Telomere Length and Symptoms of Attention Deficit and Hyperactivity Disorder in Children at 6–12 Years

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Abstract:

Objective: To explore the association between telomere length (TL) and attention deficit hyperactivity disorder (ADHD) symptoms in children at 6-12 years.
Method: Data from 1759 children belonging to the HELIX project cohorts and the Asturias, Gipuzkoa and Valencia cohorts of INMA project were included. TL was determined by blood sample using a PCR protocol. ADHD symptoms were described by parents using the Conners' Parent Rating Scale-Revised: Short Form. Multiple negative binomial regression models adjusted for potential confounders were used to estimate associations.
Results: Overall estimates showed no associations between TL and ADHD symptoms. However, we observed that a longer TL was significantly associated with a lower risk of presenting hyperactivity symptoms in children belonging to the HELIX project (IRR= 0.93, 95%CI=0.87-0.99; p= 0.022).
Conclusion: While our study did not find a consistent association between TL and ADHD symptoms across all cohorts, the significant association found within the HELIX cohort suggests that longer TL may be linked to a lower risk of hyperactivity symptoms. Further research is needed to explore this association in more detail.

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Introduction

Telomeres are nucleoprotein structures containing TTAGGG sequences that protect the ends of chromosomes (Turner et al., 2019). During the cell division process, telomeres become shorter due to DNA polymerase's inability to complete chromosomal end replication (Blackburn et al., 2015). However, when telomeres become critically short, the cell stops dividing and renewing itself, entering a state known as cellular senescence. The accumulation of senescent cells promotes the ageing process of tissues and organisms. Consequently, TL attrition is recognised as a biomarker of cellular ageing (López-Otín et al., 2013). Although TL shortening occurs throughout an individual's lifetime, research has shown that this attrition is most pronounced during the first 20 years of life because of the high number of cell divisions (Aubert et al., 2012). However, it is important to note that the rate of telomere loss is not solely determined by the rate of mitotic replication. It has been observed that other factors, such exposure to oxidative and inflammatory stressors, can influence the rate at which TL shortens, varying between individuals of the same age. Thus, TL serves as a reflection of an individual's cumulative lifetime exposure to these stressors (Correia-Melo et al., 2014; Njajou et al., 2009).

Scientific literature on TL has focused more on research on ageing-related diseases, cell regeneration, or mortality (Wang et al., 2018). Studies in the adult population have shown that shorter telomeres are related to chronic pathologies such as cardiovascular diseases (De Meyer et al., 2018), cancer (Wentzensen et al.,

2011), Alzheimer's disease (Zhan et al., 2015), and psychiatric pathologies (Darrow et al., 2016). Besides assessing the impact of TL shortening on the development of several pathologies, some studies have evaluated the role of TL on brain structure and function in healthy adults. A meta-analysis showed that longer TL was beneficial for brain structure (i.e., global brain and hippocampal volume) and cognition (i.e., global cognition, attention/velocity and executive functions) during ageing (Gampawar et al., 2022). In addition, a recent longitudinal study supports the impact of TL on the brain, finding that shorter TL was associated with smaller total brain volume, white matter volume and subcortical brain structures (e.g., thalamus, hippocampus, accumbens, putamen, pallidum) (Cao et al., 2023).

Nevertheless, limited research exists regarding the potential link between TL and children's neuropsychological development and health. To our knowledge, only a few studies have shown associations between shorter telomeres with oppositional defiant behaviours (Wojcicki et al., 2015) and autism spectrum disorder (Lewis et al., 2020; Li et al., 2014). Several studies have examined the possible relationship between TL and symptoms of attention deficit hyperactivity disorder (ADHD), but the findings have been inconsistent. Specifically, Costa and colleagues (2015) found a negative correlation between symptoms of hyperactivity and impulsivity and TL in children aged 6 to 16 with diagnosis of ADHD (Costa et al., 2015). Additionally, another study showed a significant association between shorter telomeres at 12 months and increased ADHD symptoms at 2 years (Pham et al., 2022). However, in contrast, a study conducted by Howell and colleagues (2022) reported different results, establishing an association between higher ADHD symptoms at 18 months and less telomere erosion in children aged 4 to 18 months. Similarly, a study involving young adults with ADHD diagnosis found a link between hyperactive-impulsive symptoms and longer telomeres (Momany et al., 2021).

