

## LETTERS TO THE EDITOR

# Paternal age and autism are associated in a family-based sample

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Autism is a lifelong neurodevelopmental brain disorder characterized by deficits in social interaction and communication, the presence of restricted, repetitive and stereotyped patterns of behavior, and an onset before the age of 3.<sup>1</sup> Although its etiology remains unknown, evidence suggests that autism is highly heritable, genetically heterogeneous,<sup>2</sup> and likely to be influenced by environmental factors.<sup>3</sup> A recent article by Reichenberg and co-workers<sup>4</sup> reported a significant association between paternal age at birth and a child's risk for developing autism. This association was identified in a subset of 132 161 individuals with complete information on parental age that was derived from a larger cohort of 318 506 Israeli births occurring over a 6-year period during the 1980's. When paternal ages were examined across five increasing categories, the risk of autism spectrum disorder (ASD) increased monotonically, going from 10 to 107 per 10 000 births. Given this report, we were motivated to investigate whether data from the autism genetic resource exchange (AGRE),<sup>5</sup> would also exhibit this association.

AGRE is a DNA repository and family registry created by Cure Autism Now that has been recruiting families through a variety of methods including physician referral, web site contact and family meetings and seminars, and making their DNA and diagnoses available to interested researchers since 1998. To be included, families must have at least two members that meet criteria for a diagnosis of an ASD. There are no restrictions regarding the age, ethnicity or socioeconomic status of the participants, and as long as there are two affected with ASD, parity is not considered. The families are primarily nuclear, and of the current 1419 registered families, 830 have complete clinical data and biomaterials available for investigations. The majority of the sample (75%) is Caucasian and non-Hispanic and 37% of the families are from the West coast, with most of those from California. The ASD offspring participating in the current study were all born within the last 35 years, and a majority within the last 15 years. Paternal and maternal ages, along with brief family histories and other demographic information, are collected during the screening process.

Because the ascertainment strategy used to recruit families for AGRE requires a diagnosis of ASD in at least two family members, as well as their willingness to participate in this repository, one cannot directly

estimate the risk of having an autistic child for the different paternal age categories as was done in the Reichenberg study. AGRE does, however, provide a sample of families with autism that were not selected for paternal age. These can be used to assess the evidence of an association of autism with paternal age if an independent well-matched population-based control sample of paternal ages is identified for comparison.

We were fortunate to identify a report of a US population-based sample of paternal ages in a study of early delivery<sup>6</sup> designed to assess whether there is an association between early birth and paternal age. To test this association, the authors studied the paternal age distribution of approximately 2.5 million non-twin births occurring in the US between 1995 and 2000 to first-time White, non-Hispanic married mothers less than 36 years of age from all states in the US, excluding California that was stratified by gestational age of the child at the time of delivery. Paternal ages were divided into increasing intervals from 20 to 50, with a separate category for fathers of ages over 50. The twins were excluded from this US control sample because twinning is a known risk factor for premature birth. The published raw data in Table 1 of the Basso and Wilcox paper were available to construct a paternal age distribution without regard to gestational age at delivery.

To capitalize on the availability of this published paternal age distribution, an appropriate subset of the AGRE sample was chosen for comparison. Families were selected from the AGRE sample if the parents were married, White and non-Hispanic, the birth was not multiple, the mother was less than 36 years of age, and without regard to gestational age of the child at delivery. There were two exceptions to the criteria used to construct the 'control' sample. They were made to provide an adequately sized sample for the analysis. The AGRE families from California were included, and rather than restrict this sample to births occurring between 1995 and 2000, all first time births were included, regardless of when the child was born. Our rationale for the first exception is that California has often been viewed as representative of the entire US and is not expected to differ from the US in aggregate on factors that influence paternal age at the birth of the first child. Regarding the second exception, we anticipated that including those born outside the window of 1995–2000 would make the comparison with the control group conservative because paternal ages have been increasing during that period. Surprisingly, when we examined the correlations

**Table 1** Percents in paternal age categories for singleton first child births in married non-hispanic caucasians for maternal ages < 36 years

Paternal age	US 'controls' <sup>a</sup> n = 2 492 308	AGRE autistic first child n = 312
20–29 <sup>b</sup>	55.1	41.6 <sup>c</sup>
30–39	41.9	54.7
40–49	3.0	3.7

Abbreviation: AGRE, autism genetic resource exchange.

