been associated with congenital malformation; however, a KYNU missense mutation (p.T198A) has been reported to be associated with hydroxykynureninuria (Online Mendelian Inheritance in Man [OMIM] number, 236800).[14]

Sanger sequencing confirmed variant segregation with disease (Fig. 1, and Fig. S2 in the Supplementary Appendix). In Family A, the patient was homozygous for a c.483dupT variant in *HAAO* (ClinVar accession number, SCV000540919), leading to a stop codon (p.D162*). In Family B, the patient was homozygous for a c.558G→A variant in *HAAO* (ClinVar accession number, SCV000540920), leading to a stop codon (p.W186*). In Family C, the patient was homozygous for a c.170-1G→T splicing variant in *KYNU* (ClinVar accession number, SCV000540921), leading to a stop codon downstream (p.V57Efs*21). In all three families, the unaffected parents and siblings were either heterozygous for the mutation

Table 1. Summary of Patient Clinical Features and the Identified DNA and Protein Variants.*

Variable	Family A	Family B	Family C	Family D
Defects in vertebral segmentation	Present	Present	Present	Present
Cardiac defects	Atrial septal defect	Hypoplastic left heart	Patent ductus arteriosus	Hypoplastic left heart
Renal defects	Hypoplasia, vesicoureteral reflux	Hypoplasia, dysplasia	Hypoplasia	Solitary kidney, chronic kidney disease
Limb defects	Talipes	Absent	Talipes, syndactyly, rhizomelia	Shortened long bones
Ear-related defects	Sensorineural hearing loss, Mondini defect	Sensorineural hearing loss on left side	Low-set ears	Absent
Other features	Short stature, global developmental delay, intellectual disability, laryngeal web, laryngomalacia	Palsy of left vocal cord	Anterior anus	Short stature, speech delay
Gene	*HAAO*	*HAAO*	*KYNU*	*KYNU*
DNA variants	c.483dupT (homozygous)	c.558G→A (homozygous)	c.170-1G→T (homozygous)	c.468T→A, c.1045_1051delTTTAAGC
Protein variants	p.D162*	p.W186*	p.V57Efs*21	p.Y156*, p.F349Kfs*4
Level in proband plasma vs. mean in unaffected family members				
Metabolite in kynurenine pathway	3HAA, 64 times the mean	3HAA, 385 times the mean	Not available	3HK, 161 times the mean
NAD	NAD+, 1/3rd of the mean	NAD(H), 1/4th of the mean	Not available	NAD(H), 1/7th of the mean

* 3HAA denotes 3-hydroxyanthranilic acid, 3HK 3-hydroxykynurenine, NAD nicotinamide adenine dinucleotide, NAD+ the oxidized form of NAD, and NAD(H) the sum of NAD+ and NADH, the reduced form of NAD. Details about the four families are provided in Tables S1 and S9 in the Supplementary Appendix.

or homozygous for the reference allele, indicating a recessive inheritance pattern. In Family D, the patient was compound heterozygous for *KYNU* variants c.468T→A (ClinVar accession number, SCV000540922) and c.1045_1051delTTTAAGC (ClinVar accession number, SCV000540923), which were inherited from the father and mother, respectively; each variant results in a stop codon (p.Y156* and p.F349Kfs*4, respectively). The locations of the variants in *HAAO* and *KYNU* and their corresponding protein variants are shown in Figure S3 in the Supplementary Appendix.

IN VITRO ACTIVITY OF MUTANT HAAO AND KYNU

We tested the activity of the truncated enzymes. For HAAO, we quantified the conversion of 3-hydroxyanthranilic acid (3HAA) to 2-amino-3-carboxymuconate-6-semialdehyde (ACMS). For KYNU, we quantified the conversion of 3-hydroxykynurenine (3HK) to 3HAA. (The enzyme assays are described in the Supplementary Appendix.) We tested the identified HAAO and KYNU variant proteins as well as KYNU p.T198A, which is associated with hydroxykynureninuria but not congenital malformation.[14] The specific activity of all the truncated HAAO and KYNU enzymes was 0 to 19% as high as the activity of nonmutant enzymes (Fig. S4 and Table S8 in the Supplementary Appendix). By contrast, the activity of KYNU p.T198A was 64% as high as the activity of the nonmutant enzyme.

