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Role of *p53*, Mitochondrial DNA Deletions, and Paternal Age in Autism: A Case-Control Study

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abstract

BACKGROUND: The tumor suppressor *p53* responds to a variety of environmental stressors by regulating cell cycle arrest, apoptosis, senescence, DNA repair, bioenergetics and mitochondrial DNA (mtDNA) copy number maintenance. Developmental abnormalities have been reported in *p53*-deficient mice, and altered *p53* and *p53*-associated pathways in autism (AU). Furthermore, via the *Pten-p53* crosstalk, *Pten* haploinsufficient-mice have autisticlike behavior accompanied by brain mitochondrial dysfunction with accumulation of mtDNA deletions.

METHODS: mtDNA copy number and deletions, and *p53* gene copy ratios were evaluated in peripheral blood monocytic cells from children aged 2–5 years with AU (n = 66), race-, gender-, and age-matched typically neurodeveloping children (n = 46), and both parents from each diagnostic group, recruited by the Childhood Autism Risk from Genes and Environment study at the University of California, Davis.

RESULTS: mtDNA deletions and higher *p53* gene copy ratios were more common in children with AU and their fathers. The incidence of mtDNA deletions in fathers of children with AU was increased 1.9-fold over fathers of typically neurodeveloping children, suggesting a role for deficient DNA repair capacity not driven by paternal age. Deletions in mtDNA and altered *p53* gene copy ratios seem to result from genetics (children with severity scores ≥8) and/or act in concert with environmental factors (children with 6–7 severity scores).

CONCLUSIONS: Given pro- and antioxidant activities of *p53*, and associations of genomic instability with disorders other than AU, our study suggests a link between DNA repair capacity, genomic instability in the 17p13.1 region influenced by environmental triggers, and AU diagnosis.

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Ms. Wong performed all the experiments on mtDNA and *p53*, contributed to the writing of the manuscript, and performed statistical analyses; Dr. Napoli performed some of the statistical analysis and helped in drafting and revising the manuscript; Dr. Krakowiak calculated the severity scores and provided text associated with this analysis; Dr. Tassone provided genomic DNA from individuals; Dr. Hertz-Picciotto obtained funding for the Childhood Autism Risk from Genes and Environment study and oversaw demographic data and sample collection, provided intellectual input, and contributed to the editing of the manuscript; Dr. Giulivi obtained funding for this study, conceptualized and designed the study, wrote the manuscript, and performed some of the statistical analyses; and all authors approved the final manuscript as submitted.

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WHAT'S KNOWN ON THIS SUBJECT: Developmental abnormalities have been observed in *p53*-deficient mice. Altered *p53* and *p53*-dependent pathways have been reported in autism, and via the *PTEN-p53* interplay, *Pten* haploinsufficient mice present autistic-like behavior accompanied by brain mitochondrial dysfunction with accumulation of mitochondrial DNA deletions.

WHAT THIS STUDY ADDS: Altered *p53* and mitochondrial DNA gene copy ratios segregate with autism. These outcomes seem to originate from deficient paternal DNA repair capacity (not age-related) at severity scores ≥8, whereas higher influence of gene x environment is observed at lower scores.

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The *p53* gene is responsive to a large number of environmental stressors by regulating maintenance of genomic stability,[1] changes in oxidative stress,[2–4] mitochondrial DNA (mtDNA) copy number (CN),[5,6] and mitochondrial respiration[1,6] as well as the neurotoxic response to flame retardants[7,8] and pesticide exposures.[9–12] Studies have linked *p53* to developmental abnormalities[13] (see also Supplemental Table 5), and regions adjacent to 17p13.1 encoding for proteins with important roles in brain function and neurodevelopment (eg, axonal dynein heavy chain 2 and Na^+/K^+ ATPase subunit β-2) and others have been found to be associated with autism (AU) (eg, ephrin-B3[14]). Increased *p53*/Bcl2 protein ratios had been found in brain regions of a small set of autistic subjects[15–17]; however, no characterization of posttranslational modifications or functional status of *p53* was undertaken. Neuronal *Pten* haploinsufficiency in mice results in a sustained Akt activation and decreased *p53* protein expression and function (evidenced by downregulation of *p53* targets), mitochondrial dysfunction, accumulation of mtDNA deletions,[6] and the occurrence of aberrant social and repetitive behavior associated with AU.[6,18]