ADHD is characterised by inattention, impulsivity, and hyperactivity symptoms that persist over time and negatively impact social, academic, and occupational functioning (Doernberg & Hollander, 2016). It is considered the most prevalent neurodevelopmental disorder, affecting approximately 7.6% of children worldwide (Salari et al., 2023). These symptoms contribute to a deterioration in the quality of life for both children and their families (Wanni Arachchige Dona et al., 2023).

Considering the potential role of TL in brain development, further research on the association between TL and neurodevelopment is clearly needed. TL has been suggested to have potential implications for neurodevelopmental disorders such as ADHD, particularly during childhood. Since TL highly decreases during this first stage of life, the variability in the rate of TL attrition could be associated with the development of ADHD and could potentially have long-term detrimental effects on later child health. Therefore, the main objective of this study was to examine the association between leukocyte TL and ADHD symptoms in children aged 6-12 years.

Methods

Study design and population

This cross-sectional study used data from the Human Early-Life Exposome Project (HELIX Project, http://www.projecthelix.eu/), which included six European birth cohorts: BiB (Born in Bradford, United Kingdom); EDEN (Etude des Déterminants pré et postnatals du développement et de la santé de l'Enfant,

France); KANC (Kaunas cohort, Lithuania); MoBa (The Norwegian Mother, Father, and Child Cohort study, Norway); Rhea (The Mother-Child Cohort in Crete, Greece) and INMA-Sabadell cohort (Infancia y Medio Ambiente Project, Spain). Participants from HELIX Project were selected from these larger cohorts, ensuring a representative sample of approximately 200 mother-child pairs from each of the six cohorts (Vrijheid et al., 2014). Additionally, we included data from the Asturias, Gipuzkoa, and Valencia cohorts of the INMA Project (https://www.proyectoinma.org/) (Guxens et al., 2012). All birth cohorts collected information on different exposure factors and lifestyles during pregnancy and childhood to assess their impact on the health of children and adolescents.

The present study encompassed a final sample of 1759 mother–child pair who provided complete information on the main study variables (leukocyte TL and ADHD symptoms) and potential confounders. The investigation had the approval of the institutional ethics committee involved in the study, and all families from the different cohorts gave their consent to participate.

Main variables

Leukocyte telomere length: Leukocyte TL is widely used in epidemiological studies as an indicator of TL in other parts of the body. It generally correlates with TL in most tissues of the organism (Demanelis et al., 2020), making it a suitable proxy for TL measurement. Leukocyte TL was determined by collecting a blood sample from participants aged 6 to 12 years. The mean age of children across most cohorts ranged between 7 and 9 years. However, children from the KANC and RHEA cohorts were younger, with a mean age of 6 years, while those in the EDEN cohort were the oldest, averaging 11 years. A modified fluorochrome-based quantitative polymerase chain reaction (qPCR) protocol, as described by Cawthon (2009), was used for TL determination. Measurements were performed in triplicate on a 7900HT real-time PCR system (Applied Biosystems) in 384-well format. In each cycle, a serial dilution of six DNA spots was run to assess PCR efficiency, and eight system calibrations were performed to control for variability. TL was measured using qBase software (Biogazelle, Zwijnarde, Belgium) and expressed as the ratio of telomere copy number to the number of single copy genes (T/S) relative to the average T/S of the set of all samples. In HELIX project cohorts (BiB, EDEN, KANC, MoBa, Rhea and INMA-Sabadell), a single-copy gene was used, which contained 1x QuantiTect SYBR Green PCR master mix, 300 nM 36B4u primer (CAGCAAGTGGGAAGGTGTAATCC), and 500 nM 36B4d primer (CCCATTCTATCATCAACGGGTACAA). However, in INMA-Asturias, INMA-Gipuzkoa and INMA-Valencia cohorts, a different single-copy gene was used, with a qPCR mixture containing 1x QuantiTect SYBR Green PCR master mix, 400 nM HBG1 primer (GCTTCTGACACAACTGTGTTCACTAGC), and 400 nM HBG2 primer (CACCAACTTCATCCACGTTCACC) (Martens et al., 2020).