<sup>a</sup>Categories stratified by age of delivery in the Basso and Wilcox paper were combined. Those fathers over 50 or whose ages were missing were removed from the total sample, and the percents in each age category were recalculated.

A typographical error in their paper was fixed (Table 1 age 20–24 > 36 weeks the count should read 374,305).

<sup>b</sup>Inclusive.

<sup>c</sup> $\chi^2 = 16.12$ ;  $P < 0.005$  for AGRE paternal age distribution goodness of fit to the 'control' distribution.

between the current age of the first born child over time and the paternal age at the time of birth in the AGRE sub-sample matching the study criteria, there was no evidence to support a change in paternal age at the birth of the first born child, as the correlations were not significant, regardless of whether the first child was autistic or non ASD.

A total of 312 AGRE families that met the criteria for inclusion in the study had a first-born child with ADI-R<sup>7</sup> diagnosed autism and 137 had a first-born child without autism or an ASD. The distribution of these 312 paternal ages have been categorized into increasing 10-year intervals and are reported in the second column of Table 1. Unfortunately, none of the fathers that were over 50 at the birth of a child satisfied the criteria for inclusion in the study, so we only present the distribution of paternal ages between ages 20 and 50. The first column of Table 1 reports the analogous paternal age distribution in the US 'control' sample, where those with a missing or paternal age over 50 have been removed, and a typographical error in their published table was also corrected as described in 'a', a footnote to Table 1.

The paternal age distribution of the AGRE fathers, whose first child is autistic differs significantly from that of the 'control' sample ( $P = 0.005$ ). A  $\chi^2$  goodness-of-fit test with 2 degrees of freedom was conducted using percents in the 'control' group age categories to calculate the expected values in the AGRE sample. The shift toward higher paternal ages in those with an affected first-born is seen most dramatically in the group of AGRE fathers who are 30–39 years inclusive, which is 54.7% of the distribution compared with the 41.9 % that is expected. We interpret this shifted age distribution to provide support for the recently reported finding by Reichenberg and co-workers that autism risk is associated with advancing paternal age.

There were 41 families in the AGRE sample that met all of the study criteria except that their first-born children were twins with autism. As might be expected, an upward shift in the paternal age distribution is more dramatic (24.4, 68.3 and 7.3% in each of the three paternal age categories, respectively). Although it is intriguing to think that older fathers may be more likely to have children using assisted reproduction techniques which might be related to a predisposition to autism, it is also possible to interpret this to be a result of the AGRE ascertainment criterion of two affected family members. The age distribution for the fathers of first-born non-ASD children is much closer to that of the control sample (52.5, 44.5 and 3% respectively in the 3 age-categories). Although it is tempting to also compare their age distribution to those having an autistic first-born, this comparison might be misleading as each family is required to have at least two affected to qualify for participation in AGRE. Those families who have two or more affected children after an unaffected first-born would need to have more children to qualify for the study, and might have their unaffected child at an earlier age.

Both genetic and epigenetic factors can explain the increased risk for autism with increasing paternal age. At the genetic level, spermatogonial stem cell divisions that occur over the lifetime of males can contribute to higher mutational rates in the sperm of older men, possibly including genetic mutations that result in autism. This phenomenon explains some of the increased risk of Achondroplasia in the children of older fathers. Recently, however, it was demonstrated that mutations in the sperm do not explain the paternal age effect completely.<sup>8</sup> Epigenetic phenomena have also been discussed as potential risk factors for autism, specifically within the context of assisted reproduction which is more frequent in older first-time fathers. Here, possible disrupted prenatal methylation patterns can lead to imprinting disorders that may include autism as one of their phenotypic features.<sup>9</sup> Extensive analyses of large and well-characterized study samples are needed to identify and disentangle the importance of these and other autism risk factors in the children of older fathers.