The mitochondrial electron transport chain is the major intracellular source of reactive oxygen species, and as such, mtDNA becomes oxidatively modified as it is evidenced by its relatively high mutation rate[19] and accumulation of deletions with age.[20,21] Mitochondria can compensate for these damages by responding with increased mtDNA replication without increases in oxidative phosphorylation[22–27]; however, increases in CN also have been associated with defective transcription, respiratory chain deficiency, and age-related accumulation of mtDNA deletions.[28]

Due to the involvement of *p53* in genomic stability, mitochondrial function, and protection of mtDNA from oxidative stress,[2] we hypothesized that a particular genetic background could ensue in *p53* and mtDNA damage. On exposure to environmental stressors, this damage could be compounded (second-hit hypothesis). This is demonstrated by the enhanced neurotoxic effect of excitotoxic amino acids when oxidative phosphorylation is inhibited[29–32] or the exacerbated neuronal mitochondrial toxicity to polybrominated diphenyl ethers in the presence of an AU-like background (PTEN deficiency).[33] This hypothesis provides a framework for the observed mitochondrial dysfunction reported in peripheral blood mononuclear cells (PBMCs) from children with AU,[34] deficits accompanied by increased oxidative stress, evidenced by higher rates of hydrogen peroxide production[34] and increased mtDNA deletions.[35]

The major goal of the current study was to identify changes in mtDNA CN, mtDNA deletions, and *p53* gene copy ratios in children diagnosed with AU and their biological parents. To this end, these outcomes were tested in PBMCs from children aged 2 to 5 years diagnosed with AU and race-, gender-, and age-matched, typically neurodeveloping (TD) children and their parents recruited by the Childhood Autism Risk from Genes and Environment study (CHARGE) at the University of California Davis.[36]

METHODS

Clinical Selection of Individuals and Diagnosis

Specimens and data of children aged 2 to 5 years with clinically confirmed AU (n = 66; severity scores [SSs] ≥6), and race-, gender-, and age-matched, clinically confirmed TD children (n = 46) and both parents from each diagnostic group (when available) were obtained from the CHARGE study[35,36] (Table 1). The Autism Diagnostic Observation Schedule (ADOS)-2 comparison scores were used to calculate the SSs.[37,38] This metric measures severity on a 10-point scale and equates ADOS modules across the language level and age of children. The level of autism spectrum disorder (ASD)-related symptoms based on this scale is interpreted as follows: 1 to 3 = no ASD; 4 to 5 = ASD, low level; 6 to 10 = AU, with 6 to 7 = moderate level and 8 to 10 = high level[37,39] (see also Supplemental Material).

Evaluation of mtDNA CN, Deletions in mtDNA, and p53 Gene Copy Ratios

Genomic DNA was extracted from PBMCs and changes in mtDNA CN were evaluated by quantitative polymerase chain reaction (qPCR) by using dual-labeled probes.[34,35] The gene CN of cytochrome *b* (*CYTB*), and NADH dehydrogenase subunits 1 (*ND1*) were normalized by a single-copy nuclear gene pyruvate kinase (*PK*).[34] For mtDNA CN, the ratio of *ND1*/*PK* was used, for most of the deletions are present in the major arc.[34] Deletions in mtDNA were estimated by evaluating the mitochondrial gene copy ratios of *CYTB*, located between origins of heavy-strand and light-strand mtDNA synthesis (associated with replication pausing and breakage[40,41]), normalized by *ND1*.[34,35]

