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Brief Communications

Identification of Mitochondrial DNA Variants Associated With Risk of Neuroblastoma

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Abstract

Neuroblastoma is a childhood cancer that originates in the developing sympathetic nervous system. We previously reported a crucial role of mitochondrial DNA haplogroups in the pathology of neuroblastoma. To pinpoint mitochondrial DNA variants associated with neuroblastoma risk, we applied a mitochondrial genome imputation pipeline to the single nucleotide polymorphisms array data of 2 pediatric cohorts containing a total of 2404 neuroblastoma patients and 9310 cancer-free controls. All statistical tests were 2-sided. The single nucleotide variant, rs2853493, was statistically significantly associated with neuroblastoma risk in the discovery cohort (odds ratio = 0.62, 95% confidence interval = 0.53 to 0.72, $P < .001$) and further confirmed in the replication cohort (odds ratio = 0.75, 95% confidence interval = 0.62 to 0.90, $P = .002$). Further, expression quantitative trait loci analysis indicated genotypes of rs2853493 were associated with expression levels of *MT-CYB* gene expression in neuroblastoma cells, suggesting rs2853493 may confer risk to neuroblastoma via regulating the expression level of its nearby genes.

Neuroblastoma originates from the developing sympathetic nervous system and accounts for approximately 12% of all pediatric oncology deaths (1). It is one the most fatal childhood cancers, with approximately one-half of affected individuals having disease dissemination at diagnosis and poor outcome from therapy (1,2). Survival rate for these high-risk group remains less than 50% despite aggressive multimodal therapies (1,2).

Genetic studies have uncovered a polygenic model of inheritance with the discovery of multiple genes associated with neuroblastoma through genetic studies (3-12). Besides identification of genetic risk factors from nuclear DNA, cumulative evidence suggests that mitochondrial dysfunction may link to neuroblastoma tumorigenesis (13-15). Our recent study revealed statistically significant associations between neuroblastoma risk and human mitochondrial DNA (mtDNA) haplogroup K (16). A haplogroup represents a group of similar haplotypes inherited from a common ancestor (17). The formation of mtDNA haplogroups is a result of the sequential accumulation of mutations through maternal lineages during ancient migrations of human populations (17). However, it is still unknown which haplogroup K-associated variants or mitochondrial-encoded genes may contribute to neuroblastoma risk. To extend our previous observations, we have designed studies to investigate the mitochondrial genome in more depth, using an mtDNA imputation pipeline (Supplementary Methods, available online), further pinpointing statistically significantly associated mtDNA variants by analyzing a total of 2404 neuroblastoma cases and 9310 cancer-free controls (Supplementary Methods; Supplementary Figures 1 and 2, available online). This study was approved by the Institutional Research Ethics Board of CHOP (The Children's Hospital of Philadelphia). Written informed consent was obtained from all patients by nursing and medical assistant study staff under the direction of CHOP clinicians.

A 2-sided logistic regression test indicated rs2853493, a synonymous variant in *MT-ND4*, was statistically significantly (the cutoff for statistical significance is $P < .05$) associated with

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Table 1. Association results of rs2853493 and rs2853499

				Discovery		Replication		Meta-analysis	
Risk group	RSID	POS	A1/A2	OR (95% CI)[a]	P[a]	OR (95% CI)[a]	P[a]	OR (95% CI)[b]	P[b]
All	rs2853493	11 467	G/A	0.62 (0.53 to 0.72)	<.001	0.75 (0.62 to 0.90)	.002	0.67 (0.59 to 0.76)	<.001
	rs2853499	12 372	A/G	0.69 (0.59 to 0.81)	<.001	0.74 (0.61 to 0.89)	.001	0.71 (0.63 to 0.80)	<.001
High	rs2853493	11 467	G/A	0.59 (0.47 to 0.75)	<.001	0.73 (0.55 to 0.95)	.02	0.65 (0.54 to 0.77)	<.001
	rs2853499	12 372	A/G	0.64 (0.51 to 0.82)	<.001	0.72 (0.55 to 0.94)	.02	0.68 (0.57 to 0.81)	<.001
Intermediate	rs2853493	11 467	G/A	0.55 (0.39 to 0.78)	<.001	0.69 (0.48 to 0.99)	.05	0.61 (0.48 to 0.79)	<.001
	rs2853499	12 372	A/G	0.62 (0.44 to 0.87)	.006	0.69 (0.48 to 0.98)	.04	0.65 (0.51 to 0.83)	<.001
Low	rs2853493	11 467	G/A	0.64 (0.5 to 0.82)	<.001	0.90 (0.67 to 1.21)	.48	0.74 (0.61 to 0.89)	.001
	rs2853499	12 372	A/G	0.73 (0.57 to 0.92)	.007	0.87 (0.64 to 1.17)	.34	0.78 (0.65 to 0.93)	.007