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## Mapping regulatory variants for the serotonin transporter gene based on allelic expression imbalance

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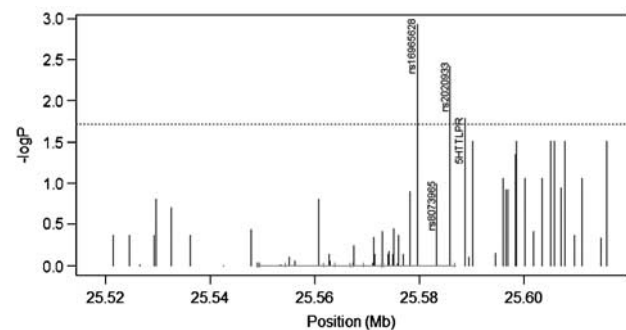
There have been a large number of inconsistent reports of an association between a functional polymorphism (*5HTTLPR*), located in the promoter of the serotonin transporter gene, and susceptibility to depression and anxiety disorders.<sup>1</sup> However, it is not known how many other sequence variants contribute to transcriptional variation of the serotonin transporter gene, nor whether their presence might confound interpretation of *5HTTLPR* genetic association studies.

We explored these questions by measuring the relative abundance of allelic transcripts of the serotonin transporter gene by analysing heterozygous-transcribed polymorphisms in cell lines genotyped by the HapMap consortium. A quantitative analysis of the transcribed polymorphism will show an allelic expression imbalance (AEI) in those individuals who are heterozygote for both the transcribed variant and the *cis*-acting variant.

Thirty-three unrelated lines from HapMap CEPH trios, heterozygous for single-nucleotide polymorphism (SNP) rs1042173, were used to measure the ratios of *SLC6A4* allelic transcripts. The CEPH HapMap trios were genotyped for the *5HTTLPR* and the  $L_G/L_A$  alleles<sup>2,3</sup> and were phased relative to the alleles of rs1042173. In cases where the phase was in the opposite direction to the common

diploypes, we inverted the ratio of the allelic expression. We then calculated the average expression ratio for each genotype group (SS, LS, LL,  $L_AL_A$ ,  $L_GS$ ,  $L_AL_A$ ,  $L_AL_G$ ). Significant AEI was observed for the LS (mean ratio = 0.76,  $n = 17$ ,  $P = 0.0043$ ) and the  $L_AL_S$  (mean ratio = 0.77,  $n = 14$ ,  $P = 0.022$ ) genotype groups, but not for other genotype groups. These results are not consistent with an effect from the  $L_G$  allele, but our conclusions are limited by a small number of observations. In the LS group ( $n = 17$ ) after inverting the ratios based on the *5HTTLPR*-rs1042173 phase, all but one ratio was less than 1, indicating a greater expression of the L relative to the S allele. Within the complete homozygote groups ( $L_AL_A$  and SS, mean ratio = 1.07,  $n = 14$ ), there were four samples (29%) showing an allelic ratio greater than 1.2.

As this result suggests that additional factors besides the *5HTTLPR* influence the *SLC6A4* transcription, we tested the extent to which the distribution of allelic transcripts ratios is explained by the genotypes (homozygotes vs heterozygotes) of 55 SNPs distributed in a 100 kb window surrounding the *SLC6A4* gene. The genotypes of two SNPs besides the *5HTTLPR* showed a significant correlation with the allelic transcript ratios, above the 5% permutation threshold (Figure 1). The most significant SNP is rs16965628 located in the middle of the first intron (mean G/C ratio = 0.47, empirical  $P = 3 \times 10^{-5}$ , maximum proportion of variance explained (PVE) = 0.52, minor allele frequency = 6%). The second SNP is rs2020933 (mean T/A ratio = 0.46, empirical  $P = 0.0014$ , PVE = 0.45) located in the first intron, 870 bp from the end of the first non-coding exon (1A). The two SNPs are in high correlation ( $r^2 = 0.79$ ) with each other but not with the *5HTTLPR*. The *5HTTLPR*



**Figure 1** Results of association mapping of AEI. The minus log of the  $P$ -value ( $-\log P$ ) for the differences between the distribution of allelic expression imbalance in homozygotes and heterozygotes is shown by the height of the vertical lines for 55 SNPs in 100 kb surrounding the *SLC6A4* gene. The horizontal dashed line is the overall 5% significance threshold empirically estimated by permutations. The location of the three significant variants, rs16965628, rs2020933 and *5HTTLPR*, is indicated. The location of the *SLC6A4* gene exons (red boxes) and introns (red connecting lines) is shown in the bottom of the plot.