LEVELS OF METABOLITES IN THE KYNURENINE PATHWAY AND NAD LEVELS

We predicted that loss of HAAO or KYNU activity would lead to increased plasma levels of metabolites upstream of these enzymes and reduced levels downstream. Patients A and B (homozygous HAAO stop codon) had upstream 3HAA levels that were 64 and 385 times the levels in unaffected heterozygous family members, respectively. Downstream of HAAO, levels of NAD+ (the oxidized form of NAD) and of NAD(H) (the sum of NAD+ and NADH, the reduced form of NAD) were one third to one quarter of the levels in unaffected heterozygous family members (Tables 1 and 2, and Table S9 in the Supplementary Appendix). Plasma was not available from Family C. Patient D (compound heterozygous for KYNU truncating mutations) had an upstream 3HK level that was 161 times the level in unaffected family members and a downstream NAD(H)

level that was one seventh of the level in unaffected family members.

MODELING OF DISEASE IN MICE NULL FOR *HAAO* OR *KYNU*

We generated mice with a null allele for *Haao* or *Kynu* (Fig. S5 in the Supplementary Appendix). Enzyme assays confirmed that the edited alleles were null (Fig. S6 in the Supplementary Appendix). Intercrosses of mice that were heterozygous null for *Haao* or for *Kynu* produced embryos in the expected mendelian ratio of genotypes. Unexpectedly, all the embryos were normal (Tables S10 and S11 in the Supplementary Appendix). We next quantified metabolites upstream and downstream of Haao and of Kynu in adult mouse serum. $Haao^{-/-}$ mice had 3HAA levels that were more than 100 times the levels in $Haao^{+/-}$ mice and wild-type mice (Table S12 in the Supplementary Appendix). Similarly, $Kynu^{-/-}$ mice had 3HK levels that were more than 70 times the levels in $Kynu^{+/-}$ mice and wild-type mice, a metabolic finding consistent with our findings in humans (Tables 1 and 2, and Table S9 in the Supplementary Appendix). By contrast, NAD(H) levels were similar in all the mice, regardless of genotype (Table S12 in the Supplementary Appendix). This suggested that elevated levels of metabolites upstream of HAAO or KYNU in humans did not cause congenital malformation and underscored a deficit in the NAD level as the cause.

NAD is produced by two pathways: one requires dietary tryptophan, and the other requires dietary niacin (Fig. 2). The NAD de novo synthesis pathway catabolizes tryptophan through the kynurenine pathway, and the NAD salvage pathway converts niacin and other precursors into NAD (independent of KYNU and HAAO). The niacin status of mice as measured by the concentration of NAD in whole blood is at least four times that in humans, possibly because mice convert tryptophan to NAD more efficiently,[15] and they consume more tryptophan or niacin per unit of body weight owing to a higher basal metabolic rate.[16] In mice, reduced niacin status occurs only when both de novo synthesis of NAD is blocked and niacin is removed from the diet.[17]

During development, embryos receive niacin from the mother and generate their own. It is therefore possible that maternal niacin has a buffering effect and protects the null mouse embryos from the development of NAD deficiency. We therefore sought to reduce niacin levels in pregnant mice that were heterozygous for a null allele ($Haao^{+/-}$ or $Kynu^{+/-}$). As a first attempt to mimic the reduced NAD levels in humans, these mice were fed a niacin-free diet during pregnancy. In litters from $Haao^{+/-}$ or $Kynu^{+/-}$ intercrosses, embryos had the expected mendelian ratio of genotypes and were phenotypically normal (Tables S11 and S13 in the Supplementary Appendix). This observation suggested that het-

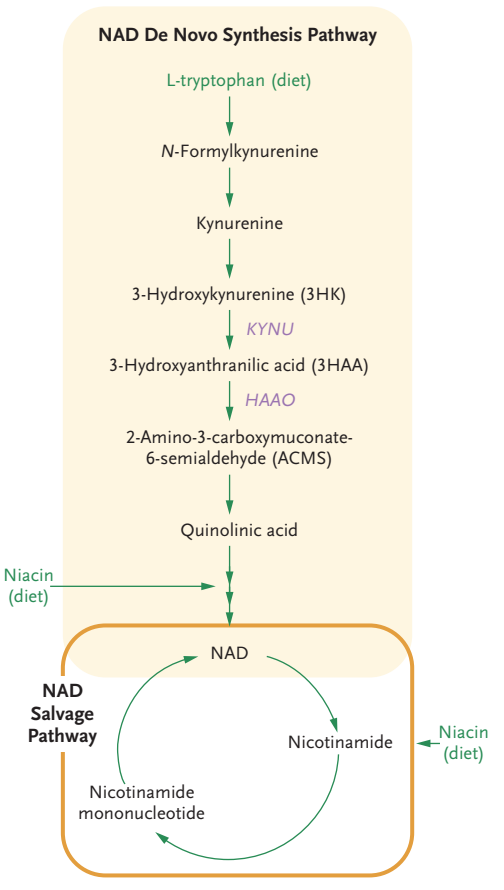

Figure 2. Synthesis of Nicotinamide Adenine Dinucleotide (NAD).