For the association analyses, telomere measurements were transformed to z-scores. This transformation involved converting the telomere measurements to a distribution with a mean equal to zero and a standard deviation of one. The purpose of this transformation was to express regression coefficients in standard deviations (SD), enabling direct comparability with the results obtained from other studies (Verhulst, 2020).

ADHD symptoms: ADHD symptoms were assessed at the same visit when the blood sample was collected for TL determination. Parents completed the Conners' Parent Rating Scale–Revised: Short Form (CPRS–R:S, Conners, 1997). The questionnaire is specifically designed for children and adolescents aged 3 to 17

years and asks parents to rate the extent to which each of the 27 symptoms has been a problem for their child during the past month. Each item on scale can be rated on a 4-point scale, with the following ratings: 0=never or rarely, 1=sometimes, 2=frequently, and 3=very often. The CPRS-R aims to assess problem behaviour in children by providing scores for three subscales: Oppositional behaviour, Cognitive problems/Inattention, and Hyperactivity. Additionally, it includes an ADHD global index, which reflects the overall severity of ADHD symptoms. The scores obtained for the subscales range from 0 to 18, with higher scores indicating increased ADHD symptoms. The ADHD global index scores range from 0 to 36, providing an assessment of the overall severity of ADHD symptoms.

Covariates

We selected sociodemographic and lifestyle variables of mothers and children that previous studies have associated with TL shortening and ADHD symptoms (Gorenjak et al., 2020; Vázquez-González et al., 2023). Also, we considered confounding variables that were included in previous studies on TL and ADHD (Costa et al., 2015; Howell et al., 2022; Pham et al., 2022; Rentscher et al., 2020). Regarding the characteristics of the mothers, we collected information on their cohort of origin, age (years), educational level (low, medium, or high), pre-pregnancy body mass index (BMI, kg/m^2) and active smoking during pregnancy (yes or no). For children, we included data on their age at clinical examination (years), the date of the blood sample extraction for telomere determination [DD/MM/AA], which was subsequently recategorised in seasons (spring, summer, autumn or winter), sex (male or female), and BMI (kg/m^2).

Statistical analysis

All statistical analyses were performed using R software version 4.3.1 (R Foundation for Statistical Computing). A statistical significance level was established at 0.05 and all contrasts were bilateral. Normal distribution of continuous variables was checked by using the Kolmogorov-Smirnov test with Lilliefors correction.

Socio-demographic characteristics and lifestyles of mothers and their children were described using frequencies and percentages for categorical variables, and median and interquartile range (IQR) for continuous variables. To compare the participants in each sample (HELIX project: BiB, EDEN, KANC, MoBa, Rhea, and INMA-Sabadell cohorts; Asturias, Gipuzkoa, and Valencia cohorts for the INMA project), we used the Chi-square test or Fisher Exact test for qualitative variables and Mann-Whitney U test for quantitative variables.

To explore the association between TL and ADHD symptoms, we performed multiple negative binomial regression models that provided incidence rate ratio (IRR) as an outcome measure. Initially, we conducted separate analyses for each cohort, including the HELIX and INMA Asturias, Gipuzkoa, and Valencia cohorts. Following these individual analyses, we combined the results using meta-analytic techniques. Models were adjusted for covariates with a p-value < 0.2 in the descriptive analysis and that changed the magnitude of the main effect by ≥ 10%. Heterogeneity between cohorts was quantified using the I^2 statistic, and pooled estimates were derived under the hypothesis of fixed-effect model (I^2 <50%) or random-effect

model (I^2 >50%), depending on the detected heterogeneity. Finally, we conducted two models of analysis: 1) a basic model adjusted for children's age at examination and sex; and 2) a main model that included, in addition to the above, maternal adjustment variables such as age, educational level, active smoking during pregnancy, and pre-pregnancy BMI, and children's BMI.