Gene copy ratios of *p53* were evaluated by qPCR (normalized to *PK*) in a segment with low incidence of mutations (<1.5%[42,43]). All individuals (regardless of diagnostic group, gender, or age) exhibited a mean *p53* haploid CN >0.8, indicative of a normal CN status (more details in Supplemental Material). All individual data are included in Supplemental Table 6. Heritability of outcomes was estimated as h^2 from coefficients for child outcomes regressed on midparent outcomes. Heritability is a ratio of variances: it is the proportion of total variance

TABLE 1 Demographics of Groups Evaluated in This Study

Outcome	TD	AU	*P*
Children, *n*	46	66	
Race, %			
Asian	7.7	7.7	.719
Hispanic	35.9	42.3	.628
White	48.7	50.0	.955
Multiracial	7.7	3.8	.636
Age, y	3.5 ± 0.1	3.9 ± 0.1	.174
Gender, %			
Girls	12.9	15.4	
Boys	87.1	84.6	.638
Test scores			
Vineland	107 ± 2	64 ± 2	<.0001
Mullen	106 ± 3	61 ± 3	<.0001
Severity	n.a.	7.7 ± 0.2	
Parents (couples; *n*)	37	48	
Age, y			
Father	34.3 ± 0.9	34 ± 1	.829
Mother	31.8 ± 0.8	35 ± 3	.819

Age of parents is shown at birth of index child. Data (age, Vineland, Mullen, and SSs) were expressed as mean ± SEM. SSs for typical children are between 0 and 3; all TD children in CHARGE were administered a screening instrument to rule out ASD symptoms and scored very low; hence n.a., not applicable.

in a population for a particular measurement (ie, mtDNA CN), taken at a particular time, attributable to variation in additive genetic or total genetic values,[44] reflecting all possible genetic contributions to populations' phenotypic variances, including epistatic effects (multigene interactions) as well as maternal and paternal effects where individuals are directly affected by their parents' phenotype.[45] To estimate h^2 in this study, the average values from spouses (midparent) versus offspring and single parent (values from either mothers or fathers) versus offspring models were used and calculated as described by Visscher et al.[44]

Statistical Analysis

Experiments were run in triplicates and repeated 3 times on different days unless noted otherwise. Normal probability plots were generated to evaluate whether the variable observations followed a normal distribution. Significant differences between means of 2 groups were evaluated with the unequal variance, 2-tailed Student's *t* test (GraphPad Prism 5 software, GraphPad software, La Jolla, CA). For frequencies, the χ^2 test was used without Yates correction. All data collected in this study, except outliers identified by the Tukey test, are shown in the Supplemental Information. Analysis of covariance (ANCOVA) was carried out to evaluate the statistical significance for the correlations reported in Fig 1.

