Apolipoprotein E Affects Serial Changes in Total and Low-Density Lipoprotein Cholesterol in Adolescent Girls: Project HeartBeat!

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Apolipoprotein E (apo E) polymorphism is a genetic determinant of lipid and lipoprotein levels and the risk for coronary heart disease. The extent to which serial patterns of change in total cholesterol and low-density lipoprotein cholesterol (LDL-C) concentrations varied by apo E genotype was therefore investigated in 247 Caucasian girls aged 8 to 14 at baseline who were participating in Project HeartBeat!, a mixed longitudinal study of cardiovascular disease (CVD) risk factor development in children. Plasma lipid concentrations were determined for each participant three times per year (every 4 months) for up to 4 years from October 1991 through August 1995. Mean total cholesterol values for individuals with ε2/3, ε3/3, and ε3/4 genotypes were 141.7, 161.6, and 165.9 mg/dL, respectively ($P < .001$). Corresponding LDL-C values for individuals with ε2/3, ε3/3, and ε3/4 genotypes were 74.6, 94.8, and 98.7 mg/dL, respectively ($P < .001$). The results of longitudinal modeling indicated that age trajectories for total cholesterol and LDL-C varied significantly by apo E genotype. Individuals with ε3/3 and ε3/4 genotypes exhibited similar patterns of change in total cholesterol and LDL-C from ages 8 to 18, while individuals with the ε2/3 genotype demonstrated a significantly different pattern of change (age^2 × genotype interaction, $P < .05$). For example, individuals with the ε2/3 genotype showed a slight increase in total cholesterol from approximately 141 to 146 mg/dL from ages 8 to 10; total cholesterol then decreased monotonically from ages 10 to 18 from 146 to 115 mg/dL. The apo E effect on total cholesterol and LDL-C and their change during adolescence is strong and may be modified by factors affecting growth, maturation, and reproductive function.

APOLIPOPROTEIN E (apo E) is a structural component of several classes of lipoprotein particles, and is a ligand for apo E–specific receptors in the liver and for low-density lipoprotein (LDL) receptors in many tissues. Human apo E is genetically variable, with three common alleles, ε4, ε3, and ε2, coding for the three major isoforms of apo E in plasma, designated E4, E3, and E2, respectively.[1]

Although the exact mechanism is poorly understood, apo E polymorphism has consistently been associated with altered total and LDL cholesterol (LDL-C) levels in many populations[1-3] and in both children[4-6] and adults.[7-8] The average effect of the ε2 allele is to decrease total cholesterol and LDL-C, while the average effect of the ε4 allele is to increase total cholesterol and LDL-C.[1] Reilly et al[9] and Hanis et al[10] have shown that the effect of apo E polymorphism is modified by gender and hormone status. Apo E genotype has been associated with the development of coronary heart disease in adults[1,11] and the presence of atherosclerotic lesions in young people.[12]

As recognized for many years, coronary heart disease has its origins in youth and early adulthood.[13-15] Understanding the longitudinal patterns of risk factor development and characterizing the effects that influence these patterns is essential for optimum identification of individuals at increased risk of disease later in life. This is particularly important in children and young adults with the advent of recent recommendations on selecting children for close surveillance and therapy for extended periods based on lipid measurements.[16] To date, researchers have not examined detailed patterns of serial change in total cholesterol and LDL-C by apo E genotype in children or adolescents. Therefore, the purpose of this investigation was to present the age trajectories of serial change in total cholesterol and LDL-C by apo E genotype in a cohort of adolescent girls participating in Project HeartBeat!, a longitudinal study of cardiovascular risk factor development in children.

SUBJECTS AND METHODS

Sample

Project HeartBeat! is a longitudinal study of cardiovascular risk factor development in children and adolescents. To assess the natural course of development of the cardiovascular disease (CVD) risk factors, three cohorts of children aged 8, 11, and 14 years at entry into the study were observed over a 4-year period from October 1991 through August 1995. Extensive assessments of CVD risk factors, including lipoprotein determinations, were conducted three times per year at 4-month intervals. The target population consisted of residents of The Woodlands community and the city of Conroe, TX. The Woodlands is a planned community in Montgomery County 30 miles north of Houston, with a population at the start of the study that was 92% white, 4% Hispanic, 2% black, and 2% other. The city of Conroe is 10 miles north of The Woodlands and is the center of the Conroe Independent School District, in which nearly all study participants in both areas were enrolled. Conroe is 75% white, 13% black, and 12% other (including Hispanic). Initial contact with potential participants was made by a letter mailed to each identified family. Families were invited to visit the project's field center for a tour and recruitment interview. Of the families contacted, 679 children enrolled in the project. The complete design and methods of Project HeartBeat! have been described previously.[17-18]