NAD is synthesized de novo from the essential amino acid L-tryptophan and salvaged from nicotinamide. Tryptophan and niacin (vitamin B_3), which is supplied as nicotinic acid and nicotinamide, represent dietary inputs. Details on NAD synthesis are provided in Figure S1 in the Supplementary Appendix.

Table 2. Alteration in Levels of Metabolites in the Kynurenine Pathway and in NAD Levels in Plasma from Patient B (Family Member II.4).*

Variable	Family B						Mean in Unaffected Family Members	Level in Patient versus Mean
	I.1	I.2	II.1	II.2	II.3	II.4		
Upstream of HAAO								
Tryptophan (μM)	52.6	39.5	62.0	48.9	85.3	63.6	57.7	1.1 times the mean
Kynurenine (μM)	1.2	1.1	1.1	1.6	1.6	1.6	1.3	1.2 times the mean
3HK (nM)	4.1	41.3	4.1	15.6	79.4	98.4	28.9	3.4 times the mean
3HAA (nM)	114.8	84.3	55.2	65.4	171.5	37,819.8	98.3	384.9 times the mean
Downstream of HAAO								
Picolinic acid (nM)	32.0	NA	31.7	26.4	35.5	33.7	31.4	1.1 times the mean
Quinolinic acid (nM)	113.5	NA	133.6	168.4	217.3	40.2	158.2	1/4th of the mean
NAD+ (nM)	24.4	25.4	22.3	21.5	27.7	3.6	24.3	1/7th of the mean
NADH (nM)	17.3	16.0	19.7	16.3	18.4	8.1	17.5	1/2 of the mean
NAD(H) (nM)	41.7	41.4	42.0	37.8	46.0	11.7	41.8	1/4th of the mean

* Data on Families A and D are provided in Table S9 in the Supplementary Appendix. Plasma was not available from Family C.

erozygous null female mice (i.e., those with one normal and one null allele of *Haao* or of *Kynu*) that receive a niacin-free diet produce sufficient NAD from dietary tryptophan to sustain normal embryonic development.

To preclude maternal NAD production from the de novo synthesis pathway in addition to the salvage pathway, null female mice (*Haao*$^{-/-}$ or *Kynu*$^{-/-}$) were mated with *Haao*$^{+/-}$ or *Kynu*$^{+/-}$ male mice and fed a niacin-free diet. All the embryos died, regardless of genotype (Tables S11 and S14 in the Supplementary Appendix). Death also occurred when the niacin-free diet was limited from embryonic day 0.5 to embryonic day 4.5, 5.5, or 6.5 (Tables S11 and S14 in the Supplementary Appendix). We determined that a niacin-free diet supplemented with 5 mg of nicotinic acid per liter of drinking water between embryonic day 7.5 and embryonic day 12.5 better sustained embryogenesis in null mothers. Despite a large number of resorptions, live null embryos were present (Tables S11 and S15 in the Supplementary Appendix). All the *Haao*$^{+/-}$ and *Kynu*$^{+/-}$ embryos were normal. By contrast, all the *Haao*$^{-/-}$ and *Kynu*$^{-/-}$ embryos had multiple defects, including defects in vertebral segmentation, heart defects, small kidney, cleft palate, talipes, syndactyly, and caudal agenesis (Fig. S7 and Tables S15 and S16 in the Supplementary Appendix). NAD levels in null mouse embryos were one half the levels in unaffected heterozygous littermates at embryonic day 9.5 (Fig. 3). This observation indicates that in both mice and humans, loss of embryonic NAD leads to embryo defects and death. It is the embryonic NAD deficit that causes defects rather than the maternal deficit, because null mothers produce normal heterozygous embryos (Table S15 and Fig. S7 in the Supplementary Appendix).