Results

Sociodemographic and lifestyle characteristics of the included participants are presented in Table 1. Among the 1759 participants, the mothers had a median age of 31.0 years (IQR= 28.0-34.0). Over 80.0% of these mothers had a medium to high level of education. Their median preconception body mass index (BMI) was 23.4 kg/m² (IQR= 21.1-26.6), and 20.9% of them reported smoking during pregnancy. We observed differences in these characteristics between the participants from the HELIX project and the INMA-Asturias, Gipuzkoa, and Valencia cohorts. Specifically, a higher percentage of mothers from the HELIX project had a high level of education compared to those from the INMA sample (50.3% vs. 41.3%). Additionally, mothers from the HELIX project had a higher median preconception BMI (23.9 vs. 22.6 kg/m²). Conversely, a higher percentage of mothers from the INMA sample reported smoking during pregnancy (29.7% vs. 15.0%). Regarding the children, slightly more than half of them were boys, with a median age of 7.8 years (IQR= 7.0-8.4). Their median BMI was 16.5 kg/m² (IQR= 15.3-18.3), and approximately 53.2% of blood samples were collected during the autumn and winter months. Children from the HELIX sample had a modestly higher median age (8.0 vs. 7.7 years) and a lower BMI (16.3 vs. 17.0 kg/m²) when comparing to those from the INMA sample.

Table 2 displays the outcomes regarding the association between TL z-scores and ADHD symptoms among children aged 6-12 years. Overall estimates (n=1759) did not show significant associations. However, when analysing the results separately for both samples (HELIX project cohorts and INMA Asturias, Gipuzkoa and Valencia cohorts), we observed differences in the estimates. The results of the main models showed a possible association between a longer TL and a lower risk of hyperactivity symptomatology (IRR= 0.93; 95%CI=0.87-0.99; p=0.022) in the children of the HELIX project cohorts (n= 1086). However, no associations were observed in the children of the INMA project (n= 673).

The results of the association between TL and hyperactivity symptoms for each cohort are graphically represented in Figure 1. With the exception of the MoBa cohort, the HELIX cohorts showed a positive association between longer TL and a lower risk of hyperactivity symptoms. This association was stronger and with more precise intervals for the KANC- Lithuania (IRR= 0.58; 95%CI=0.29-1.14; p=0.117), Rhea-Greece (IRR= 0.57; 95%CI=0.28-1.17; p=0.129) and INMA Sabadell-Spain (IRR= 0.47; 95%CI=0.18-1.18; p=0.109) cohorts. However, the results of the INMA-Gipuzkoa and Valencia, as well as the overall INMA cohorts, showed an inverse association between longer TL and higher risk of hyperactivity, although these associations were not statistically significant. As displayed, overall estimates from all cohorts showed an inconclusive association.

Discussion

The present study explored the association between TL and ADHD symptoms in a multi-cohort study of European children aged 6-12 years. In the overall population, no significant association was observed.

However, when conducting a stratified population analysis, an association between longer TL and fewer hyperactivity symptoms was observed among participants from the HELIX project, mainly driven by the KANC (Lithuania), Rhea (Greece) and INMA-Sabadell (Spain) cohorts.

