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Advanced parental age as risk factor for childhood acute lymphoblastic leukemia

Results from studies of the Childhood Leukemia International Consortium

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ABSTRACT

Advanced parental age has been associated with adverse health effects in the offspring including childhood (0-14 years) acute lymphoblastic leukemia (ALL), as reported in our meta-analysis of published studies. We aimed to further explore the association using primary data from 16 studies participating in the Childhood Leukemia International Consortium. Data were contributed by 11 case-control (CC) studies (7919 cases and 12942 controls recruited via interviews) and five nested case-control (NCC) studies (8801 cases and 29690 controls identified through record linkage of population-based health registries) with variable enrollment periods (1968-2015). Five-year paternal and maternal age increments were introduced in two meta-analyses by study design using adjusted odds ratios (OR) derived from each study. Increased paternal age was associated with greater ALL risk in the offspring (OR_{CC}:1.05, 95% CI:1.00-1.11; OR_{NCC}:1.04, 95% CI:1.01-1.07). A similar positive association with advanced maternal age was observed only in the NCC results (OR_{CC}:0.99, 95% CI:0.91-1.07, heterogeneity I^2=58%, p=0.002; OR_{NCC}:1.05, 95% CI:1.01-1.08). The positive association between parental age and risk of ALL was most marked among children aged 1-5 years and remained unchanged following mutual adjustment for the collinear effect of the paternal and maternal age variables; analyses of the relatively small numbers of discordant paternal-maternal age pairs were not fully enlightening. Our results strengthen the evidence that advanced parental age is associated with increased childhood ALL risk; collinearity of maternal with paternal age complicates causal interpretation. Employing datasets with cytogenetic information may further elucidate involvement of each parental component and clarify underlying mechanisms.

Key words: maternal age; paternal age; acute lymphoblastic leukemia; childhood; risk factors; case-control.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) accounts for 25-30% of all cancers in children [1]; yet, our understanding on the etiology of the disease is rather limited [2]. Individual studies and large consortia, such as the Childhood Leukemia International Consortium (CLIC) [3], are exploring a constellation of factors related to the perinatal origins of the disease [4-6] including birth anthropometrics [7], early immune stimulation [8], prenatal vitamin supplementation [9], and pre-labor cesarean delivery [10, 11].

Sharply increasing trends of parents with advanced age at first delivery, have attracted scientific interest due to the reported consequences on offspring's health [12-14]. Indeed, advanced maternal age has been linked to several adverse pregnancy outcomes [15] including an increase in the risk of chromosomal abnormalities in the offspring [16]. Albeit less studied, advanced paternal age has also been associated with single gene mutation birth defects, chromosomal abnormalities and neurodevelopmental disorders in offspring [17]. Genomic sequencing studies have shown higher numbers of *de novo* mutations in the offspring of older parents [18, 19] and decreased DNA methylation patterns [20], potentially increasing offspring vulnerability to carcinogenesis [21, 20].

In the context of the current CLIC study, a meta-analysis was undertaken [22] showing positive associations of advanced age of both parents at birth of the index child with ALL in the offspring irrespective of study design. Subsequently, registry-based, record-linkage nested case-control (NCC) studies from the US and Denmark also reported an increased ALL risk with advanced maternal age, whereas the positive associations with older paternal age were marginally significant [23-26]. Incomplete control for confounding, variable treatment of the paternal and maternal age variables collinearity between maternal and paternal age and limited power preclude, however, firm conclusion [27].

To this end, we used primary data from 16 CLIC studies conducted in 12 countries around the world to explore the association of parental age with childhood ALL. Given indications of non-representative controls selection in CC studies, resulting in potentially misleading effect estimates regarding parental age [27], we compared data from 11 CC studies with those derived from 5

population-based cancer registries linked with birth and health registries following a nested case-control design (NCC).

METHODS

Study designs and availability of data

Primary data were contributed by 15 studies participating in CLIC following data transfer agreements of individual studies with the Nationwide Registry for Childhood Hematological Malignancies and Solid Tumors (NARECHEM-ST) (Supplementary Table 1). Specifically, 11 were of CC design entailing subject contact, recruitment and telephone or in-person interviews to obtain exposure and disease related information from Brazil, Costa Rica, Egypt, Germany, Greece, Italy, New Zealand, UK, US-California, US- COG-E15 and US-Texas and the additional four of NCC design with population-based linked cancer and birth/health registry data from which controls were drawn (Canada-Quebec, Denmark, Finland, Washington State).

Lastly, the Californian State NCC contributed only maximally adjusted summary estimates for the meta-analyses, and not primary data, due to regulatory constraints of the California Cancer Registry, making a total of 16 studies for analyses. Cases and controls were aged <15 years at diagnosis/recruitment. Down syndrome (~1.3% of cases), a well-established risk factor of childhood ALL strongly associated with maternal age at birth, were and excluded from the analysis, was an exclusion criterion for selection of controls in CC studies and were excluded in the analyses [28]. Parents are usually the legal guardians whose age is reported but not necessarily the biological parents; it is rather unlikely, however, that the negligible proportion of non-biological parents, could impact on the results of the parental age association with childhood ALL [23]; indeed, available information in the nationwide Danish study shows that only 0.6% of children are adopted. Data collection and harmonization is detailed in a Supplementary Materials file.

Statistical analysis

To examine the relationship between paternal and maternal age and risk of childhood ALL, fractional polynomials were used to ascertain the best-fitting curves across the pooled dataset; additionally (Figure 1), restricted cubic spline models were applied using meta-analysis-derived effect estimates [29]. Since linear relationships could not be improved upon (p>0.10) for either maternal or paternal age when examined separately or concurrently (data not shown), we primarily included paternal and maternal age variables in 5-year increments. To address collinearity between the two main variables of interest, paternal and maternal age were included in alternative models one by one and simultaneously. In addition, concordant and discordant pairs of three by three parental age categories (<25 [reference], 25-34, ≥35 years) were created; out of these nine cells, due to small numbers two of the discordant cells had to be collapsed in order to run meta-analyses of multiple logistic regression derived estimates of individual studies, as appropriate.

Two separate meta-analyses were undertaken by study design (CC, NCC) employing random-effects models; heterogeneity across studies was evaluated with the Cochran Q and I^2 statistics (statistical significance set at p-value <0.10, derived from the Cochran Q test). The individual risk estimates were calculated in multiple logistic regression maximally adjusted models (variables with >20% missing values in individual studies were excluded from the study-specific multivariate models). Conditional or unconditional analyses depended in individual study design, whereas maternal and paternal age variables were initially concurrently included in the models for these analyses. Furthermore, sensitivity analyses were undertaken by excluding one study per analysis to assess the effect of on maternal and paternal age.

Pooled multivariate logistic regression analyses using primary data of the 15 studies along with meta-analyses by parental sex were also employed. Based on the availability of covariates across individual studies, a partially (child's age, sex, ethnicity, time period at diagnosis/recruitment, birth weight and maternal education) adjusted and a maximally (additionally controlling for maternal smoking during pregnancy, pre-term birth, birth order and multiple pregnancy) adjusted model were constructed with further analyses by study site. Breastfeeding was not included in the main models, as it was 100% missing in Denmark and Finland and 75% missing in Washington State, thus making the analysis of

the NCC studies not meaningful; in an additional sensitivity analysis, we further included breastfeeding including only studies, in which this variable was available.

Subgroup meta-analyses by child's age group (<1, 1-5, 6-14 years), sex, time period of diagnosis/recruitment and child's ethnicity to assess specific impacts of these variables on the reported effect were conducted only among the NCC studies unlikely to be subject to selection bias. Likewise, to assess the effect of potentially unmeasured confounding, the E-value was estimated [30], based on maximally adjusted effect estimates for categories of maternal and paternal age on the risk for childhood ALL. E-values indicate the size of the effect estimate that potentially unmeasured or uncontrolled confounding would require to totally attenuate the observed associations. Statistical analyses were conducted with SAS 9.4 version and STATA 14.1 version.

RESULTS

Baseline characteristics

The 11 CC studies contributed data for 7919 cases and 12942 controls, whereas the 5 NCC studies for 8801 cases and 29690 controls. The enrollment periods at diagnosis of cases or recruitment of controls ranged within almost 50 years (1968-2015) and widely within and across studies. The distribution of study variables by case-control status and study design is presented in Table 1. The majority of subjects were of Caucasian origin, notably ~80% in the CC studies and ~60% in the NCC studies, among which the Californian investigation weighted more heavily. The distribution of maternal and paternal age at birth of the controls was highly variable across studies as shown in the Supplementary Figure 2.

Meta-analysis by study design (CC=11 and NCC=5)

Figure 1, shows results from random effects meta-analyses on the association of parental age (5-year increments) with childhood ALL derived from separate models of CC and NCC studies. Regarding the paternal age association, similar results were observed regardless of study design (OR_{CC}:1.05, 95%

CI:1.00-1.11, I^2: 29%, p=0.17 and OR_{NCC}:1.04, 95% CI:1.01-1.07; I^2: 0%, p=0.86). The heterogeneous results for the maternal age association derived from CC studies (OR_{CC}:0.99, 95% CI:0.91-1.07; heterogeneity I^2: 64%, p=0.002) were differed than expected and those actually derived from NCC studies (OR_{NCC}:1.05, 95% CI:1.01-1.08; I^2: 0%, p=0.64).

The categorical meta-analyses (Supplementary Figure 3) demonstrated similar results. These meta-analysis-derived associations also followed linear patterns, as indicated by the spline models (Figure 2), with higher effect estimates when parental ages >35 years were compared to those <25 years. Similar results were also obtained when analyses were repeated introducing only the "maternal" or only the "paternal" age variable into the models (data not shown).