DNA analyses in Project HeartBeat! were restricted to the sample of 331 girls who underwent baseline examinations. Eight participants were excluded due to missing lipid data (n = 6) or excessively elevated

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baseline lipid values (n = 2). Participants with excessively elevated baseline and subsequent lipid values (total cholesterol > 250 mg/dL) were believed to represent children with familial hypercholesterolemia, and thus may have demonstrated a different pattern of change in cholesterol values as compared with the rest of the sample.

The present analyses were restricted to Caucasian participants; thus, 76 non-Caucasian girls were excluded from analysis (63 black, 10 Hispanic, two Asian, and one American-Indian). It is important to note that the results did not differ when white and black groups were combined or analyzed separately (results not shown). Thus, the population for analysis consisted of 247 Caucasian adolescent girls. Informed consent and parental consent were documented for each participant, and Project HeartBeat! was approved by The University of Texas-Houston, Committee for the Protection of Human Subjects.

Plasma Lipid and Lipoprotein Analyses

Lipid concentrations were determined in plasma after an overnight fast using standard enzymatic methods.[19-21] Samples were obtained and processed as previously reported[18] in the Lipid Research Laboratory at Baylor College of Medicine. A Cobas Fara II analyzer (Montclair, NJ) was used for the enzymatic process of cholesterol determination. Values are reported in milligrams per deciliter. LDL-C was calculated using the equation, LDL-C = [total cholesterol − (triglycerides/5 + HDL-C)].[19]

Apo E Genotyping

Apo E genotyping was performed following polymerase chain reaction–assisted amplification of a region of the apo E gene using primers described by Emi et al[22] and digestion with *Hha*I.[23] Following polyacrylamide gel electrophoresis and ethidium bromide staining, the gels were scored independently by two laboratory personnel.

Statistical Analysis

Routine statistical analyses were conducted using SPSS (Version 6.0) and Stata (Version 4.0) statistical computer packages.[24-25] Longitudinal multilevel modeling of the age trajectories of total cholesterol and LDL-C by apo E genotype was performed using the MLn software.[26] The MLn regression analysis program computed maximum likelihood estimates of the parameters for mixed linear models. To indicate statistical significance, the parameter estimate divided by its standard error was assumed to follow a t distribution, such that if the test statistic was greater than 1.96, then P was less than .05.

Data from this study were inherently hierarchical; therefore, a multilevel linear model was used as the major statistical method for the analyses. This type of model is a further development of the generalized linear model,[27] which takes into account the hierarchical characteristics of the data structure and has the general form, $Y = X\beta + ZU + \epsilon$, where $X\beta$ is the fixed part of the model that describes the mean response as a linear combination of the unknown coefficients, and ZU and ϵ constitute the random part of the model. With Project HeartBeat! data, ZU describes the interindividual variation (level 2 variation) in contrast to the mean response described by the fixed part of the model, and ϵ describes the intraindividual variation among different repeated measurements (level 1 variation). Thus, level 1 of the hierarchy comprises the repeated measurements on the response variables (total cholesterol or LDL-C) within subjects, with level 2 being the subjects.

Specifically, repeated measurements of total cholesterol and LDL-C were regressed on age, age^2, age^3, apo E genotype, and age × genotype interaction terms. The inclusion of quadratic and cubic terms in age in describing the trajectory for total cholesterol and LDL-C concentrations was considered necessary, due to the hypothesis that the age-related trajectory of lipid values is polyphasic from ages 8 to 18.[18] The basic model-building strategy consisted of including one-by-one the different terms of age (age and quadratic and cubic terms of age), followed by apo E (dummy-coded), and then adding the age × genotype interaction terms to the model. Changes in -2 logarithmic (likelihood) statistics were used to indicate whether the improvement or reduction in the goodness-of-fit of the model was significant after including or excluding a predictor variable. The change in -2 log (likelihood) follows approximately a chi-squared distribution with degrees of freedom equal to the difference in dimension of the parameter spaces of the models being compared. Age was centered first by subtracting the mean from the original value before entering it into the total cholesterol and LDL-C multilevel models. The primary hypothesis of interest pertained to the significance of the apo E and age × genotype interaction terms.