The existing evidence on the association between TL and ADHD symptoms in childhood has yielded inconsistent findings. Two previous studies reported significant associations between shorter telomeres and ADHD symptoms, aligning with our initial hypothesis. However, direct comparisons between these studies and ours are not possible due to methodological differences. For instance, the study by Costa et al. (2015) focused on children diagnosed with ADHD rather than children from the general population sample, as in our case. Moreover, their analysis was based on correlating the hyperactive-impulsive dimension of ADHD with TL ($r = -0.339$, $p = 0.008$) in Brazilian children aged 6 to 16 years, rather than employing a multiple adjusted association analysis, which could account for potential confounding variables. An Australian birth cohort study (n=676) observed that TL at 12 months was inversely associated with Attention Problems ($\beta = -0.56$; $p = 0.05$) and the Attention Deficit/Hyperactivity Problems ($\beta = -0.66$; $p = 0.004$) at 2 years (Pham et al., 2022). However, this study examined the association between TL at 1-year-old infants and later development of ADHD symptoms measured at 2 years old, while in our study, both variables were measured concurrently. Other studies have reported contrasting results, showing an association between longer telomeres and ADHD symptoms in toddlers (Howell et al., 2022) and young adults (Momany et al., 2021). However, these studies differ significantly from ours, as TL was measured from a buccal saliva sample rather than a blood sample.

One possible explanation for the absence of global association between telomeres and ADHD symptoms in our study may be attributed to our dataset, which primarily consisted of healthy children with minimal variability in ADHD symptoms. In fact, only approximately 10.0% of the children in our sample exhibited problematic scores indicative of symptoms compatible with ADHD (scores above the 80th percentile). We observed the highest percentages of the presence of problematic ADHD symptomatology in the children of the KANC (15.4%) and INMA-Sabadell (13.8%) cohorts, which coincides with the statistically significant associations. This limited variability in ADHD symptom presentation across the sample may have influenced our ability to detect significant associations.

Most studies exploring neurocognitive outcomes and TL have focused on adults, where known differences in brain ageing among healthy individuals of the same age exist. Some individuals may experience a more pronounced decline in cognitive functions and brain volume as they age (Cole et al., 2019). Consequently, associations between shorter telomeres and this decline in brain functions and structures are more likely to be observed in adults, given the greater inter-individual variability at older ages (Cao et al., 2023; Gampawar et al., 2022). This research highlights the variability in cognitive decline and brain volume among healthy adults, underscoring the importance of understanding how TL dynamics may contribute to individual differences in neurocognitive ageing.

For their part, several neuroimaging studies have revealed structural alterations, such as an overall brain volume reduction of 2.5%, alongside functional and neurochemical brain differences in individuals diagnosed with ADHD (Baroni & Castellanos, 2015; Doernberg & Hollander, 2016; Vázquez-González et al., 2023). These structural and functional changes may reflect underlying neurobiological processes

influenced by neuroinflammation, a hallmark feature of several neurodevelopmental disorders. Neuroinflammation plays an important role in the development of disorders such as ADHD, involving different mechanisms such as glial cell activation, increased oxidative stress, loss of neuronal function and neurodevelopmental changes (Alvarez-Arellano et al., 2020; Corona, 2020).

Considering these factors, our initial hypothesis was formulated based on the idea that the activation of neuroinflammatory processes could trigger the active proliferation of leukocytes and cause telomere shortening (Scarabino et al., 2020; Zhang et al., 2016). However, as discussed above, we did not find consistent evidence of this association in our sample of children, where most parents did not report symptoms of inattention and hyperactivity. Additionally, it is essential to note that TL exhibited very little variability in our study, and we only had one measurement. Therefore, obtaining multiple measurements over time for both variables would be valuable to better observe any potential associations.

When analysing the associations separately for each project, we found a significant association between TL and hyperactivity symptoms in children from the HELIX project. However, this association was not observed in children from the INMA project. One possible explanation for the differences between the cohorts may be attributed to specific factors or lifestyle characteristics within the socio-economic and cultural context in which the children develop (Rentscher et al., 2020). Despite accounting for multiple variables as confounding factors, our results revealed a high heterogeneity in the observed associations across cohorts. It is plausible other undetected aspects could be influencing the association. Existing research underscores the multifactorial aetiology of ADHD, involving genetic and environmental factors. Candidate genes associated with ADHD, such as dopaminergic genes, have been identified, alongside peri-, pre- and postnatal events implicated in the development of ADHD, including maternal stress during pregnancy, vitamin D intake, exposure to environmental pollutants, smoking, among others (Vázquez-González et al., 2023). Moreover, it is important to note that, in this study, telomere determinations were performed using different normalizations between the HELIX and INMA cohorts. However, the PCR methodology applied was the same in both cases and the z-score TL measurement for each cohort was used for the analyses.