After excluding one study at a time, the incremental effect of both paternal and maternal age on the risk for childhood ALL remained essentially the same in all analyses among the NCC studies but did not reach statistical significance after excluding the large Californian NCC study (OR for maternal age:1.04, 95%CI: 0.98-1.10; OR for paternal age: 1.05, 95%CI: 0.99-1.10; Supplementary Figure 4).

Pooled analyses and meta-analyses of 15 studies contributing primary data

Pooled analyses were also contacted for the 15 studies with primary data (Supplementary Table 3), notably all apart from the Californian NCC which contributed only effect estimates for the meta-analyses. Regarding paternal age, the linearly increasing risk of ALL was evident (5-year increment; maximally adjusted OR: 1.08, 95% CI: 1.04-1.11) and maximized (17%) for paternal age ≥35 years (OR:1.17, 95% CI:1.04-1.32). Similar patterns were found in the categorical maximally adjusted analyses as well as the partially adjusted models with higher numbers of cases and controls.

Advancing maternal age (5-year increment) was associated with a statistically significant decreased risk for childhood ALL (maximally adjusted OR:0.92, 95% CI:0.89-0.96). Further adjustment for study site, as well as alternative introduction of the maternal or paternal age variables in the models, and further adjustment for breastfeeding including only studies availing this variable, did not essentially change the results (data not shown).

The meta-analysis for all studies with primary data, i.e., except the Californian NCC (Supplementary Table 3-right panel and Supplementary Figure 5) confirmed the increased risk for childhood ALL with advancing paternal age ($OR_{5\text{-year increment}}$:1.05, 95% CI:1.02-1.09; no heterogeneity), but not with advancing maternal age ($OR_{5\text{-year increment}}$:1.00, 95% CI: 0.95-1.06; statistically significant heterogeneity).

Combined maternal and paternal age effects

Due to the discrepant results for the maternal age derived from CC, all further analyses were conducted only among the five NCC studies. In Table 2, we further assessed the individual and/or combined effects of maternal and paternal age at birth of children within different maternal-paternal age combinations, relative to children whose both parents aged 25-34 years at birth of the index child. The highest statistically significant OR was observed for children with both parents aged ≥35 years (OR:1.16, 95% CI:1.04-1.28) as contrasted to those with both parents <25 years (OR:0.84, 95% CI:0.77-0.91), with comparison to the baseline group of 25-24 years in both instances. ORs for other age combinations did not indicate notable changes in ALL risk. There is a suggestion, however, that older paternal age across all maternal age categories was associated with increased disease risk in the offspring, whereas the same pattern is not clear for advanced maternal age across the paternal age categories.

The maximally adjusted effect estimates for maternal and paternal age ≥35 years (ORs:1.16 and 1.18, respectively) in the registry-based studies meta-analyses, corresponded to E-values of 1.59 and 1.64, respectively; the respective E-values for the low 95% confidence intervals were 1.28 and 1.24.

Subgroup analyses: Age at diagnosis, sex, ethnicity, and diagnosis time period

The associations of ALL with maternal and paternal ages were most marked for children diagnosed at ages 1-5 years (Table 3). Associations with maternal age were equally present for male and female children, more marked among non-Caucasian children. Associations with paternal age were more marked among males and Caucasian children. Associations with time period of diagnosis/recruitment

were all modestly increased; not all ORs were statistically significantly, albeit any increased risk with maternal age seemed to have proceeded that with paternal age timewise.

DISCUSSION

We found a linearly increasing and statistically significant risk for childhood ALL with advanced paternal age. The same size association with advanced maternal age was evident only in the NCC studies as opposed to a decreasing risk estimate derived from both the CLIC CC and pooling analyses. There are some indications that the effect is mainly conferred by advanced paternal age possibly through different parental gender related mechanisms as implied in differential age, gender and ethnic group associations

Reasons for the contradictory results, confined only to the maternal age association, may include selection bias resulting in non-representative distribution of controls in the CC studies as noted in the previously published German study [27] and also evident in the distribution of maternal age across several of the large size included CC studies. Indeed, the maternal age distribution of the UK study also suffered a deficit among control mothers of younger age. The mean maternal age of controls in the Californian CC study was ~2 years older compared to that of the NCC study (29.3 vs. 27.4 years) in the same State; no such difference (28.2 vs. 27.8 years) was noted, though, among the CC cases, which comprised a fraction of the NCC study cases, collected over a lengthier study period.

Not all CLIC case-control studies are subject to the same bias, however. For example, the Greek NARECHEM-ST maternal age distribution among controls seemed to follow the nationwide estimates. Likewise, the Italian SETIL study followed the population pattern and seemed to yield results similar to those of the previously published cohort study in the same area [31]. Unlike NCC studies, CC studies can additionally be subject to recall bias; it is considered implausible, however, that there might be differential recall in the age of both parents at index child's birth [32].

The distribution of maternal and paternal age varied widely across CLIC studies (Figure 1). The heavy weight towards the older age in some studies, possibly reflects socioeconomic and cultural variations in the underlying populations. This may have resulted in a deficit of variance for parental age

distribution in some CC studies, such as NARECHEM-ST or SETIL, which could possibly explain the null maternal age associations noticed in these two studies. Temporal variations of the age distributions within individual CLIC studies reflected dramatic increases in parental age at first delivery in the recent decades [13, 14], they were more prominent in studies with lengthy collection periods and were, therefore, taken into account in the analyses.

The recently described E-value was used to assess unmeasured confounding among the NCC studies [30]. In order to sufficiently explain the observed effect estimates for both maternal and paternal age, an unmeasured confounder may impact the risk of childhood ALL with an effect estimate of a level of 1.6, which is considered quite high, given the magnitude of the observed associations with the perinatal factors that have already been described in the literature.

Whether solely advanced paternal, solely advanced maternal age or both contributed to the observed positive association with ALL (Pearson coefficient: ~71%), is difficult to tease out as the results remained nearly the same in all analyses. Moreover, the numbers in the extreme parental age discordant cells were rather limited to allow firm conclusions on the seemingly higher contribution of the advanced paternal compared to the maternal age on ALL risk. Lastly, information on genetic markers and maternal risk factors such as alcohol consumption [33] or maternal diabetes [34] was not currently contributed by the majority of studies to further enlighten underlying pathophysiological mechanisms. Similarly, information on breastfeeding, a proposed protective factor against childhood ALL [35] was actually missing in 3 out of 5 NCC studies, thus precluding meaningful analyses; nevertheless, sensitivity analyses restricted to studies availing this information showed similar results.

The sub-analyses revealed a more marked effect of both paternal and maternal age in the age group 1-5 years. This might be expected given that infant leukemia (<1 year at diagnosis) is characterized by distinct clinical and cytogenetic features and is assumed to have a distinct etiology compared to leukemia in older children [36, 37], whereas in older children the potential effect of perinatal factors on leukemogenesis might be attenuated. Furthermore, we found the effect of paternal age to be rather confined to males. Although gender differences and a higher susceptibility of males to childhood leukemia have previously been described [1], this finding requires further investigation.

Several outcomes, including chromosomal abnormalities [16, 17], neurodevelopmental disorders [38, 39], psychiatric diseases or conditions [40, 41] and cancer [25] in the offspring have been associated with older parental age. Indeed, accumulation of *de novo* genetic mutations in the germ cells of older fathers [18, 19] could increase childhood cancer risk in the offspring [42, 43]. Related to older maternal age was a DNA methylation processed in the offspring and correlated with cancer as shown in an epigenome-wide association study [20]. Moreover, the well-established association of older maternal age with chromosomal abnormalities and birth defects [44, 45] as well as ALL [46-48] could possibly mediate the observed effect. Of note, in the current study, children with Down syndrome, which are more likely to develop the disease were excluded. Lastly, we have also shown in previous publications of CLIC studies that cesarean delivery, and specifically elective and not emergency cesarean section, which is more likely among children born to older mothers and consequently fathers, is associated with childhood ALL [49, 50].

The sharp increase of advanced parental age at childbearing worldwide during the last decades seems to have attracted scientific interest due to its public health implications [13, 14]. Following previous studies investigating whether the temporal increase in childhood ALL rates in the developed countries [51-53, 1] could be partially attributed to advanced paternal age patterns [31, 25], several CLIC studies participating in the current analyses have published individual data since the expression of interest and the meta-analysis on published studies [23, 24, 26, 27].

The strengths of the present study include access to large numbers of primary case and control data and most requested covariates along with availability of two efficient study designs that allowed to explore robustness of the observed associations in several sub-analyses testing a hypothesis on the etiology of a rare disease [54]. Limitations of the study include the divergent data collection methods for cases and controls; the lengthy and variable, by individual study, periods of data collection, by individual studies, for the main variables of interest which showed increasing trends over time within each individual study; the high levels of missing values in several essential covariates, which led to considerable decrease of the efficient sample size in the maximally adjusted analyses and the efforts to disentangle the collinear paternal and maternal age and possibly led to heterogeneous results in some

instances. Lastly, the missing ALL immune-phenotype and cytogenetic data on the part of the majority of the studies, especially the NCC studies precluded further analyses.

In conclusion, this is the largest study to-date using primary data aiming to further explore the association of parental age at birth with childhood ALL. Our results confirm those from the meta-analysis on published studies and more recent reports demonstrating that advanced parental age is associated with increased disease risk and showed that the associations are mostly marked in the age group 1-5 years. It is possible that advanced parental age confers the effect through different parental gender related mechanisms as indicated by the differential parental gender by age, gender and ethnic group of the index child associations. Indeed, *de novo* genetic mutations in the fathers' germ cells and epigenetic alterations in the offspring born to older mothers could explain the observed associations. Subtype analysis on cytogenetic characterizations and immunophenotype could further refine our understanding on the mechanisms through which advanced parental age is implicated in leukemogenesis among children.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Table 1. Distributions of cases with acute lymphoblastic leukemia (ALL) and controls by study variables and study design.