RESULTS

Table 1 shows the mean ± SD and r^2 values for baseline age, plasma cholesterol, and triglyceride values by apo E genotype. The mean levels of total cholesterol and LDL-C were significantly different among apo E genotypes. Individuals with the ϵ2/3 genotype had lower average total cholesterol and LDL-C levels, whereas individuals with an ϵ3/4 genotype had higher average total cholesterol and LDL-C levels. Individuals with ϵ2/3, ϵ3/3, and ϵ3/4 genotypes had mean total cholesterol values of 141.7, 161.6, and 165.9 mg/dL, respectively. Corresponding LDL-C values for ϵ2/3, ϵ3/3, and ϵ3/4 individuals were 74.6, 94.8, and 98.7 mg/dL, respectively. The variation in apo E genotype accounted for 8.2% of the variation in total cholesterol and 10.5% of the variation in LDL-C. The frequency of ϵ2, ϵ3, and ϵ4 alleles was 6.5%, 79.1%, and 14.4%, respectively. These proportions did not vary significantly from the expectation under Hardy-Weinberg equilibrium.

Table 2 shows the parameter estimates from longitudinal modeling of the age trajectories for total cholesterol and LDL-C by genotype. The trajectories for total cholesterol and LDL-C by genotype at ages 8 to 18 years were based on 2,092 determinations of plasma cholesterol concentrations. Multilevel

Table 1. Mean ± SD and r^2 Values for Baseline Age, Cholesterol, and Triglyceride by Apo E Genotype: Project HeartBeat!, 1991-1995

Parameter	Genotype 2/3 (n = 23)	3/3 (n = 157)	3/4 (n = 54)	Total (N = 247)	r^2*	P†
Age (yr)	10.9 ± 2.5	10.8 ± 2.5	11.4 ± 2.5	10.9 ± 2.5	.005	.32
Cholesterol (mg/dL)						
Total	141.7 ± 22.0	161.6 ± 22.2	165.9 ± 25.1	161.0 ± 23.8	.082	<.001
LDL	74.6 ± 20.0	94.8 ± 19.6	98.7 ± 25.3	94.0 ± 22.5	.105	<.001
HDL	51.3 ± 12.9	50.1 ± 10.7	49.1 ± 11.6	49.9 ± 11.0	.002	.71
Triglycerides (mg/dL)	79.5 ± 28.8	83.4 ± 41.6	90.6 ± 42.1	85.4 ± 41.2	.000	.44

NOTE. Two, 5, and 6 girls had apo E genotypes 2/2, 2/4, and 4/4, respectively.

*Percent of variance accounted for by apo E genotype.

†Determined from 1-way ANOVA using F ratio.

Table 2. Multilevel Longitudinal Models for Total Cholesterol and LDL-C by Apo E Genotype in 247 Girls: Project HeartBeat!, 1991-1995

Parameter	Total Cholesterol Estimate	Total Cholesterol Standard Error	LDL-C Estimate	LDL-C Standard Error
Fixed parameters				
Constant	142.00*	4.48	71.67*	4.02
Age	−4.49*	1.05	−4.93*	0.93
Age^2	−0.74*	0.23	−0.48*	0.21
Age^3	0.10*	0.02	0.05*	0.02
3/3 Genotype	17.16*	4.80	18.83*	4.31
3/4 Genotype	21.55*	5.36	23.12*	4.81
Age × 3/3 genotype	0.79	1.06	1.15	0.93
Age × 3/4 genotype	0.09	1.19	0.38	1.05
Age^2 × 3/3 genotype	0.78*	0.25	0.58*	0.22
Age^2 × 3/4 genotype	0.71*	0.27	0.73*	0.24
Between-subjects variance/covariance				
Constant	395.50*	41.90	318.70*	33.59
Age/constant	−6.54	6.07	−5.99	4.78
Age	5.97*	2.05	4.18*	1.59
Within-subject variance				
Error	159.20*	5.60	128.50*	4.52

*$P < .05$ (significance of estimate determined as estimate/standard error > 1.96, $P < .05$).

modeling is presented for total cholesterol and LDL-C because of the well-known effects of apo E on total cholesterol and LDL-C metabolism.[1] Since the primary hypothesis for this investigation concerned the anticipated interaction between age and genotype, the regression modeling strategy was used specifically to test this hypothesis. Results were similar for the total cholesterol and LDL-C models.