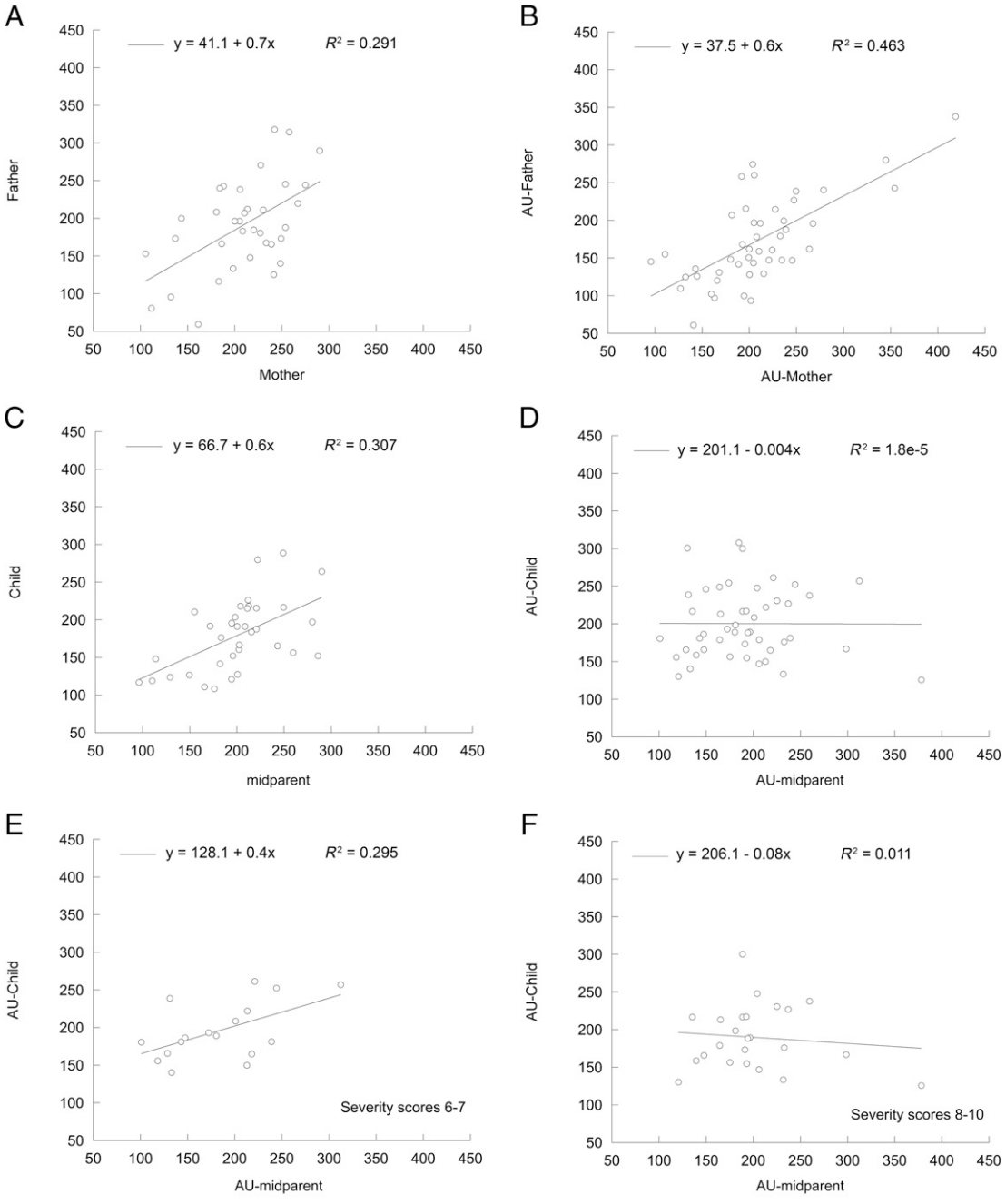
RESULTS

Increased mtDNA CN/PBMC in Children With AU and Higher Incidence of mtDNA Deletions

This study was conducted on a sample of the population-based case-control CHARGE study, in which the major discriminators are diagnosis (AU versus TD, by design), and their accompanying adaptive behavior (Vineland scores) and cognitive function (Mullen Scales of Early Learning scores; Table 1). The diagnostic groups were matched for age, gender, and race. The mean mtDNA CN per lymphocyte, evaluated by qPCR, in children with AU was 212 ± 8 (mean ± SEM; n = 66), significantly higher than that of TD children (184 ± 7; n = 46; P = .012; Table 2). The incidence of children with AU with high mtDNA CN (where high was considered with a z score ≥2) was 1.4-fold that of TD children (P = .256; Table 3). We tested whether the higher average mtDNA CN was accompanied by increased mtDNA deletions. The mean mitochondrial gene ratio for TD children was 1.00 ± 0.02, not different from that of children with AU (0.98 ± 0.02; Table 2); however, the incidence of children with AU with mtDNA deletions was 2.6-fold of TD children (P = .026; Table 3). The mtDNA deletions detected in this study should not be considered pathogenic per se, for a mitochondrial defect is evidenced when a gene is deleted by >60%.[46] Taken together, these results indicated that both the mtDNA CN (~1.4-fold) and the incidence of mtDNA deletions were higher (~3.0-fold) in children with AU than TD children. Increases in mtDNA CN and accumulation of deletions, without concomitant increases in mitochondrial mass and function, are consistent with increased oxidative stress triggered by environmental factor(s) and/or to an idiopathic form of chronic oxidative stress, confirming and extending our previous studies.[35] Increases in mtDNA CN also have been associated with mitochondrial dysfunction and defects in the nucleoid structure.[28]