Study design	**Case-control Interview-based (*n*=11)**					**Nested Case Control Registry-based (*n*=5)**			
Variables	**ALL Cases (N=7989)**		**Controls (N=13482)**			**ALL Cases (N=8801)**		**Controls (N=29690)**	
	N	**%**	**N**	**%**		**N**	**%**	**N**	**%**
Index child's age at diagnosis/recruitment (years)[a]									
<1	328	4.1	1018	7.5		283	3.2	1210	4.1
1-4	4257	53.8	6404	47.5		4788	54.4	15756	53.0
5-9	2236	28.2	3795	28.2		2527	28.7	8306	28.0
10-14	1098	13.9	2265	16.8		1203	13.7	4418	14.9
Index child's sex									
Male	4422	55.8	7417	55.0		4924	55.9	16433	55.3
Female	3497	44.2	6055	45.0		3877	44.1	13257	44.7
Time period of diagnosis/recruitment									
1968-1993	2952	37.3	5298	39.3		2627	29.8	8221	27.7
1994-2003	2958	37.3	5849	43.4		3560	40.5	12025	40.5
2004-2015	2009	25.4	2335	17.3		2614	29.7	9444	31.8
Index child's ethnicity									
Caucasian	6166	78.0	10890	80.9		5349	60.9	17705	59.8
Non-Caucasian	1741	22.0	2578	19.1		3435	39.1	11920	40.2
Missing		*0.2*		*0.1*			*0.2*		*0.2*
Birth weight (g)									
<2500	423	5.5	847	6.4		399	4.7	1551	5.4
2500-2999	1130	14.8	2140	16.3		1094	12.9	3992	14.0
3000-3499	2814	36.8	4883	37.2		2999	35.4	10277	36.0
3500-3999	2321	30.4	3867	29.5		2699	31.9	8920	31.3
≥4000	960	12.5	1388	10.6		1273	15.1	3787	13.3
Missing		*3.4*		*2.7*			*3.8*		*3.9*
Maternal education									
Low	2013	25.6	3241	24.3		922	13.9	3435	15.7
Intermediate	4060	51.7	7044	52.9		3872	58.3	12638	57.8
High	1786	22.7	3037	22.8		1844	27.8	5797	26.5
Missing		*0.8*		*1.2*			*24.6*		*26.3*
Maternal smoking during pregnancy									
No	6201	78.9	10515	78.5		5735	89.7	19288	91.4
Yes	1655	21.1	2889	21.5		658	10.3	1815	8.6
Missing		*0.8*		*0.6*			*27.4*		*28.9*
Pre-term birth[b]									
No	6174	92.4	10676	92.5		7150	91.3	24004	91.5
Yes	507	7.6	866	7.5		682	8.7	2243	8.5
Missing		*15.6*		*14.4*			*11.0*		*11.6*
Multiple pregnancy									
No	6917	97.6	12045	98.0		7740	97.5	25716	97.4

Yes	171	2.4	251	2.0		198	2.5	693	2.6
Missing		*10.5*		*8.8*			*9.8*		*11.1*
Birth order									
1	3455	45.7	5833	45.1		3585	41.5	11925	40.9
2	2531	33.4	4446	34.3		2885	33.4	9575	32.9
≥3	1580	20.9	2667	20.6		2166	25.1	7620	26.2
Missing		*4.5*		*4.0*			*1.9*		*1.9*
Maternal age at birth (years)									
<20	537	6.9	824	6.2		597	6.8	2340	7.9
20-24	1864	23.8	3074	23.1		1939	22.0	6977	23.5
25-29	2588	33.1	4570	34.4		2804	31.9	9250	31.2
30-34	1953	25.0	3330	25.1		2216	25.2	7184	24.2
≥35	878	11.2	1498	11.3		1241	14.1	3927	13.2
Missing		*1.3*		*1.4*			*0.05*		*0.04*
Mean ± SD	27.65 ± 5.55		27.78 ± 5.46			28.10 ± 5.77		27.74 ± 5.83	
Paternal age at birth (years)									
<25	1154	15.4	1858	14.7		1478	16.9	5641	19.3
25-29	2075	27.8	3736	29.7		2565	29.4	8493	29.0
30-34	2226	29.8	3809	30.2		2480	28.4	8128	27.7
35-39	1273	17.1	2066	16.4		1411	16.2	4597	15.7
≥40	739	9.9	1137	9.0		796	9.1	2445	8.3
Missing		*5.7*		*6.5*			*0.8*		*1.3*
Mean ± SD	31.10 ± 6.57		30.94 ± 6.38			30.72 ± 6.54		30.35 ± 6.57	

[a]Infants comprised 36% of the Brazilian study´ the Italian included children aged 0-10 years

[b]gestational age: <37 weeks

Table 2. Meta-analysis derived Odds Ratios (OR) and 95% Confidence Intervals (95% CI) from the five registry-based case-control studies (Canada-Quebec; Denmark; Finland; US, California State, CCLRP; US, Washington State) on the association of the combined effect of maternal and paternal age at birth of the index child with childhood (0-14 years) acute lymphoblastic leukemia.

ALL cases/ controls' OR (95% CI)[a]	**Paternal age <25 years**	**Paternal age 25-34 years**	**Paternal age ≥35 years**
Maternal age <25 years	1181/4318 **0.84 (0.77-0.91)** I^2: 0%, p=0.53	1036/3357 0.96 (0.82-1.12) **I^2: 55%, p=0.07**	87/279 1.17 (0.77-1.77) I^2:45%, p=0.12
Maternal age 25-34 years	192/678 0.88 (0.74-1.04) I^2: 0%, p=0.71	3382/10122 Reference	1114/3343 1.05 (0.97-1.13) I^2: 0%, p=0.80
Maternal age ≥35 years		264/793 1.07 (0.92-1.24) I^2: 0%, p=0.64	906/2582 **1.16 (1.04-1.28)** I^2: 11%, p=0.34

Bold indicates statistical significance (p<0.05 for effect size and p<0.10 for heterogeneity). Maternal and paternal age are simultaneously introduced in all models.

[a] Random-effect meta-analysis of maximally adjusted Odds Ratios from individual studies for any of the following variables that were available with <20% missing values in the total dataset: index child's age (categorical; <1, 1-4 [reference], 5-9, 10-14 years), sex, ethnicity (Caucasian vs. non-Caucasian), birth weight (continuous; 500 gr increment), maternal education (categorical; low, intermediate [reference], high) pre-term birth (yes vs. no), maternal smoking during pregnancy (yes vs. no), multiple pregnancy (yes vs. no) and birth order (continuous; 1, 2, ≥3).

Table 3. Meta-analysis[a] derived Odds Ratios (OR) and 95% Confidence Intervals (95% CI) on the association of parental age at birth of the index child with childhood (0-14 years) acute lymphoblastic leukemia in sub-analyses by index child's age group, sex, ethnicity, and time period of diagnosis/recruitment, as determined by the 5 registry-based case-control studies (Canada-Quebec; Denmark; Finland; US, California State, CCLRP; US, Washington State).

Variable	N ALL cases	N Controls	Paternal age (5-year increment)		Maternal age (5-year increment)	
			OR (95% CI) [b]	Heterogeneity I^2, p	OR (95% CI) [b]	Heterogeneity I^2, p
Index child's age group (years)						
<1	272	860	1.09 (0.92-1.29)	0%, 0.53	0.98 (0.81-1.18)	0%, 0.53
1-5	5270	16302	**1.05 (1.01-1.09)**	0%, 0.83	**1.04 (1.00-1.09)**	0%, 0.89
6-14	2621	8304	1.03 (0.90-1.19)	**74%, 0.004**	1.06 (0.97-1.16)	30%, 0.22
Index child's sex						
Males	4576	14293	**1.07 (1.03-1.11)**	0%, 0.64	**1.04 (1.00-1.09)**	0%, 0.54
Females	3586	11180	1.00 (0.96-1.05)	0%, 0.96	**1.05 (1.00-1.11)**	0%, 0.83
Index child's ethnicity						
Caucasian	4771	13898	**1.06 (1.01-1.08)**	0%, 0.82	1.04 (0.99-1.08)	0%, 0.67
Non-Caucasian	3348	11522	1.02 (0.97-1.06)	0%, 0.38	**1.06 (1.01-1.11)**	0%, 0.36
Time period of diagnosis/recruitment						
1968-1993	2152	6076	1.01 (0.75-1.08)	0%, 0.95	1.01 (0.89-1.15)	**56%, 0.06**
1994-2003	3446	10939	1.04 (0.99-1.09)	0%, 0.97	**1.07 (1.00-1.15)**	20%, 0.29
2004-2015	2564	8458	**1.06 (1.00-1.11)**	0%, 0.40	1.03 (0.98-1.10)	0%, 0.50

Bold indicates statistical significance (p<0.05 for effect size and p<0.10 for heterogeneity). Maternal and paternal age are simultaneously introduced in all models.

[a] Random-effect meta-analysis of maximally adjusted Odds Ratios from individual studies for any of the following variables that were available, apart if stratified for the specific variable: index child's age (categorical; <1, 1-4 [reference], 5-9, 10-14 years), sex, ethnicity (Caucasian vs. non-Caucasian), birth weight (continuous; 500 gr increment), maternal education (categorical; low, intermediate [reference], high) pre-term birth (yes vs. no), maternal smoking during pregnancy (yes vs. no), multiple pregnancy (yes vs. no) and birth order (continuous; 1, 2, ≥3).