Age, age^2, and age^3 and genotypes 3/3 and 3/4 (dummy-coded) were added consecutively to the total cholesterol and LDL-C multilevel models. For both models, the independent contributions of age^3, apo E genotype, and age^2 × genotype interactions were significant explanatory variables of the change in total cholesterol and LDL-C (indicated by -2 log [likelihood] test, $P < .05$). In the total cholesterol and LDL-C multilevel models in Table 2, age, age^2, genotypes 3/3 and 3/4, and the interaction between age^2 and genotype (3/3 and 3/4) were statistically significant ($P < .05$). The mean total cholesterol based on the fixed part of the model can be estimated as $Y = 142.00 - 4.49(\text{age}) - 0.74(\text{age}^2) + 0.10(\text{age}^3) + 17.16(3/3) + 21.55(3/4) + 0.79(\text{age} \times 3/3) + 0.09(\text{age} \times 3/4) + 0.78(\text{age}^2 \times 3/3) + 0.71(\text{age}^2 \times 3/4)$. The standard deviation for total cholesterol measurements can then be estimated as $SD = \{[395.5 - 2(6.54)(\text{age}) + 5.97(\text{age}^2)] + [159.2]\}^{1/2}$. Note that age used in this calculation refers to age as a centered variable. The same strategy can be used to estimate the mean ± SD for LDL-C. In general, the multilevel modeling results in Table 2 indicate that the age trajectories for total cholesterol and LDL-C varied significantly by genotype.

Figure 1A and B illustrates the genotype-specific age trajectories for total cholesterol and LDL-C. In Fig 1A, the genotype-specific age trajectory of total cholesterol shows that girls with ε3/3 or ε3/4 genotypes had higher total cholesterol values throughout ages 8 to 18 in comparison to those with the ε2/3 genotype. Individuals with the ε3/4 genotype had the highest total cholesterol and those with the ε2/3 genotype had the lowest total cholesterol from ages 8 to 18. Individuals with ε3/3 and ε3/4 genotypes demonstrated similar patterns of change in total cholesterol such that the total cholesterol values were about 170 mg/dL from ages 8 to 10, decreased from 170 to near 155 mg/dL from ages 10 to 15, and then began to increase again at about age 17. Individuals with the ε2/3 genotype had consistently lower total cholesterol throughout the age range, and also showed a different pattern of change in total cholesterol from ages 8 to 18 in comparison to girls with ε3/3 and ε3/4 genotypes. Individuals with the ε2/3 genotype showed a slight increase in total cholesterol from about 141 to 146 mg/dL from ages 8 to 10; total cholesterol then decreased monotonically from ages 10 to 18 from 146 to 115 mg/dL. Individuals with the ε2/3 genotype clearly showed a different pattern of change in total cholesterol in comparison to those with ε3/3 and ε3/4 genotypes, as evidenced by the significant age^2 × genotype interaction in the multilevel model (Table 2).

Figure 1B shows the genotype-specific age trajectories in LDL-C. Individuals with ε3/3 and ε3/4 genotypes had consistently higher LDL-C than those with the ε2/3 genotype from ages 8 to 18. Individuals with the ε3/4 genotype had the highest LDL-C and those with the ε2/3 genotype had the lowest LDL-C