[a]Two-sided logistic regression test. A1 = minor allele (coded allele); A2 = major allele; CI = confidence interval; OR = odds ratio; POS = genomic coordinates; RSID = rsID number of reported mitochondrial variants.
[b]Two-sided meta-analysis.

neuroblastoma risk in the discovery cohort of 1474 case patients and 5699 cancer-free controls (odds ratio [OR] = 0.62, 95% confidence interval [CI] = 0.53 to 0.72, $P < .001$; Table 1; Supplementary Table 1, available online). This association was further replicated in the replication cohort comprised of 930 cases and 3611 controls (OR= 0.75, 95% CI = 0.62 to 0.90, $P = .002$; Table 1; Supplementary Table 1, available online) and confirmed by a sensitivity analysis (Supplementary Methods, available online). Meta-analysis of 2 studies yielded a *P* value that extends well beyond the statistical significance threshold (OR = 0.67, 95% CI = 0.59 to 0.76, $P < .001$; Table 1) and additionally detected a synonymous variant, rs2853499, in *MT-ND5* (OR = 0.71, 95% CI = 0.63 to 0.8, $P < .001$; Table 1). Consistent with our previous observation, minor alleles of both rs2853493 (11467G) and rs2853499 (12372A) are listed as top-level haplogroup markers of haplogroup K, which should be present at 80% or more in the top-level haplogroups (18). In our data, the fractions of haplogroup K carriers possessing minor alleles of rs2853493, rs2853499, or both were 91.2%, 97.2%, and 90.8% respectively. We next conditioned our analysis on rs2853493 (Figure 1; Supplementary Table 2, available online) and found the association between rs2853499 and neuroblastoma disappeared (OR = 0.73, 95% CI = 0.18 to 2.46, $P = .68$). Likewise, the association between rs2853493 and neuroblastoma was not statistically significant (OR = 0.89, 95% CI = 0.24 to 3.34, $P = .86$) after conditioning on rs2853499 indicating a single signal residing in this region. Our results also indicated the previously described association between risk of neuroblastoma and haplogroup K was explained by these 2 variants, because this association was not statistically significant (OR = 1.01, 95% CI = 0.91 to 1.11, $P = .93$) when genotypes of rs2853493 and rs2853499 were included as covariates. Moreover, we replicated this signal in another independent cohort (rs2853493, OR = 0.65, 95% CI = 0.49 to 0.89, $P = .008$) of 344 European cases from the United Kingdom and 2752 ancestry-matched controls from CHOP. Although the reported variants were not statistically significant in the African American cohort of 332 cases and 2748 controls, the estimated odds ratios for both variants (rs2853493, OR = 0.71, 95% CI = 0.39 to1.27, $P = .26$; rs2853499, OR = 0.69, 95% CI = 0.38 to 1.22, $P = .21$) were very similar to the estimates given above for Europeans. This suggests that this signal may overlap in both racial groups.

We next tested rs2853493 and rs2853499 for association with genotypes at 7 known neuroblastoma loci (3,4,7,8,19). No statistically significant interaction was detected (Supplementary Table 3, available online), suggesting the neuroblastoma risk attributed to this mitochondrial locus was independent from other loci. Also, no additional variants were identified as associated with a specific risk group of neuroblastoma (Table 1) (20). Cochrane's Q tests for heterogeneity of the odds ratios over the 3 risk groups were not statistically significant (rs2853493, $P = .90$; rs2853499, $P = .93$). Further survival analysis based on the log rank test detected no statistically significant differences between patients with minor (G) and major (A) alleles of rs2853493 (Supplementary Figure 3, available online). In addition, no statistically significant association was detected between the reported variants and demographic variables.

We next analyzed the RNAseq data of neuroblastoma primary tumor tissues (Supplementary Methods, available online) and found genotypes of rs2853493 were statistically significantly associated with expression levels of *MT-CYB* (Supplementary Figure 4, available online). We also conducted an expression quantitative trait loci analysis of rs2853493 based on the Genotype-Tissue Expression Program v8 data to explore the potential regulatory effect of rs2853493 in normal human tissues (Supplementary Methods, available online). Statistically significant associations between rs2853493 and nearby genes were detected (Supplementary Table 4, available online), including *MT-CYB*, *MT-ND4*, *MT-ND4L*, and *MT-ND5*. However, none of the reported mtDNA variants resides in the regulatory region according to the epigenetic data (Supplementary Methods, available online).