FIGURE LEGENDS

Fig1 Forest plots from the meta-analyses of case control (CC, interview-based) and nested case-control (NCC, registry-based, record-linkage) studies on the association of (A) paternal and (B) maternal age (5-year increments) with childhood (0-14 years) acute lymphoblastic leukemia.

Random-effect meta-analysis of maximally adjusted Odds Ratios from individual studies for any of the following variables that were available (<20% missing values in the total dataset): index child's age (categorical; <1, 1-4 [reference], 5-9, 10-14 years), sex, ethnicity (Caucasian vs. non-Caucasian), birth weight (continuous; 500 gr increment), maternal education (categorical; low, intermediate [reference], high) pre-term birth (yes vs. no), maternal smoking during pregnancy (yes vs. no), multiple pregnancy (yes vs. no) and birth order (continuous; 1, 2, ≥3). Studies are presented in ascending order according to the mean maternal and paternal age. Maternal and paternal age are simultaneously introduced in all models.

Fig2 Curves depicting the association of (A) paternal and (B) maternal age with childhood (0-14 years) acute lymphoblastic leukemia, as derived from meta-analysis restricted cubic spline models encompassing the five registry-based case-control studies (Canada-Quebec; Denmark; Finland; US, California State, CCLRP; US, Washington State).

The solid line depicts the effect estimate, whereas dash-lines correspond to 95% confidence intervals.

ELECTRONIC SUPPLEMENTARY MATERIAL

Data collection and harmonization

The study variables contributed by individual studies were reviewed and harmonized. In particular, a maximum of 4 randomly chosen controls per case, frequency matched on age at diagnosis (<1, 1-4, 5-9, 10-14 years) and sex were selected in nested case-control (NCC) studies which contributed higher case to control ratios. Covariates included in the multivariate model were determined a priori based on the associations between the available variables described in the literature and graphically presented in a conceptual directed acyclic graph (DAG; Supplementary Figure 1). The availability of each variable by study is presented in Supplementary Table 2.

Treatment of variables in the analyses

Data on covariates were categorized as follows: child's age (categorical; <1, 1-4 [reference], 5-9, 10-14 years); sex (male vs. female); child's ethnicity (Caucasian vs. non-Caucasian), birth weight (500 g increments), maternal education (categorical; low=secondary education not completed, intermediate=secondary education completed [reference], high=college, university or higher degree), maternal smoking during pregnancy (yes vs. no), pre-term birth (gestational age <37 weeks: yes vs. no), multiple pregnancy (yes vs. no), birth order (1, 2, ≥3) and time period at diagnosis/recruitment (categorical; 1968-1993, 1994-2003 [reference], 2004-2015).

Baseline characteristics: parental age distribution among controls

The Italian study reported the lowest proportion of mothers <25 years at birth of the index control (12%), whereas the highest was reported in the Costa Rican (46%) and the Brazilian (52%) studies. More than 15% of controls reported maternal age ≥35 years in the Californian NCC, Finnish, Greek, and Italian studies, whereas the lowest frequencies were reported in the Canadian-Quebec (6%) and the US, COG-E15 studies (8%). Proportions of controls with paternal age at birth ≥35 years ranged from <20% in the

Canadian-Quebec and the COG-E15 studies to >35% in the Egyptian, Greek and Italian studies. Paternal age <25 years also varied from 3% (Italy) and 7% (Greece) to 32% (Texas) and 34% (Brazil).

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Supplementary Table 1. Description of studies participating in the Childhood Leukemia International Consortium analysis of parental age and acute lymphoblastic leukemia (ALL).

Study location, Name	Recruitment	Cases source	Controls source/recruitment type	ALL cases, N	N controls
Brazil	1998-2015	Hospitals	Hospitals/ Interviews	428	540
Canada, Quebec, QCLS	1980-2000	All pediatric hospitals (province-wide) mandated by the Quebec government to treat children with cancer	Government Health Insurance Files population-based registry (province-wide)	752	790
Costa Rica, CRCLS	2001-2003	Nationwide Cancer registry	Nationwide Birth registry/ Interviews	251	454
Denmark	1968-2012	National Cancer registry	National Central Population Register/ Electronic linkage	1325	4722
Egypt	2008-2012	Hospital	Population-based recruitment/Interviews	296	351
Finland	1989-2011	National Cancer registry	National Central Population Register/ Electronic linkage	857	3277
Germany, GCCR	1991-1994	National Cancer registry	German Registries of residents (regional with national coverage)/ Interviews	640	2054
Greece, NARECHEM-ST	1996-2015	Nationwide Clinical cancer registry	Hospitals/Interviews	1260	1435
Italy, SETIL	1998-2001	Nationwide cancer registry	National health system rosters/Interviews	554	1044
New Zealand, NZCCS	1989-1994	National Cancer registry, Children's Cancer registry; Hospital Admission/Discharge system	Nationwide Birth registry/ Interviews	97	121
UK, UKCCS	1991-1997	Nationwide General practitioners' registry	Nationwide General practitioners' registry/ Interviews	1437	3399
US, California State, CCLS	1995-2008	Hospitals	State Birth registry/ Interviews	816	1225
US, California State, CCLRP[1]	1988-2011	Statewide Cancer registry	Linked State birth-hospital discharge records	4921	17272
US, COG[2]-E15	1989-1993	CCG clinical trials	Random digit dialing/ Interviews	1886	2516
US, Texas State	1997-2015	Hospital	Hospital/ Interviews	254	343
US, Washington State	1974-2014	Cancer registry (regional 1974-1993; statewide 1994-onwards)	Linked State birth-hospital discharge records	946	3629

[1] Effect estimates were provided for the meta analyses of record-linkage studies; [2]Children's Cancer Group.

Supplementary Table 2. Proportion of missing values (% for both cases and controls) of the study variables by participating study.

Study location, Name	Maternal age at birth	Paternal age at birth	Child's age	Child's sex	Child's ethnicity	Birth weight	Maternal education	Maternal smoking during pregnancy	Pre-term birth	Birth plurality	Birth order
Brazil	0.8	9.3	0.0	0.0	0.0	4.3	0.1	0.7	62.4	0.0	23.2
Canada, Quebec, QCLS	0.0	0.3	0.0	0.0	0.0	0.3	0.1	0.0	0.4	0.3	0.0
Costa Rica	0.4	15.7	0.0	0.0	0.0	14.3	0.7	1.3	0.6	100.0	0.4
Denmark	0.0	1.2	0.0	0.0	0.0	13.6	45.7	58.6	53.8	0.0	0.0
Egypt	0.0	0.2	0.0	0.0	0.0	54.1	0.0	0.0	100.0	100.0	0.0
Finland	0.2	1.4	0.0	0.0	0.0	15.7	0.0	17.5	16.0	100.0	15.8
Germany, GCCR	0.2	1.0	0.0	0.0	0.0	0.5	6.4	0.5	1.5	0.0	0.0
Greece, NARECHEM-ST	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	39.2	0.0	0.0
Italy, SETIL	0.4	2.9	0.0	0.0	0.0	0.1	0.1	0.8	37.5	0.0	0.0
New Zealand, NZCCS	0.0	2.3	0.0	0.0	0.0	1.4	0.0	0.9	0.0	0.0	0.0
UK, UKCCS	0.6	7.3	0.0	0.0	0.5	1.1	0.5	0.6	0.8	0.5	0.5
US, California State, CCLS	0.3	2.7	0.0	0.0	0.0	0.5	0.1	0.1	6.0	2.2	2.0
US, California State CCLRP	0.0	5.8	0.0	0.0	0.0	0.1	24.0	25.6	6.5	0.0	0.2
US, COG-E15	0.1	8.0	0.0	0.0	0.0	0.2	0.0	0.7	0.0	0.0	0.0
US, Texas State	37.9	48.2	0.0	0.0	0.0	4.0	2.2	6.0	11.2	100.0	100.0
US, Washington State	0.2	7.0	0.0	0.0	1.8	0.5	41.6	23.3	10.8	0.1	1.8

Supplementary Table 3. Odds Ratios (OR) and 95% Confidence Intervals (95% CI) derived from multiple logistic regression analysis of the pooled data or random-effects meta-analysis for the association of maternal and paternal age with childhood (0-14 years) acute lymphoblastic leukemia (ALL).

Variable	Pooled analysis (partially adjusted model) [a]			Pooled analysis (maximally adjusted model) [b]			Meta-analysis[c]			
	N ALL cases	N controls	OR (95% CI)	N ALL cases	N controls	OR (95% CI)	N ALL cases	N controls	OR (95% CI)	Heterogeneity I^2, p
Maternal and paternal age included as continuous variables										
Maternal age (5-year increment)	9749	19803	**0.93 (0.90-0.96)**	7173	13054	**0.92 (0.89-0.96)**	10361	18667	1.00 (0.95-1.06)	**58%, 0.002**
Paternal age (5-year increment)			**1.07 (1.04-1.10)**			**1.08 (1.04-1.11)**			**1.05 (1.02-1.09)**	9%, 0.36
Maternal and paternal age included as categorical variables										
Maternal age (years)										
<25	2673	5158	Reference	2037	3430	**Reference**	2826	4949	Reference	
25-34	5905	12171	**0.91 (0.85-0.98)**	4327	8084	**0.90 (0.83-0.98)**	6287	11489	1.00 (0.88-1.15)	**64%, <0.001**
≥35	1171	2474	**0.83 (0.74-0.92)**	809	1540	**0.84 (0.74-0.95)**	1248	2229	0.98 (0.84-1.15)	**40%, 0.05**
Paternal age (years)										
<25	1396	2789	Reference	1115	1944	Reference	1443	2587	Reference	
25-34	5773	12009	1.03 (0.95-1.12)	4260	7936	1.05 (0.95-1.15)	6154	11407	1.06 (0.96-1.18)	13%, 0.31
≥35	2580	5005	**1.16 (1.05-1.28)**	1798	3174	**1.17 (1.04-1.32)**	2764	4673	**1.18 (1.03-1.36)**	24%, 0.19