A strength of the present study is its multi-country cohort, which includes participants from diverse geographical and socio-economic backgrounds. However, it is important to note that this diversity is primarily geographical, as specific data on ethnicity or racial background were not collected. Given the European origins of the included cohorts, the study population is likely predominantly of European/Caucasian descent, which limits the generalizability of the findings to populations with other ethnic or racial backgrounds. Future research should aim to replicate these findings in more ethnically diverse cohorts to ensure broader applicability. . In addition, the study benefits from a larger sample size compared to previous studies on this topic (n= 1795 vs <700). Moreover, being a follow-up study population, we had access to information on covariates during pregnancy and postnatal follow-up visits, minimising the possible occurrence of recall and sample selection biases. However, there are some limitations to acknowledge in this investigation. First, ADHD symptoms were reported by the children's parents, which may introduce some inaccuracies, although any such inaccuracies are likely to be non-differential in nature. Second, while TL was measured using a standard and widely used method in

epidemiological studies (Lindrose et al., 2021), it is important to consider that this methodology provides an average measurement across all samples, and potential measurement errors should be taken into account (Nettle et al., 2019). Third, despite adjusting regression models for possible confounders, the presence of unknown or residual confounders cannot be ruled out. In addition, because of the cross-sectional nature of the study, establishing causality in the observed association is not possible.

In conclusion, the overall results of our study do not show a consistent association between TL and ADHD symptoms in children aged 6 to 12 years. However, the significant association found within the HELIX cohorts suggests that longer TL may be linked to a lower risk of hyperactivity symptoms. This finding highlights the potential for TL as a biomarker for hyperactivity. We recommend that future research should examine this relationship longitudinally, considering multiple time points and including a higher percentage of children diagnosed with ADHD. Longitudinal studies would provide valuable insights into the potential role of TL as a biomarker of neurodevelopmental problems in children. Additionally, given that TL has been implicated as a potential biomarker for various health issues in adulthood, further exploration of TL in the context of childhood neurodevelopment is necessary. Continued research in this area may contribute to our understanding of the underlying mechanisms and potential clinical implications of TL in child health.

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Table 1. Sociodemographic characteristics and lifestyles of mothers and children belonging to the HELIX and INMA projects (n= 1759).

	All (n= 1759)	HELIX's sample[a] (n= 1086)	INMA's sample[b] (n= 673)	p- value[c]
Mother's characteristics				
Age (years), median (IQR)	31.0 (28.0-34.0)	31.0 (27.2-34.0)	31.0 (29.0-34.0)	0.040
Educational level, n (%)				<0.001
Low	275 (15.6)	153 (14.1)	122 (18.1)	
Medium	660 (37.5)	387 (35.6)	273 (40.6)	
High	824 (46.8)	546 (50.3)	278 (41.3)	
Pre-pregnancy BMI (kg/m^2), median (IQR)	23.4 (21.1-26.6)	23.9 (21.3-27.1)	22.6 (20.7-25.6)	<0.001
Active smoking during pregnancy, n (%)				<0.001
No	1396 (79.4)	923 (85.0)	473 (70.3)	
Yes	363 (20.6)	163 (15.0)	200 (29.7)	
Child's characteristics				
Sex, n (%)				0.169
Female	815 (46.3)	489 (45.0)	326 (48.4)	
Male	944 (53.7)	597 (55.0)	347 (51.6)	
Age at clinical assessment (years), median (IQR)	7.8 (7.0-8.4)	8.0 (6.0-9.0)	7.7 (7.6-8.1)	0.006
BMI (kg/m^2), median (IQR)	16.5 (15.3-18.3)	16.3 (15.1-17.9)	17.0 (15.7-18.8)	<0.001
Season blood drawn, n (%)				0.215
Winter	494 (28.1)	323 (29.7)	171 (25.4)	
Spring	440 (25.0)	259 (23.8)	181 (26.9)	
Summer	381 (21.7)	233 (21.4)	148 (22.0)	
Autumn	444 (25.2)	271 (24.9)	173 (25.7)	

