The Mitochondrial DNA A3243A>G Mutation Must Be An Infrequent Cause Of Asperger Syndrome

To the Editor:

It is generally accepted that the etiology of Autistic Spectrum Disorder (ASD) has a major genetic component, involving up to 15 discrete interacting genetic loci.[1] Despite a major international effort, it has not yet been possible to identify the underlying gene mutations. There is increasing evidence that impaired mitochondrial respiratory chain function contributes to the pathophysiology.[2] Pons and colleagues recently described three children with ASD as a presenting feature of one of the most common pathogenic mitochondrial DNA (mtDNA) mutations: 3243A>G.[3]

The 3243A>G mutation is associated with a diverse clinical phenotype, ranging from migraine and diabetes in mildly affected persons, to a severe encephalomyopathy with stroke-like episodes, dementia, and epilepsy, often associated with hypertrophic cardiomyopathy and sensorineural deafness.[4] Psychiatric and behavioral disturbances may be the presenting features of the 3243A>G mutation.[5] There is therefore a strong rationale for investigating mitochondrial genetic mechanisms in the neurodevelopmental conditions that fall within the autistic spectrum.

Asperger syndrome (AS) lies at one extreme of the autistic spectrum. To determine whether the 3243A>G mutation is a common cause of AS we screened 129 persons fulfilling *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, criteria for AS.[6] DNA samples were isolated from buccal swabs. Persons with the 3243A>G mutation harbor a mixture of mutated and wild-type mtDNA (heteroplasmy), often with low percentage levels of mutated mtDNA in easily accessible tissues. We therefore used a sensitive fluorescent assay for the 3243A>G mutation, which can detect down to 2.6% mutated mtDNA (Figure; available at www.jpeds.com). None of the AS cases had the 3243A>G mutation.

In two of the ASD cases reported by Pons et al, the 3243A>G mutation was not detected in accessible tissues in the affected child, but it was detectable in tissues in the mother, including maternal blood in one case. This raises the possibility that the causative mutation was transmitted from mother to child, but nonrandom segregation during development led to the loss of mutated mtDNA from accessible tissues (including blood, buccal mucosa, and hair follicles) and the persistence of mutated mtDNA in the central nervous system. We therefore screened maternal buccal swab mtDNA from the mothers of 138 patients with AS using the same sensitive assay. None of the mothers had the 3243A>G mutation.

We conclude that the 3243A>G mtDNA mutation is a rare cause of isolated AS (exact 95% confidence interval 0%-2% for probands, and 0%-2% for mothers of probands). Although we cannot exclude the possibility that the 3243A>G mutation was present in other tissues in the cohort we studied, the percentage level of mutated mtDNA is usually higher in buccal mucosa than in blood or hair follicles,[3,7,8] making this less likely. Our observations support the conclusion of Pons et al[3] that mitochondrial dysfunction should only be considered in children with autistic features when there are additional neurologic features or there is a relevant maternal family history.