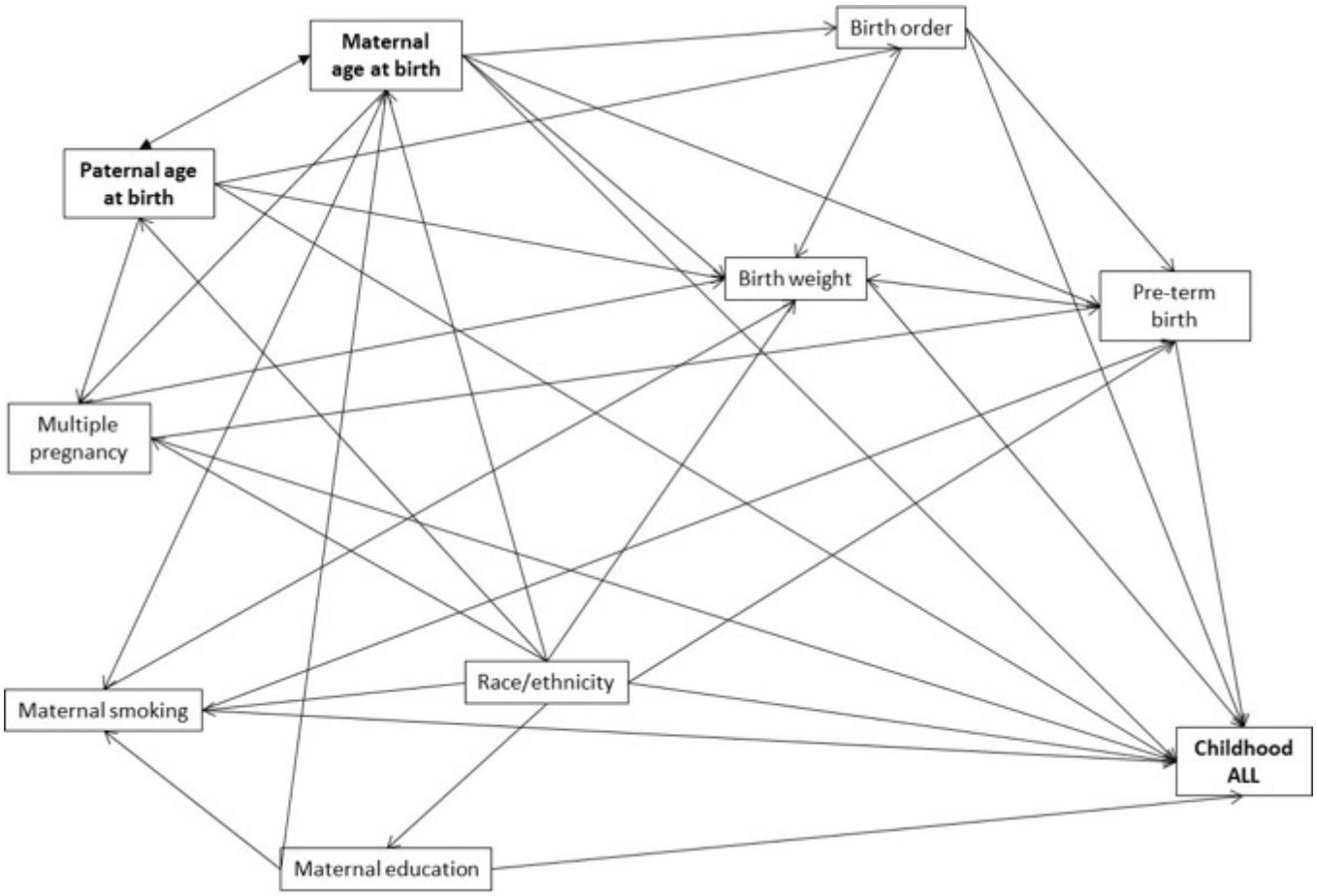
Bold indicates statistical significance (p<0.05 for effect estimate and p<0.10 for heterogeneity). Maternal and paternal age are simultaneously introduced in all models.

[a] Model 1: Odds Ratios are partially adjusted for index child's age (categorical; <1, 1-4 [reference], 5-9, 10-14 years), sex, ethnicity (Caucasian vs. non-Caucasian), birth weight (500 gr increment), maternal education (categorical; low, intermediate [reference], high) and study period (<1994, 1994-2003, 2004+).

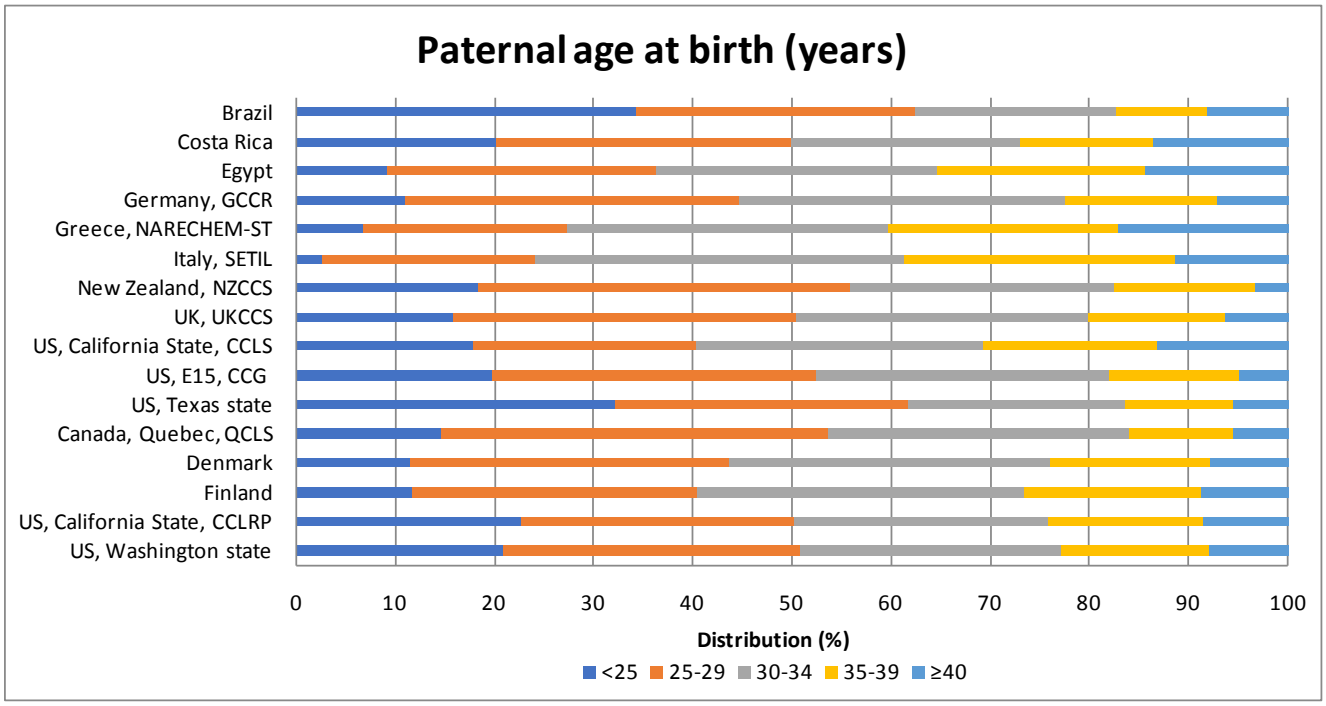
[b] Model 2: Odds Ratios are maximally adjusted for the same variables as in model 1 plus pre-term birth (yes vs. no), maternal smoking during pregnancy (yes vs. no), multiple pregnancy (yes vs. no) and birth order (1, 2, ≥3).