IQR, Interquartile Range; BMI, Body Mass Index.
a HELIX cohorts: BiB (United Kingdom), EDEN (France), KANC (Lithuania), MoBa (Norway), Rhea (Greece) and Sabadell (Spain).
b INMA cohorts: Asturias, Gipuzkoa and Valencia (Spain).
c The Chi-square test or Fisher's Exact test was used for categorical variables and Mann-Whitney U test for continuous nonparametric variables.

Table 2. Association between TL (z-score) and ADHD symptoms in children aged 6- 12 years from the HELIX and INMA projects (n= 1759).

	All participants *(n= 1759)*			HELIX's participants[a] *(n= 1086)*			INMA's participants[n] *(n= 673)*		
	IRR (95% CI)[c]	**p-value**	**I^2 (%)**	**IRR (95% CI)**	**p-value**	**I^2 (%)**	**IRR (95% CI)**	**p-value**	**I^2 (%)**
CRSR-27									
Oppositional									
Basic model	0.99 (0.96; 1.04)	0.839	0.6	0.97 (0.93; 1.02)	0.293	0.0	1.04 (0.97; 1.12)	0.241	0.0
Main model	1.00 (0.96; 1.04)	0.847	1.8	0.98 (0.93; 1.03)	0.490	0.0	1.05 (0.97; 1.13)	0.177	0.0
Cognitive problems/ Inattention									
Basic model	0.99 (0.94; 1.04)	0.710	10.3	0.98 (0.92; 1.04)	0.521	21.3	1.00 (0.93; 1.10)	0.820	10.8
Main model	1.00 (0.95; 1.05)	0.939	6.8	0.99 (0.93; 1.06)	0.867	17.0	1.01 (0.92; 1.11)	0.774	17.2
Hyperactivity									
Basic model	0.97 (0.90; 1.06)	0.540	60.6	0.91 (0.85; 0.97)	0.005	0.0	1.08 (0.95; 1.22)	0.247	58.0
Main model	0.98 (0.91; 1.06)	0.667	55.3	0.93 (0.87; 0.99)	0.022	0.0	1.07 (0.93; 1.22)	0.332	61.9
ADHD Index									
Basic model	0.99 (0.93; 1.06)	0.826	55.2	0.96 (0.89; 1.04)	0.355	54.8	1.05 (0.98; 1.12)	0.137	0.0
Main model	1.00 (0.95; 1.07)	0.749	50.7	0.98 (0.91; 1.06)	0.695	51.4	1.06 (0.98; 1.14)	0.144	19.3

TL, Telomere Length; ADHD, Attention deficit/hyperactivity disorder; IRR, Incidence Rate Ratio, CI, Confidence Interval; CRSR, Conner's Rating Scale-Revised.
[a] HELIX cohorts: BiB (United Kingdom), EDEN (France), KANC (Lithuania), MoBa (Norway), Rhea (Greece) and Sabadell (Spain).
[b] INMA cohorts: Asturias, Gipuzkoa and Valencia (Spain).
[c] IRR is expressed for a 1-unit increment in Z-Score TL.
Basic models were adjusted by children's age at examination (years) and sex (male or female).
Main models were adjusted by mother's age (years), educational level (low, medium or high), active maternal smoking during pregnancy (yes or no), pre-pregnancy body mass index (continuous), children's age at examination (years), sex (male or female) and body mass index (continuous).

Figure 1. Cohort estimates (effects expressed with 95% confidence intervals and for a 1-unit increase in the Z-Score TL) of the association between TL and subscale hyperactivity symptoms in children aged 6-12 years in the HELIX and INMA projects.