Higher Incidence of mtDNA Deletions in Fathers of Children With AU, Not Related to Age, Opposite to Mothers' Trend

Fathers of TD children exhibited a mean mtDNA CN of 195 ± 9 (n = 36), not different from that of fathers with AU (180 ± 8; n = 47; Table 2). No difference in the average mtDNA CN between TD children and their fathers was observed; within the AU group, however, the fathers had significantly lower mean mtDNA CN than their affected children (P = .007). The possibility that few outliers in the AU father group had influenced the mean mtDNA CN was excluded because only 2 fathers, 1 from each diagnostic group, were



Plot	Pearson's *P*-value	Slope comparison	ANCOVA *P*-value
A	<.001	A vs. B	.750
B	< 1e-0.4	C vs. D	.007
C	<.001	E vs. F	.058
D	.977	C vs. E	.384
E	.024	C vs. F	.005
F	.613		

FIGURE 1
mtDNA CN in PBMC from TD children and children with AU and their parents. The mtDNA CN per cell was determined as explained in the Methods section and evaluated in PBMC from children, mothers, and fathers. Statistical analyses were obtained by Pearson correlation and ANCOVA. A, Correlation between TD-mothers and TD-fathers. B, Correlation between AU-mothers and AU-fathers. C, Correlation of TD-midparents and TD children. D, Correlation between AU-midparents and AU-children. E, Correlation between AU-midparents and AU-children with SSs of 6 to 7. F, Correlation between AU-midparents and AU-children with SSs of 8 to 10. See other details in the text.

TABLE 2 mtDNA CN/Cell, mtDNA Deletions, and *p53* Gene Copy Ratios in TD and AU Trios

Outcome	Children			Mothers of			Fathers of		
CN	TD	AU	*P*	TD	AU	*P*	TD	AU	*P*
mtDNA (±SEM)	184 (±7)	212 (±8)	.012	205 (±9)	207 (±9)	.875	195 (±9)	180 (±8)	.237
n	46	66		36	48		36	47	
Deletions in mtDNA (±SEM)	1.00 (±0.02)	0.98 (±0.02)	.458	0.83 (±0.01)	0.87 (±0.01)	.206	0.86 (±0.02)	0.88 (±0.02)	.520
n	46	66		35	46		37	48	
p53 gene copy ratio (±SEM)	1.00 (±0.02)	1.05 (±0.01)	.055	1.09 (±0.04)	0.93 (±0.04)	.009	1.07 (±0.02)	0.98 (±0.02)	.055
n	44	60		36	47		37	46	

mtDNA deletions and *p53* gene copy ratio values were normalized to TD children. Statistical analysis was performed with the 2-tailed Student's *t* test.

TABLE 3 Incidence of Changes in mtDNA CN, mtDNA Deletions, and *p53* Gene Copy Ratios in TD and AU Trios

Outcome	Children, %		*P*	Mothers of, %		*P*	Fathers of, %		*P*
	TD	AU		TD	AU		TD	AU	
mtDNA CN	10.9[a]	15.2[a]	.256	8.1	6.3	.357	10.8	12.5	.409
n	46	66		36	48		36	47	
mtDNA deletions	8.7	22.7[b]	.026	8.6	2.2	.087	5.4	10.4[b]	.202
n	46	66		35	48		37	48	
p53 gene copy ratio	9.3[a]	25.0[a]	.017	13.9	21.3	.193	8.1	19.6	.070
n	44	60		36	47		37	46	

The incidence of mtDNA deletions represents the number of individuals with deletions at the segment encoding for *CYTB* (*z* score ≤ −2 for adults and ≤ −1 for children). Incidence of *p53* gene copy ratio was calculated as the percentage of individuals with high copy ratio (*z* score ≥ 2) or low gene copy ratio (*z* ≤−2). mtDNA deletions and *p53* gene copy ratio values were normalized to TD children's values and the *z* scores were also calculated by using the mean and SD from TD children. All TD versus AU comparisons were performed within each group (children, mothers, and fathers) by using 1-tailed χ^2 test.