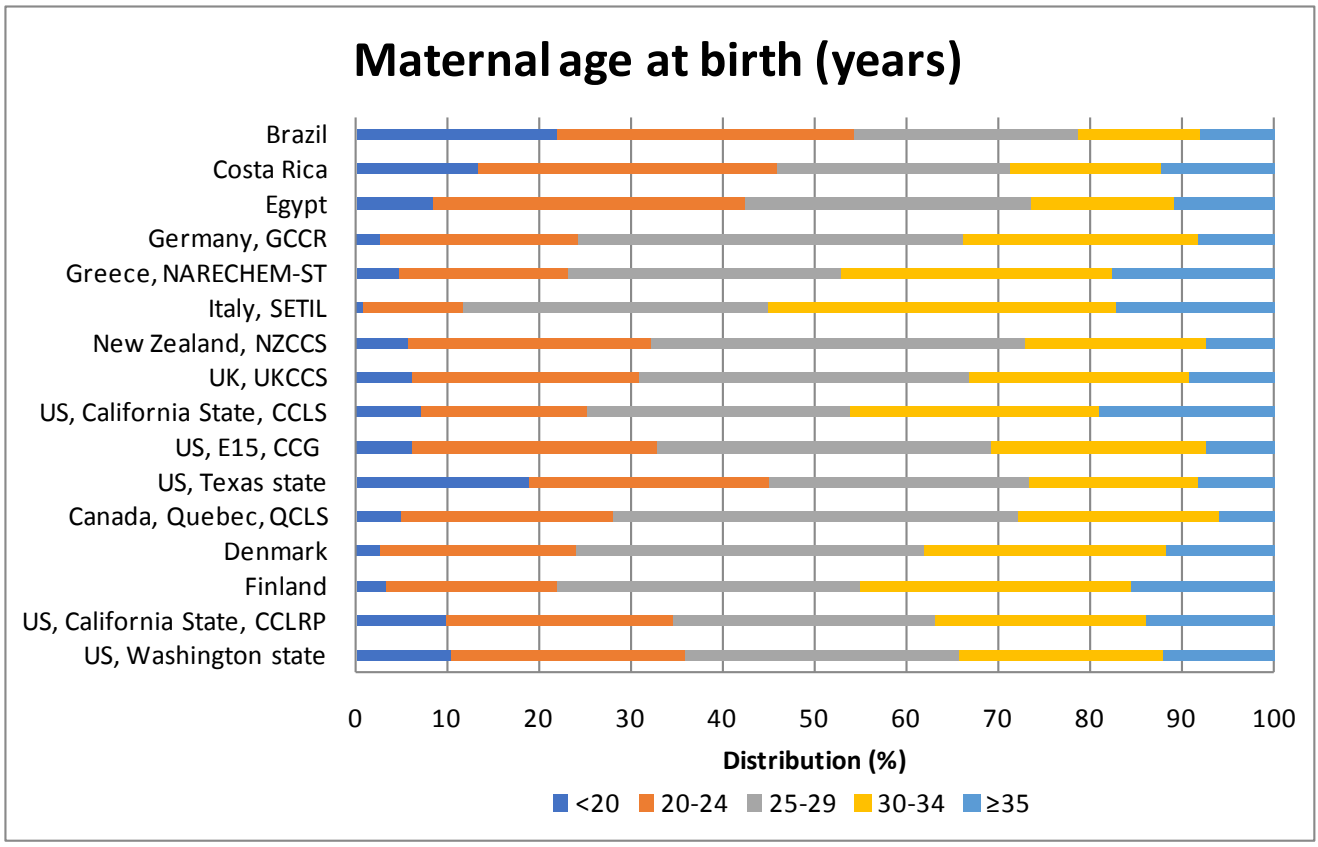
[c] Random-effects meta-analysis of maximally adjusted Odds Ratios from individual studies for any of the following variables that were available (<20% missing values in the total dataset): index child's age (categorical; <1, 1-4 [reference], 5-9, 10-14 years), sex, ethnicity (Caucasian vs. non-Caucasian), birth weight (500 gr increment), maternal education (categorical; low, intermediate [reference], high) pre-term birth (yes vs. no), maternal smoking during pregnancy (yes vs. no), multiple pregnancy (yes vs. no) and birth order (1, 2, ≥3).

Supplementary Figure 1. Conceptual model: associations of maternal-paternal age and commonly studied covariates on the risk of childhood (0-14 years) acute lymphoblastic leukemia (ALL).



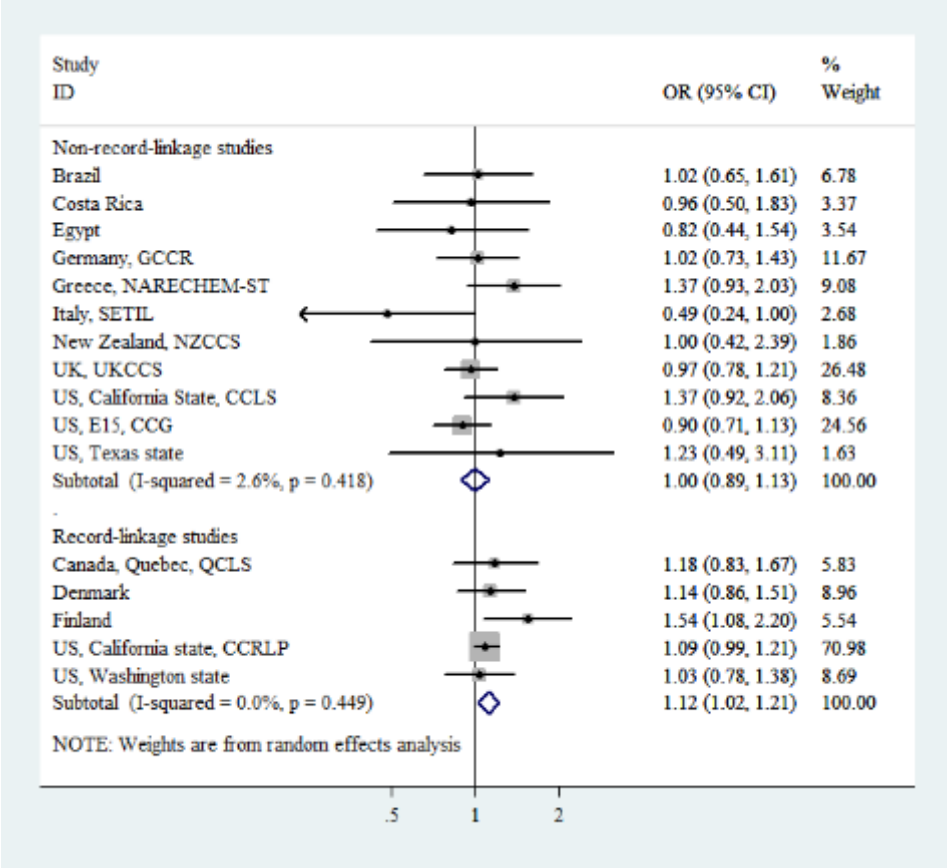
Supplementary Figure 2. Distribution of paternal and maternal age at birth of controls across the participating studies (ranked by case-control followed by nest case-control studies)**.**



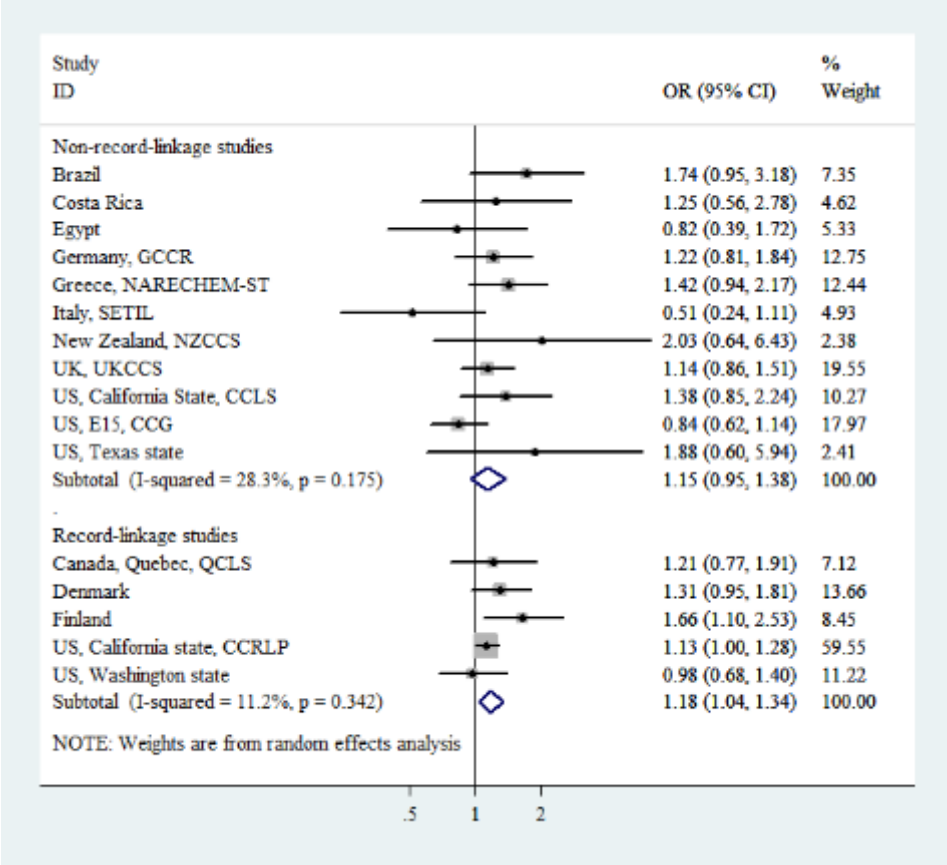


Supplementary Figure 3. Forest plots from the meta-analyses on the association of (A-B) paternal and (C-D) maternal age, as categorical variables [<25 (ref), 25-34, ≥35 years) with childhood (0-14 years) acute lymphoblastic leukemia in subgroup analyses by study design (interview-based case-control and record-linkage nested case-control studies).