The genomic region containing *MT-ND4* and *MT-ND5* has been reported as a genetic hotspot region for multiple cancers and neurological disorders (21-25). Triska et al. (23) reported that mtDNA mutations of pediatric tumors clustered at a statistically significant hotspot region in *MT-ND4* and *MT-ND5*. Jaberi et al. (21) observed that the *MT-ND5* region is prone to mutations in hematological cancers and in dopaminergic neurons involved in Parkinson disease. High incidence of *MT-CYB* mutations has also been reported in esophageal cancer (25) and glioblastoma (26). Consistently, we found 2 regions (Supplementary Table 5, available online) within *MT-ND5* and *MT-CYB* exhibited a statistically significant enrichment of mutations indicating the presence of potential mutation hotspots in these regions (Supplementary Methods, available online).

A limitation of this study is that the imputation analysis is limited by poor coverage of mtDNA variants in the genotyping arrays, because only 485 of 3617 mtDNA variants were successfully imputed. Future studies using whole-genome sequencing are likely to detect additional mtDNA variants near this region associated with neuroblastoma.

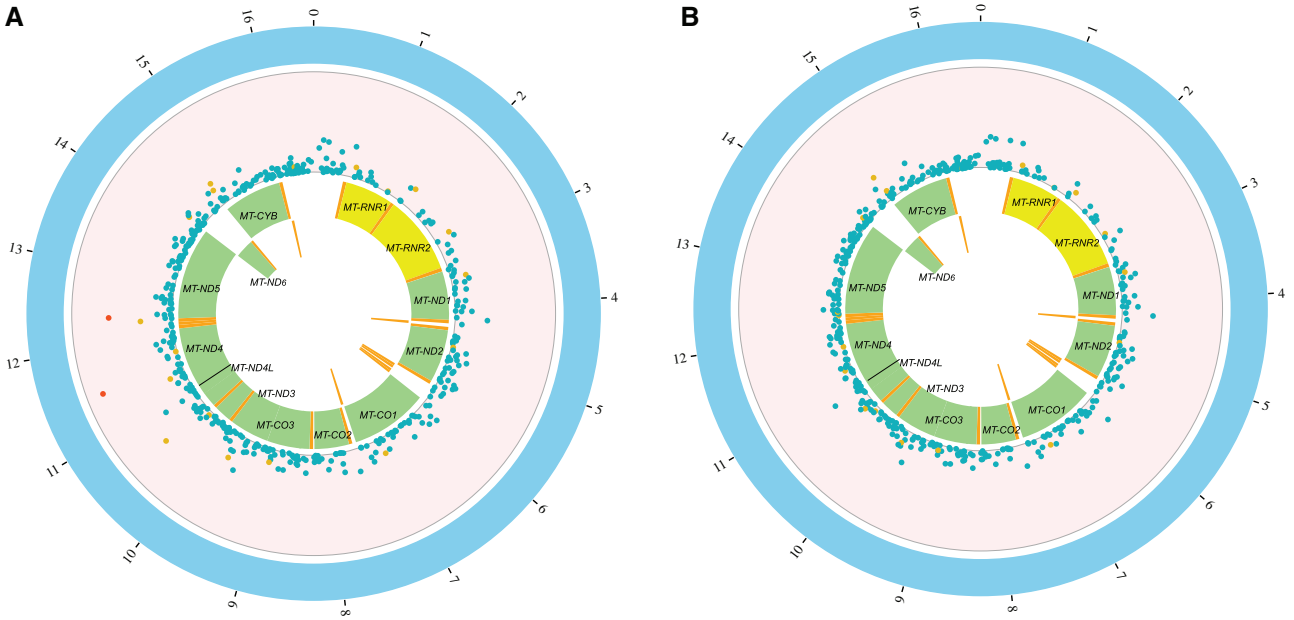

Figure 1. Mitochondrial DNA variants associated with neuroblastoma. **A)** Circos plot of the association results. **Circles** from the outside to the inside indicate the following: position of a variant on the mitochondrial genome; statistical significance of tested mitochondrial DNA variants (the radial axis corresponds to the −log10-transformed *P* values, 2 genome-wide statistically significant variants are colored in **red**, haplogroup K markers are colored in **yellow**); and regions corresponding to the different mtDNA genes (**green**, coding region; **yellow**, ribosomal RNAs; **orange**, transfer RNAs). **B)** Circos plot of the association results conditioned on rs2853493.

This study presents an extension of our previous findings by uncovering mtDNA variants and candidate genes for future functional studies on mitochondrial mechanisms in the development of neuroblastoma.

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Notes

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Data Availability

Individual-level genotyping data are available in dbGAP, and can be accessed with accession phs000124.v3.p1.

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