To prove that NAD deficiency was disrupting embryogenesis, pregnant null mice were fed a niacin-free diet as before, and water was supplemented with 10 or 15 mg of nicotinic acid per liter. Because mice consume 1.3 liters of water per kilogram of food,[18] we calculated that with this regimen mice would consume 14% of the niacin equivalent in complete mouse chow (90 mg per kilogram). Litters from these mice contained embryos with genotypes in the expected mendelian ratio. With 10 mg of nicotinic acid per liter, null embryos were normal except for kidneys that were 30% smaller than those in heterozygous controls; with 15 mg of nicotinic acid per liter, all the embryos were normal (Table S15 in the Supplementary Appendix). We also observed a dose-dependent increase in embryonic NAD(H) levels in response to maternal niacin supplementation (Fig. 3), a finding consistent with the ab-

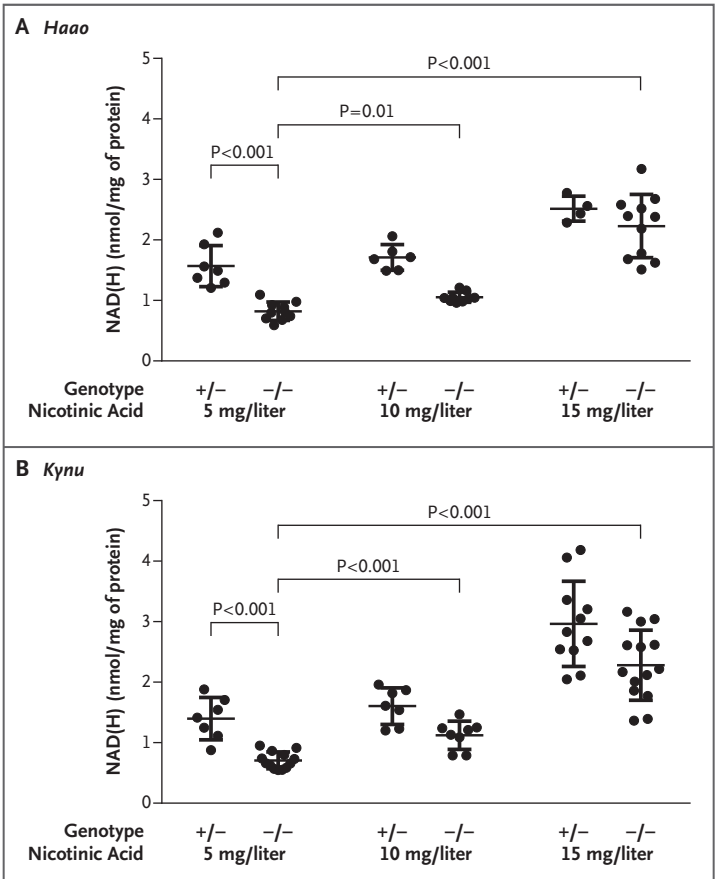

Figure 3. Effect of Niacin Supplementation on Levels of NAD in Null Mouse Embryos.

Female mice that were homozygous null for *Haao* or *Kynu* were mated with heterozygous male mice. Pregnant mice were fed a niacin-free diet supplemented with 5, 10, or 15 mg of nicotinic acid per liter of drinking water from embryonic day 7.5 to 9.5. Embryos were harvested at embryonic day 9.5, and levels of NAD(H) (the sum of NAD+ and NADH) were quantified. The difference between groups was tested with the use of a one-way analysis-of-variance test based on $\log_2$-transformed data, followed by Dunnett's multiple-comparison test (all other groups vs. null mice receiving 5 mg of nicotinic acid per liter). The middle lines of the I bars indicate mean values, and the I bars ±1 SD.

sence of a phenotype. These data show that embryo death and defects were specifically due to a deficit of NAD in embryos and that niacin supplementation prevented the disruption of embryogenesis.

DISCUSSION

We identified mutations blocking de novo NAD synthesis that cause multiple congenital malformations, including those found in the VACTERL association. The NAD de novo synthesis pathway catabolizes tryptophan, leading to the production of NAD. Although metabolite levels upstream of the block are elevated and the metabolites have postnatal functions,[19] we found that it is the deficiency in embryonic NAD, downstream of the block, that is disrupting embryogenesis. We found that in mice, supplementing dietary niacin during gestation prevents embryo defects caused by NAD deficiency. We evaluated the pathogenicity of the *HAAO* and *KYNU* variants according to American College of Medical Genetics and Genomics guidelines.[20] On the basis of the predicted deleterious effects that were confirmed by in vitro enzyme assays, an absence or extremely low frequency of minor alleles, and the recessive inheritance pattern, we conclude that all the *HAAO* and *KYNU* variants that were identified in this study are pathogenic (Table S17 in the Supplementary Appendix).