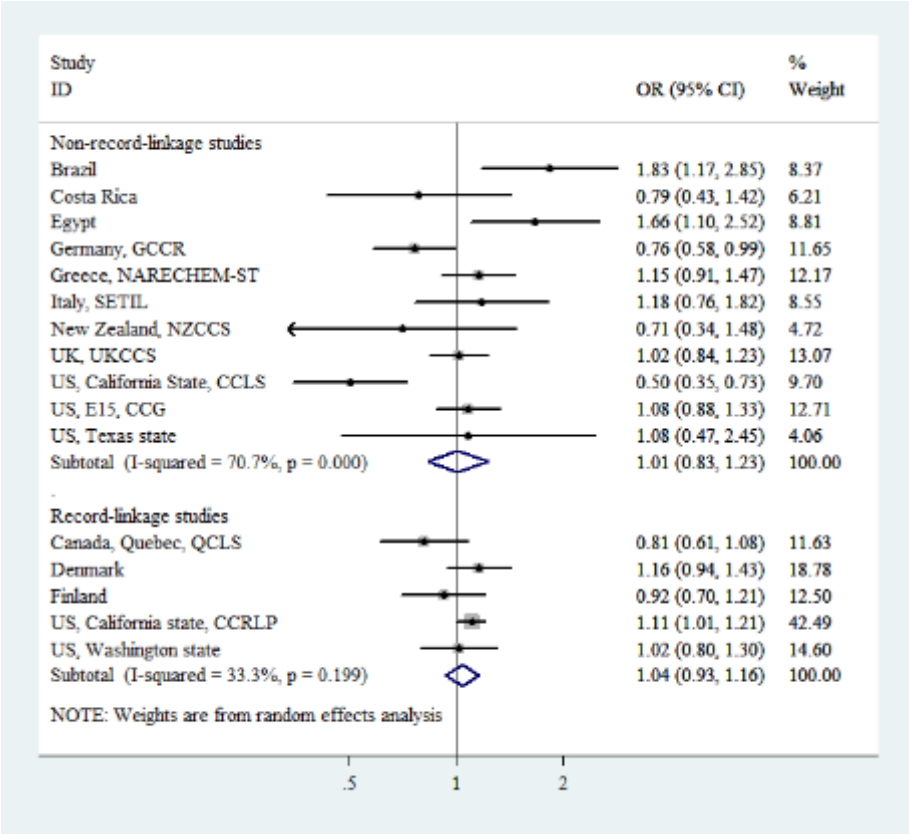
A. paternal age; 25-34 vs. <25 years



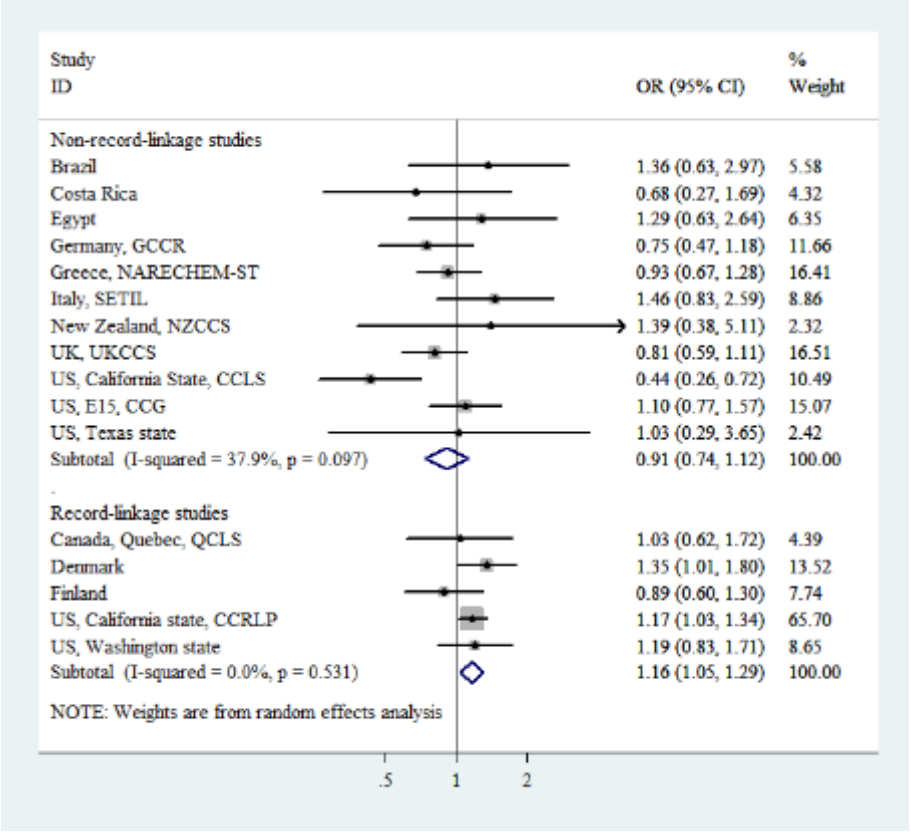
B. paternal age; ≥35 vs. <25 years



C. maternal age; 25-34 vs. <25 years



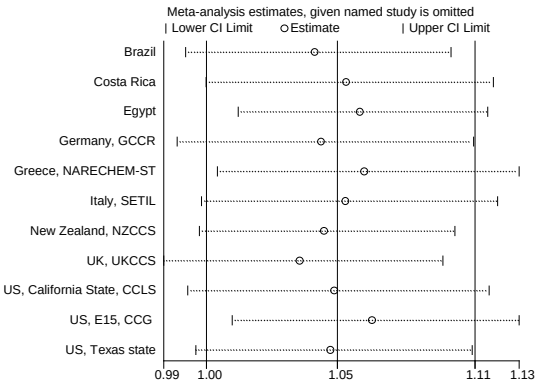
D. maternal age; ≥35 vs. <25 years



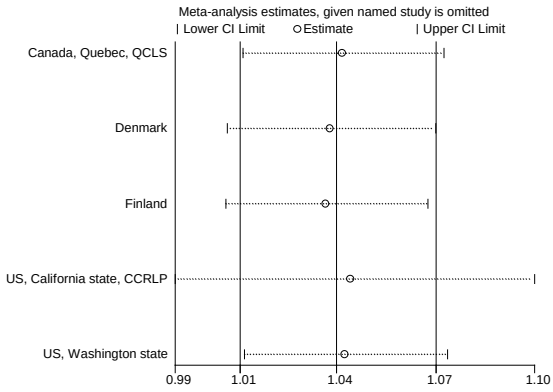
Supplementary Figure 4. Sensitivity meta-analyses by study design excluding one study per analysis for the effect of (A) paternal and (B) maternal age (5-year increment) on childhood (0-14 years) acute lymphoblastic leukemia (ALL).

A.

Paternal case-control studies

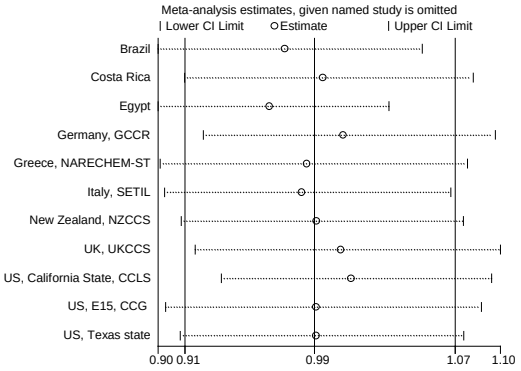


Paternal nested case-control studies

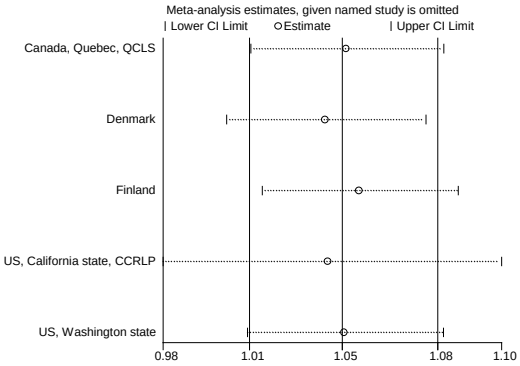


B.