[a] High mtDNA CN was considered as values with a *z* score ≥2.

[b] Similar trend in outcomes between child and parents.

considered to have mtDNA depletion (with mtDNA CN <60%).

The mtDNA deletions in TD and AU parents were within the same range (14%–17%), whereas in children, regardless of diagnosis, were <2% (*P* = .012 all parents versus all children; for all 6 groups: parents versus children of any diagnostic group *P* < .0001; Table 2). These results were consistent with an age-dependent imbalance between mtDNA repair and oxidative stress, not necessarily related to the diagnosis of the child.

As reported before, both children with AU and fathers with AU exhibited a higher incidence of mtDNA deletions compared with their respective TD group (2.6-fold children with AU versus TD children, *P* = .026; 1.9-fold fathers with AU versus TD fathers, *P* = .202; Table 3 and Napoli et al[35]). When stratified into decades of paternal age, the ratio of fathers with AU with mtDNA deletions versus TD fathers was 1.0 during the third, increasing to 1.4 at the fourth (*P* < .05) and 1.5 at the fifth (*P* < .05). Given that fathers' mean age within each decade of life was statistically not different between AU and TD, a residual effect of age as a confounding factor is precluded. These results instead support age as an effect modifier of the relationship between paternal mtDNA deletions and risk for ASD. Thus, combined with the existing literature on paternal age and ASD risk,[47–49] our findings are consistent with the concepts that (1) in fathers older than 30, defective repair systems governed by *p53* confer an increased risk of the child developing ASD, independently of age; and (2) deficits in mtDNA repair systems may serve as one of the various processes (along with de novo mutations in nuclear DNA, epigenetic alterations, and so forth) or pathways by which advancing fathers' age influences ASD risk.[47–49]

No differences in the mean mtDNA CN were observed between AU and TD mothers (207 ± 9; *n* = 48 and 205 ± 9; *n* = 36, respectively), consistent with the observations in fathers. No differences were observed in the mtDNA CN between children and mothers, regardless of their diagnostic group. The mean mitochondrial gene ratio in TD mothers was 0.83 ± 0.01 (*n* = 35), similar to that of mothers with AU (0.87 ± 0.01; *n* = 46) but both, as observed with fathers, lower than those from children (*P* < .0001), consistent with an age-dependent accumulation of deletions. Despite having similar mtDNA deletions (or similar mean mitochondrial gene ratios), the incidence of mothers with AU with damaged mtDNA was lower (26% of TD mothers; *P* = .087; Table 3).

Taken together, these results indicated that (1) the incidence of mtDNA deletions was higher in both children with AU and their fathers than their respective age-matched TD groups (approximately threefold and twofold, respectively), similar to the main paternal origin of de novo genomic point mutations[49]; and (2) the incidence of mothers with AU with mtDNA deletions was lower (one-third) than that expected for their age- and gender-matched group.