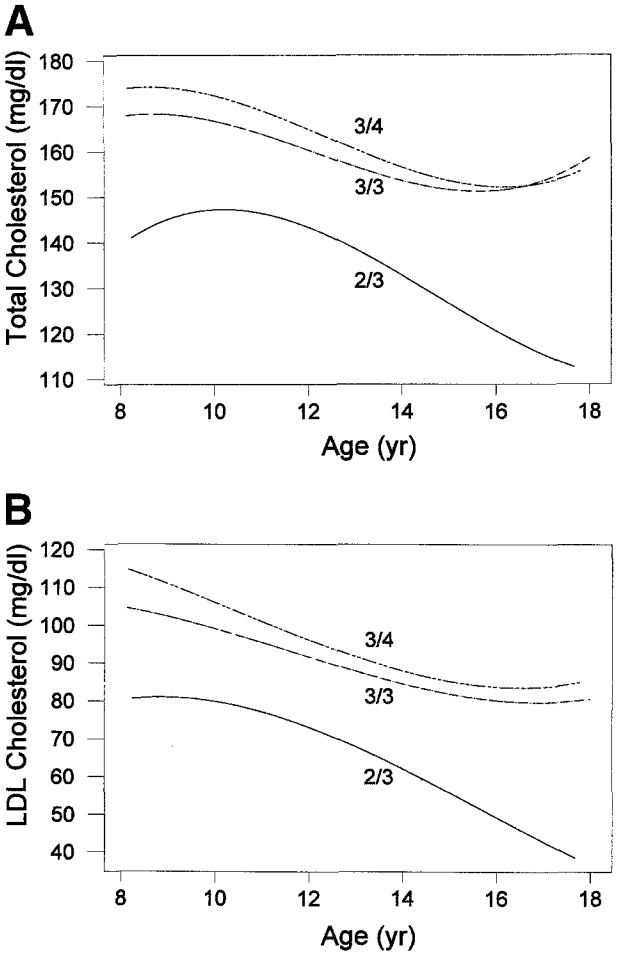

Fig 1. Genotype-specific age trajectories for (A) total cholesterol and (B) LDL-C in girls participating in Project HeartBeat!.

values. Again, there was a significant age^2 × genotype interaction (Table 2) in the multilevel model, indicating that the age trajectory in LDL-C among individuals with the ε2/3 genotype was significantly different from the pattern found in those with ε3/3 and ε3/4 genotypes. Among ε3/4 individuals, LDL values decreased from 115 to 86 mg/dL from ages 8 to 15 and then stabilized from ages 15 to 18 at 86 mg/dL. Individuals with the ε3/3 genotype showed a similar pattern of change in LDL-C in comparison to those with the ε3/4 genotype, as their LDL decreased from 104 to 83 mg/dL from ages 8 to 15 and then stabilized near 82 mg/dL from ages 15 to 18. Individuals with the ε2/3 genotype showed a different pattern of change versus those with ε3/3 or ε3/4 genotypes, as they had stable LDL-C values of 80 mg/dL from ages 8 to 10, followed by a monotonic decrease in LDL from 80 to 40 mg/dL from ages 10 to 18.

DISCUSSION

In this study, we observed significant variation in genotype-specific age trajectories for total and LDL-C in adolescent girls participating in Project HeartBeat!. Individuals with the ε2/3 genotype had lower total cholesterol and LDL-C values from ages 8 to 18 but also demonstrated a different pattern of change in cholesterol compared with ε3/3 and ε3/4 girls. This is the first study to examine longitudinally the serial changes in total cholesterol and LDL-C values during adolescence by apo E genotype. The synthetic cohort design of Project HeartBeat! and the extensive schedule of 4-month assessments illustrate the dynamics of change in total cholesterol and LDL-C during adolescence and present the differences in the pattern of change in cholesterol by apo E genotype.

Findings from the present study are consistent with previous reports of the frequency of apo E alleles in US children and adults. We observed ε2, ε3, and ε4 allele frequencies among Caucasian girls in our study population of 7%, 79%, and 14%, respectively. These findings are comparable with other reports of apo E frequencies in Caucasian children.[4,6] Likewise, the effects of apo E polymorphism are consistent with those observed in many other studies: the average effect of the ε2 allele was to decrease total cholesterol and LDL-C and the average effect of the ε4 allele was to increase total cholesterol and LDL-C.[1] Variation in the apo E genotype explained 8.2% of the variance in total cholesterol and 10.5% of the variance in LDL-C. These findings are comparable with others[4] and are consistent with reports that approximately 6% to 7% of the variance in LDL-C is accounted for by the variation in apo E genotype.