NAD deficiency is linked to disease through genetic and environmental means. Severe niacin deficiency causes pellagra, characterized by dermatitis, diarrhea, dementia, and death.[21] Although pellagra is rare today, niacin deficiency that is caused by dietary inadequacies, malabsorption of nutrients, and drug interference is still observed.[21] Moreover, niacin deficiency is common during pregnancy.[22] Niacin deficiency can also be inherited. Hartnup disease, characterized by dermatitis and neurologic and behavioral defects, is caused by mutation of *SLC6A19*, which is required for the transport of neutral amino acids, including tryptophan.[23] NAD synthesis is also reduced by pathophysiological factors such as type 2 diabetes, obesity, and inflammation.[22,24-26] These may have been confounding factors in Families B, C, and D.

Our findings increase understanding of the genetic and environmental causes of congenital malformation. Our discovery that the genetic disruption of NAD synthesis causes congenital malformations suggests that mutation of many genes might have the same effect (Fig. S1 in the Supplementary Appendix). These include genes required for de novo synthesis of NAD, such as genes encoding tryptophan transporters (*SLC7A5*, *SLC7A8*, and *SLC6A19*), genes encoding enzymes of the kynurenine pathway (*TDO2*, *IDO1-2*, *AFMID*, *KMO*, *KYNU*, and *HAAO*), and genes encoding enzymes that convert quinolinic acid to NAD

(*QPRT*, *NMNAT1-3*, and *NADSYN1*). Furthermore, genes encoding enzymes of the NAD salvage pathway (*NAMPT* and *NMNAT1-3*) and genes required for the transport of nicotinic acid (*SLC5A8* and *SLC22A13*) or the uptake of dietary niacin (nicotinic acid, nicotinamide, or nicotinamide riboside) or its entry into NAD-producing pathways (*NAPRT*, *PNP*, and *NMRK1,2*) could also be candidates for causing congenital malformations. It is noteworthy that mutation of *NMNAT1* causes the retinal degenerative condition Leber's congenital amaurosis type 9 (OMIM number, 608553). Here, the almost-normal enzymatic activity of mutant proteins would preclude a broad phenotype such as congenital malformation.[27]

NAD is a cofactor with broad cellular effects that is involved in ATP production, macromolecular synthesis, PARP-dependent DNA repair, and sirtuin-dependent protein deacetylation.[28] NAD+ or NADH participates in approximately 400 redox reactions, NADP+ or NADPH participates in approximately 30 redox reactions, and NAD+ is consumed in approximately 50 reactions involving PARP-dependent DNA repair or sirtuin-dependent deacetylation.[28] Given the various roles of NAD, there are many possible ways by which a deficit of it might disrupt embryogenesis.

First, NAD is central to energy metabolism, serving as an essential coenzyme in glycolysis, the tricarboxylic acid cycle, and the mitochondrial electron transport chain, and is also central to the production of macromolecules, including nucleotides. Embryogenesis is characterized by dynamic, well-orchestrated cellular proliferation and differentiation. NAD deficiency is therefore likely to most affect tissues undergoing the greatest change.

Second, NAD levels regulate sirtuin-mediated deacetylation, which modulates transcription factors and metabolic enzymes that promote mitochondrial oxidative function and enhance cell survival under stress conditions.[29-31] Sirtuin activity is required during embryogenesis and affects the fate of stem cells and progenitor cells; loss of activity causes multiple organ defects.[32]

Third, DNA repair is active during rodent embryogenesis and is required for normal development.[33] There is evidence that NAD deficiency impairs DNA repair[28] and that a DNA fragmentation disorder in humans, Fanconi's anemia, can result in multiple congenital malformations defining the VACTERL association.[3]

In the patients we describe here, there was consistency in the tissues affected but variability in phenotype. Given the phenotypic spectrum and the essential role of NAD, it is possible that additional variable phenotypes may yet be discovered. Such variability is probably due to a range of factors, including gene modifiers in both the mother and the affected child; maternal physiology that is influenced, for example, by diabetes; diet, including the intake of niacin and tryptophan; and other factors affecting NAD production, salvage, and consumption. We theorize that supplementation with high-dose niacin (140 mg per day, which is 10 times the U.S. recommended daily allowance for women) before and during pregnancy might prevent recurrence of disease in these four families. It is also possible that niacin supplementation may benefit the speech and developmental delays in the surviving patients.

In conclusion, many genetic and environmental factors have the potential to cause NAD deficiency during gestation. We propose that cases of congenital malformation that occur because of a deficit in NAD be collectively referred to as congenital NAD deficiency disorders.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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