Maternal case-control studies

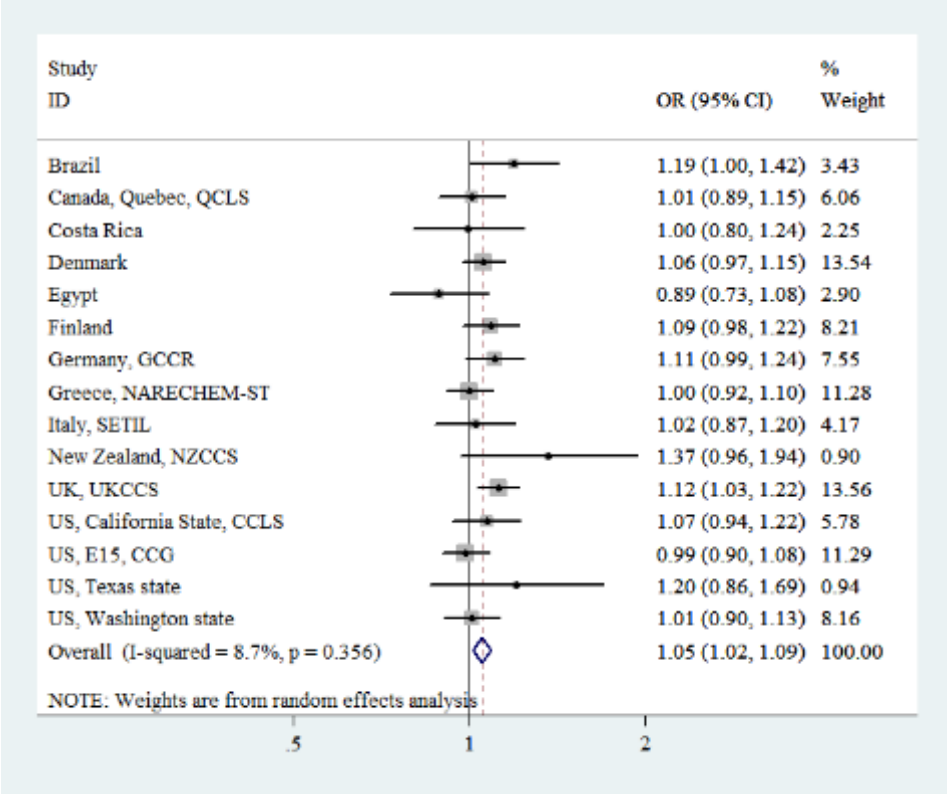


Maternal nested case-control studies

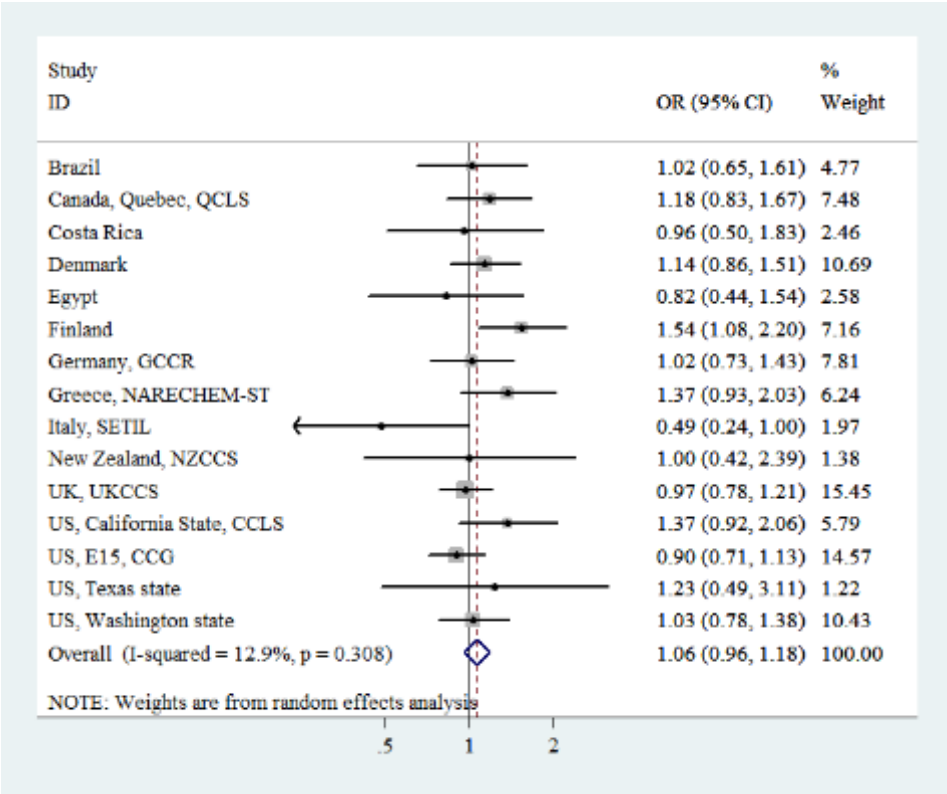


Supplementary Figure 5. Forest plots from the meta-analysis of 15 studies on the association of (A-C) paternal age and (D-F) maternal age (5-year increment and categorical, <25 (ref), 25-34, ≥35 years) with childhood (0-14 years) acute lymphoblastic leukemia. Effect estimates are derived from maximally adjusted random-effects analyses of the individual participating studies.

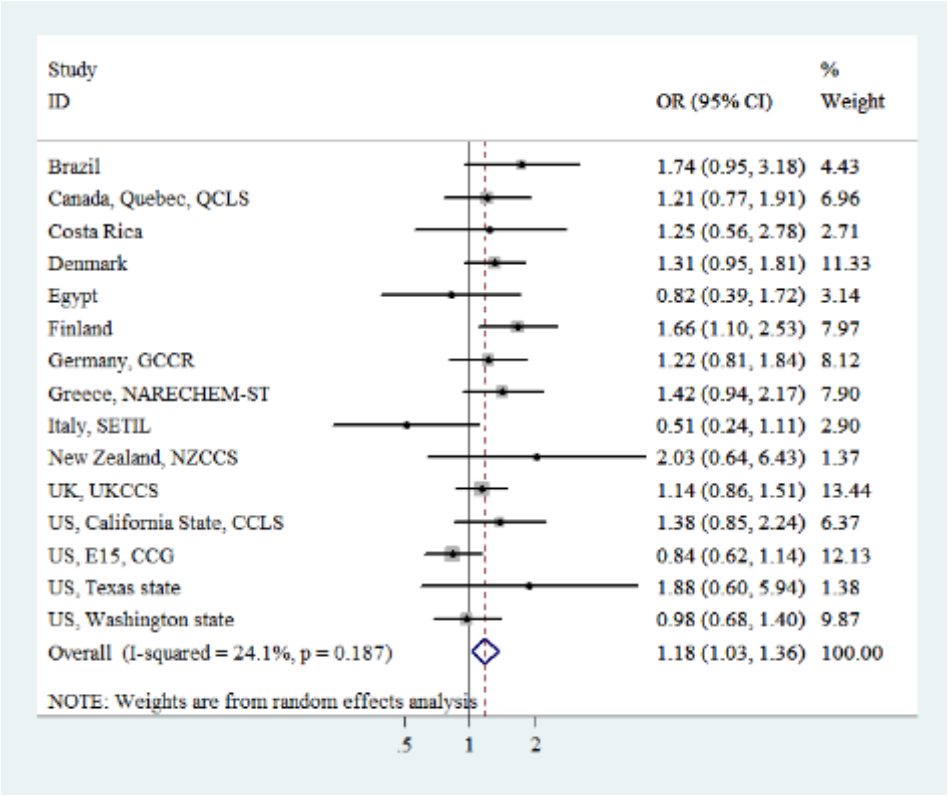
A. paternal age (5-year increment)



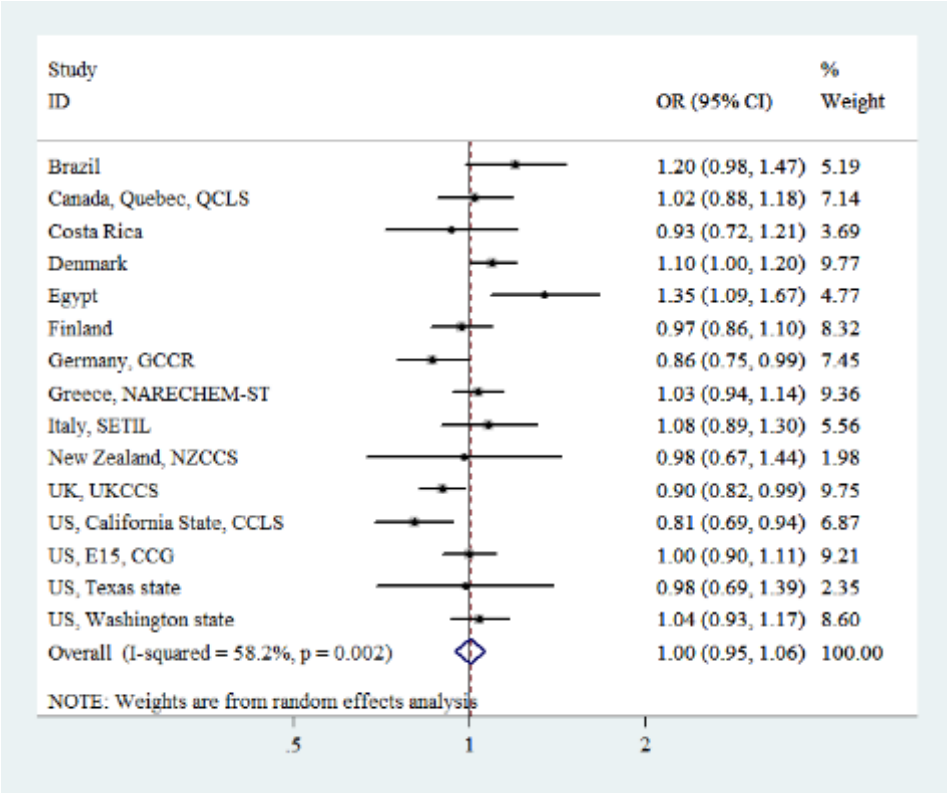
B. paternal age; 25-34 vs. <25 years



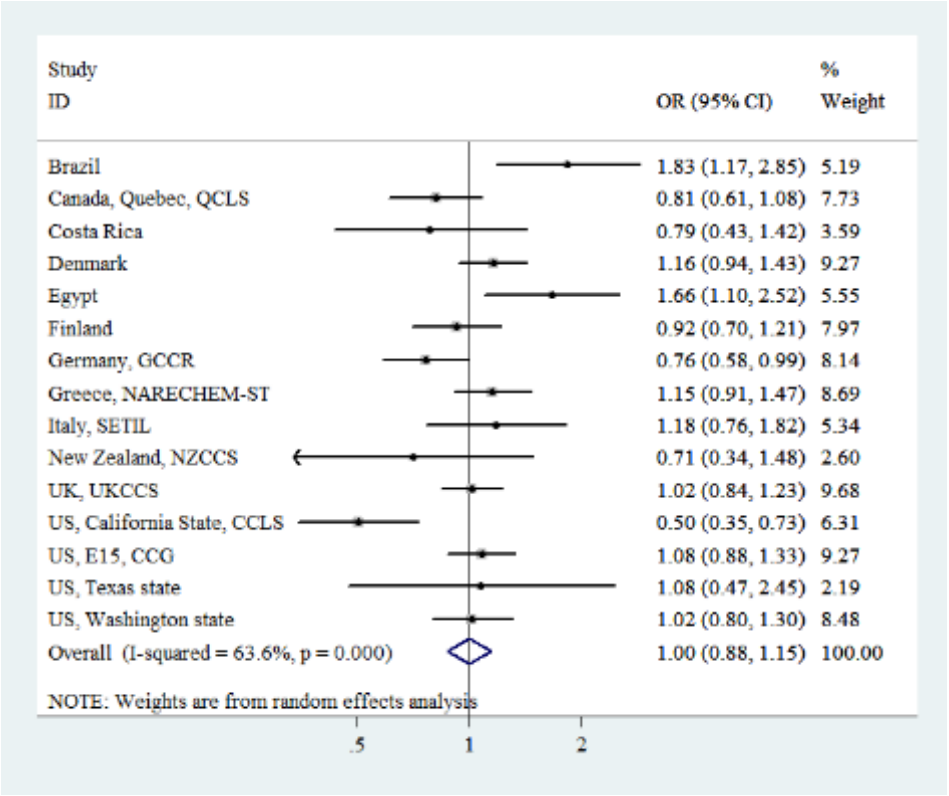
C. paternal age; ≥35 vs. <25 years



D. maternal age (5-year increment)



E. maternal age; 25-34 vs. <25 years



F. maternal age; ≥35 vs. <25 years