Genotype-specific age trajectories for total cholesterol and LDL-C have not been reported in the literature; therefore, it is difficult to compare our findings with others. The synthetic cohort design of Project HeartBeat! permitted construction of genotype-specific growth curves that have not existed previously. Most previous studies in children have been cross-sectional, with only two reports being longitudinal with 3-year[6] and 6-year[5] intervals between measurements. Porkka et al[6] did not construct growth curves for cholesterol values, but apo E genotype was associated with intraindividual variability in total cholesterol and LDL-C in males, indicating that apo E genotype was associated with the tracking of total cholesterol and LDL-C levels in male children. Lehtimaki et al[5] observed among Finnish youth that changes in total cholesterol and LDL-C by apo E phenotype were stable over a 6-year follow-up period, and the mean serum cholesterol concentration in the whole study population decreased 6.3%. Gueguen et al[28] observed among adults that 5-year changes in triglycerides were influenced by the interaction between apo E and changes in weight, such that individuals with an ε4 allele had a significantly greater increase in triglycerides accompanying an increase in weight versus individuals with no ε4 allele.

The patterns and trajectories of growth in anthropometric measurements of height and weight have been used extensively to assess normal variation in growth and maturation in children and adolescents.[29] Growth curve methodologies have been used to study serial patterns of change in disorders such as Turner's syndrome,[30] adolescent substance use behaviors,[31] and CVD risk factor development.[8,32] Application of growth curve methods to the analysis of longitudinal patterns of change has applications and advantages beyond the obvious analyses of stature or weight, and may provide the investigator with an estimate of both the magnitude and rate of change of a variable over time, which may not be analogous to the age-related changes observed in a series of cross-sectional analyses.[29]

The polyphasic pattern of change in total cholesterol and LDL-C with age has been observed recently in female and male adolescents,[18] confirming cross-sectional findings previously suggesting such a pattern of change.[16] Among the girls in our sample, for all genotypes beginning around age 11, there was a distinct monotonic decrease in total cholesterol and LDL-C until approximately age 16. This dramatic change in cholesterol values during adolescence may be explained by hormonal variations or possibly other factors. Puberty is triggered by a greatly increased secretion of gonadotropins leading to an increase in sex hormones (ie, estrogen in girls). Also, an increase in growth hormone secretion by the pituitary gland triggered by increasing estrogen production is the main cause of the adolescent growth spurt in girls. Changes in weight have been associated with changes in total cholesterol and LDL-C in young men[33] and adults.[34] Investigators from the Bogalusa Heart Study observed that children with E2/2 or E2/3 phenotypes had a significantly lower percent body fat than children with the E3/3 phenotype, 18.4% versus 21.4%, respectively.[35] However, Labarthe et al[18] recently observed that serial changes in cholesterol among girls were not associated with concurrent changes in body composition. It is therefore important to investigate further how growth, maturation, and endocrine function may interact with apo E genotype.

Investigators have recently observed that the effect of apo E polymorphism may be gender-specific,[9] leading to the hypothesis that the potential biological mechanism(s) influencing the effect of apo E on lipid and lipoprotein profiles may be influenced by reproductive hormones. Using animal models, investigators have shown that estrogen-treated MRL/lpr mice showed a shift in cholesterol carried on LDL that was correlated with an increase in apo E,[36] and baboons treated with estrogen alone or in combination with progesterone had significantly decreased plasma apo E compared with controls.[37] Apo E is present in human follicular fluid and decreases dramatically as the follicle approaches ovulation,[38] and postmenopausal women treated with estrogen showed a significant decrease in apo E in the LDL + HDL fraction.[39] Further, Hanis et al[10] observed that

the contribution of apo E polymorphism to interindividual cholesterol variation was greater in premenopausal versus postmenopausal women, 28% versus 4%, respectively. Thus, further elucidation of the metabolic pathways involved in cholesterol metabolism, fat deposition, and their interaction or synergism with reproductive hormones is warranted to clarify our understanding of the complex physiologic changes that occur during adolescence.

In summary, apo E genotype was associated with age-related serial changes in total cholesterol and LDL-C in Caucasian girls participating in Project HeartBeat!, a longitudinal investigation of cardiovascular risk factor development in children and adolescents. Further examination of serial changes in cholesterol by apo E genotype in boys and non-Caucasian girls is warranted to support our findings. In addition, the roles that reproductive hormones, body composition, and sexual maturation play in understanding the changes in cholesterol that occur during adolescence require further investigation.

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