Contribution of Gene × Environment Interactions on the mtDNA CN in Children With AU

Considering that mtDNA CN is determined by genetic[50] and environmental factors,[51,52] the mtDNA CN was analyzed in AU and TD trios to elucidate the contribution of genetics and epigenetic-environmental factors (Fig 1). The correlation for the nongenetic, shared-environment or assortative-mating relationship between mtDNA CN of fathers and mothers was 0.72 for TD and 0.65 for AU (Fig 1 and Supplemental Table 8). Based on the lack of significant differences between the linear fits of AU and TD couples (as judged by ANCOVA analysis; Fig 1), the concept of assortative mating in either group is precluded. The significant and similar concordance between couples' mtDNA CN for both diagnostic groups (and considering that they are not genetically related) supports the notion that environmental factors (eg, couples sharing at least some dietary habits, features of lifestyle, and household environment) have a strong influence on the mtDNA CN maintenance in PBMCs, as it has also been reported before.[24,52]

Heritability estimate (h^2 or narrow sense heritability) for mtDNA CN was significant in TD child-midparent (0.56) but not for AU child-midparent (–0.004). Correlations for mtDNA CN between TD father-child (0.50) and TD mother-child (0.34) were significant, whereas those for AU were not, unless the SSs were taken into account (Supplemental Table 8). Further analysis based on the level of ASD-related symptoms (quantified as AU SSs) showed a clear pattern of h^2 for mtDNA CN in children with AU: the h^2 of mtDNA CN from children with AU with SSs of 6 to 7 was significant with midparent (0.37) and their mothers (0.36), reaching almost significance with their fathers as well (0.27), whereas the only correlation significant at high SSs was that of AU child-father (0.34) (Supplemental Table 8).

Taken together, these results suggest that (1) all mtDNA CN correlations involving children (0.3–0.4) were lower than the range of nongenetic, shared-environment observed between spouses (0.6–0.7), suggesting a larger variation contributed by the epigenetic/environmental influence at defining the mtDNA CN in children versus parents, possibly based on the habit differences between these 2 age groups; (2) a combination of parental and environmental influence was observed for the mtDNA CN in children with AU with moderate levels of severity (0.27–0.37), similar to that observed for TD children (0.34–0.56); and (3) the mtDNA CN in children with AU with higher SSs has a large paternal influence.

With regard to mtDNA deletions, the correlations were significant only between spouses for both diagnostic groups (0.36 and 0.39 for TD and AU, respectively), albeit at a lower level than the correlations for mtDNA CN, suggesting similar environmental influences (eg, shared habits or similar age) contributing to both mtDNA CN and deletions (Supplemental Table 9).

Higher Incidence of Altered p53 Gene Copy Ratio in Children With AU and Their Fathers

Several *p53*-associated proteins have been found mutated in ASD,[49] lower *p53* expression, and increased mtDNA CN and deletions have been observed in *Pten* haploinsufficient mice with ASD-like behavior.[6] Further, the *p53* gene is responsive to a large number of environmental stressors[1–4] implicated in the maintenance of mtDNA CN[5,6,28,53] and mitochondrial respiration.[1,6] To this end, *p53* gene CNs were evaluated by qPCR in the trios.

The mean *p53* gene copy ratios of children with AU were on average 5% higher than TD (Table 2), and the incidence of high *p53* gene copy ratio was higher in children with AU than TD children (2.7-fold; Table 3). In parents of children with AU, the mean *p53* gene copy ratio was lower (P = .009 for mothers with AU, and P = .055 for fathers with AU) than TD parents (Table 2). A higher incidence of low *p53* gene copy ratio also was observed in parents with AU (approximately twofold on average; Table 3).

Taken together, the incidence of high *p53* gene copy ratio was higher in children with AU versus TD children and lower in parents with AU than TD parents.

AU Risk and Outcomes

The contribution of the previously described outcomes at influencing AU incidence was estimated with the odds ratio (OR). The risk of AU diagnosis in a child was threefold when bearing mtDNA deletions or high *p53* gene copy ratio in PBMCs (Table 4). The risk of having a child with AU for fathers with mtDNA deletions or low *p53* gene copy ratio was also two- to threefold.