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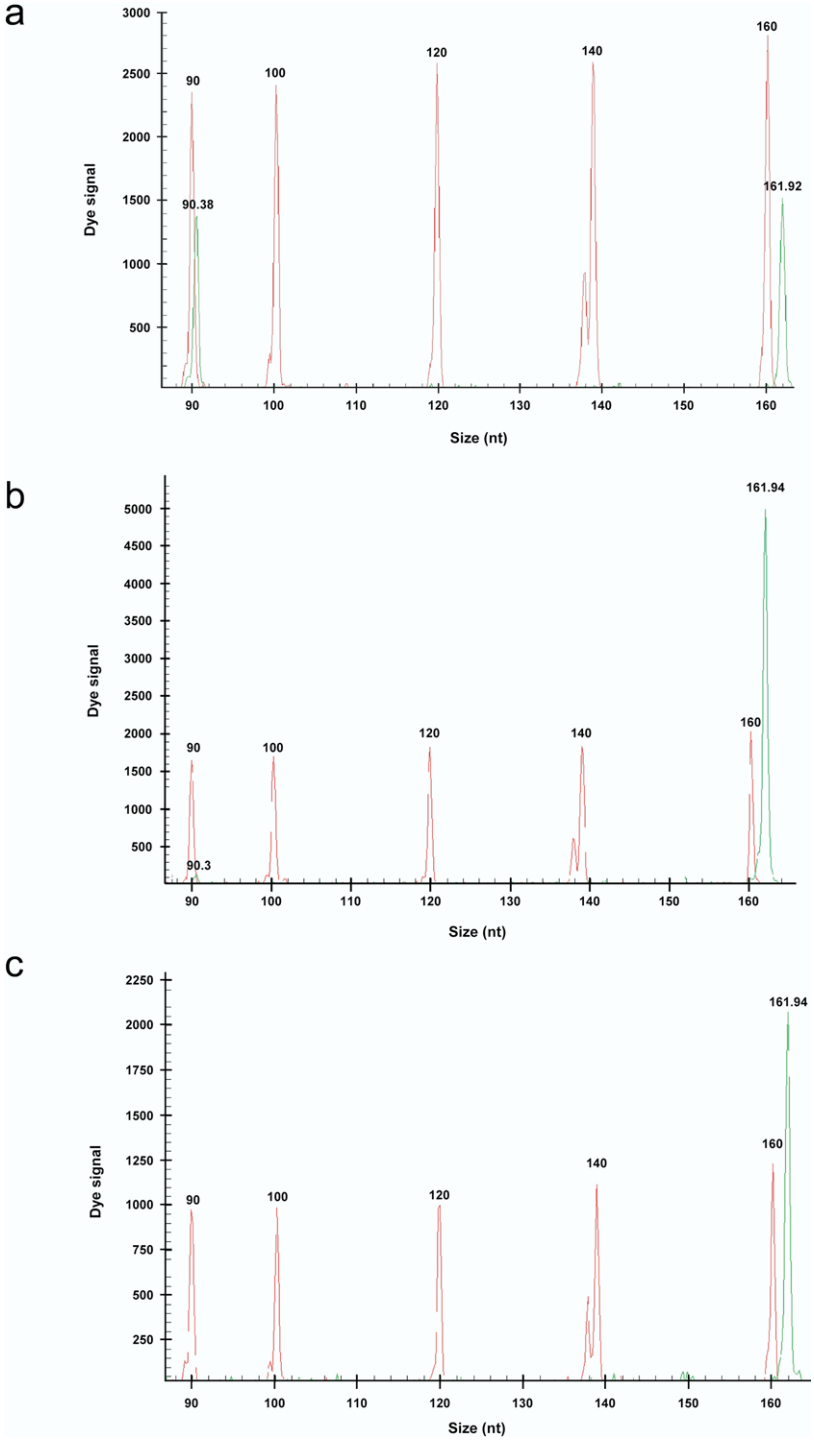

Figure. Fluorescent assay for the 3243A>G mitochondrial DNA (mtDNA) mutation. MtDNA encompassing the mtDNA Leu$^{(UUR)}$ tRNA gene was amplified using a fluorescent tagged forward primer (5'-cac aaa gcg cct tcc cc-3') and a nontagged reverse primer (5'-gcg att aga atg ggt aca at-3'), and standard thermal cycling conditions (Tm 61°C). The polymerase chain reaction products were digested with *Hae* III and the products separated and sized on a Beckman CEQ 8000 capillary DNA analyser (Foster City, CA). The undigested polymerase chain reaction product is 199 bp, the wild-type digested product 162 bp, and the mutated digested product 90 bp. Cloned products from a patient with high percentage levels of the 3243A>G mutation were used to generate a set of serial dilutions of the 3243A>G mutation to use as positive controls. (**a**) Trace showing wild-type and mutated peaks from a clone mix of 41.6% mutated mtDNA; (**b**) trace showing wild-type and mutated peaks from a clone mix containing 2.6% mutated mtDNA; (**c**) trace from a blood DNA template from an AS case showing 100% wild-type mtDNA. Y-axis = fluorescent signal intensity. X-axis = size (bp).