DISCUSSION

For the first time, *p53* gene copy ratio and mtDNA, as molecular stress markers of nuclear and mitochondrial damage, respectively, have been studied in PBMC from a well-defined population of children with AU and their families. Although abnormalities in PBMCs may not reflect those in the brain, the impaired immune response observed in children with AU,[54] the effect of neonatal immunity on neurodevelopment,[55,56] and the central

TABLE 4 Contribution of *p53* and mtDNA Outcomes to AU Diagnosis in Children and Their Parents

OR of AU Diagnosis Versus TD	OR	95% Confidence Interval
High *p53* gene copy ratio in child	3.4	1.04–11.13
Deletions in child mtDNA	3.1	1.0–10.0
Low paternal *p53* gene copy ratio	2.8	0.7–11.0
Deletions in paternal mtDNA	2.0	0.4–11.0

Outcomes shown have a $P < .10$. Cutoff values were indicated in Table 3.

role of mitochondria in immunity[57–59] highlight the relevance of using these cells in the context of AU. The higher incidence of an altered *p53* gene copy ratio (Table 3) and increased mtDNA deletions in children with AU (Table 3) and their fathers (see text for age-stratified comparisons made previously and in Napoli et al[35]), compared with age- and gender-matched TD, is suggestive of a gender-dependent gene × environment interaction, exacerbated in children with AU, leading to the disruption of the link between *p53* and mtDNA maintenance, possibly via increased oxidative stress and defective repair/antioxidant defenses. Contrary to other reports, our study supports the notion that increased paternal cumulative deletions (compared with age-matched group), rather than older age, confers the increased risk to AU diagnosis in the offspring.[47]

Increased mitochondrial reactive oxygen species production accompanied by accumulation of defective mitochondria is observed when *p53* function is affected, either directly by allele loss (murine brain) or indirectly via *Pten* (murine brain and striatal neurons),[6] resulting in lower transcription of genes encoding for antioxidant defenses.[60] This is relevant in the central nervous system, given that the oxidative stress threshold required for the *p53*-induced pro-oxidant effects in neurons is lower than those reported in any other cell type.[61] Taken together, instability in the *p53* genomic region (haploinsufficiency, or altered CN) might set a background of heightened susceptibility for other detrimental triggers (environmental exposures,[33,62] use of maternal medications and/or metabolic status,[63,64] other diseases[65–68]) influencing the progeny outcome, especially during highly susceptible periods of development, such as perinatal stages.

Although >30 types of deletions are reported for the *C*-terminus of *p53* (http://p53.iarc.fr), and CN variations in chromosome 17p13.1 (*p53*) and adjacent regions have been reported in cases of AU, mental retardation, and developmental delay (Supplemental Table 5), their impact on AU has not yet been fully elucidated with the exception of 1 report on ring chromosome 17.[69] It is difficult to ascertain cause-effect in a case-control study; however, altered *p53* gene copy ratios might underlie some of the mitochondrial defects observed before in children with AU with high SSs,[34] as well as the reported genomic instability.[70–72] The stronger paternal influence on mtDNA and *p53* in children with AU at higher SSs is consistent with the concept that most of the de novo variants have a paternal origin.[73,74] The environmental influence in children with lower SSs is consistent with the higher incidence in AU of damaging mutations in genes linked to epigenetic modification of histones,[73] chromatin remodeling, and cell lineage determination.[75,76,–

Given the antioxidant and pro-oxidant activities of *p53*,[77,78] and that genomic instability also has been linked to disorders other than AU,[79] future studies would need to address the role of *p53* in nonimmune cells, and more importantly to define the mechanisms by which environmental factors shape this genetic susceptibility resulting in AU.

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ABBREVIATIONS

ADOS: Autism Diagnostic Observation Schedule
ANCOVA: analysis of covariance
ASD: autism spectrum disorder
AU: autism
CHARGE: Childhood Autism Risk from Genes and Environment study
CN: copy number
CYTB: cytochrome *b*
mtDNA: mitochondrial DNA
ND1: NADH dehydrogenase subunits 1
OR: odds ratio
PBMC: peripheral blood mononuclear cell
PK: pyruvate kinase
qPCR: quantitative polymerase chain reaction
SS: severity score
TD: typically neurodeveloping

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