Advanced maternal and paternal age as risk factors for childhood acute lymphoblastic leukemia: results from studies of the Childhood Leukemia International Consortium (CLIC)

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ABSTRACT

Advanced parental age has been linked to adverse health effects in the offspring including childhood acute lymphoblastic leukemia (ALL), as shown in a large scale meta-analysis of published data. We aimed to further explore the association using primary data from 11 case-control studies (7919 cases; 12942 controls) and 5 nested case-control studies (8801 cases; 29690 controls) of the Childhood Leukemia International Consortium (enrollment periods 1968-2015). Following application of fractional polynomials, maternal and paternal age - with and without mutual adjustment - were introduced as 5-year continuous variables in pooled unconditional logistic regression analyses, as well in meta-analyses by study design using maximally adjusted odds ratios (OR) derived from each of the 16 individual study results. Increasing paternal age was associated with higher risk for childhood ALL (OR_{pooled}:1.08 per 5-year increment, 95% CI: 1.04-1.11; $OR_{case\text{-}control}$: 1.05, 95% CI:1.00-1.11; OR_{nested}:1.04, 95% CI: 1.01-1.07). By contrast, a similar positive association with advanced maternal age was found only in the nested case-control studies (OR_{pooled}:0.92, 95% CI: 0.89-0.96; $OR_{case\text{-}control}$:0.99, 95% CI: 0.91-1.07, heterogeneity I2=58%, p=0.002; OR_{nested}:1.05, 95% CI: 1.01-1.08). The findings were confined to ages 1-5 years and practically unchanged by mutual adjustment for the collinear parental age variables; analyses of discordant parental age pairs from nested case-control studies was limited by the relatively small numbers. In conclusion, the most valid nested case-control studies add evidence regarding the role of advanced parental age in ALL risk; further analyses by cytogenetic subtypes could further disentangle the effect and underlying mechanisms.

Key words: maternal age; paternal age; childhood acute lymphoblastic leukemia; collinearity; unmeasured confounding; study design

INTRODUCTION

Acute lymphoblastic leukemia (ALL) accounts for 25-30% of all cancers in children (1). Several treatment breakthroughs in the last 50 years have contributed to an >80% increase in survival of this most common childhood malignancy (2). Less progress has been made, however, in understanding the genetic, environmental and lifestyle risk factors of leukemogenesis (3); individual studies and large consortia, such as the Childhood Leukemia International Consortium (CLIC) (4), are currently exploring the constellation of these factors with progress on the role of birth anthropometrics (5), indicators of early immune stimulation (6), prenatal maternal supplementation with folic acid and vitamins (7), as well as pre-labor cesarean delivery (8, 9).

Stipulations on a prenatal origin of childhood ALL have been expressed in the past and already explored (10-12), whereas several studies have also examined the potential role of parental reproductive factors. In particular, maternal and paternal age at birth represent several potential factors, including socio-economic components, life decisions, fertility issues and cultural dynamics (13). Over the recent decades, the increasing trends of parental age at first delivery worldwide, on account mainly of career pursuit and awareness of fertility treatment availability, have attracted intense scientific interest due to reported consequences on offspring's health (14, 15). Indeed, advanced maternal age has been linked to a series of adverse pregnancy outcomes (16) and a dramatic increase in the risk of chromosomal abnormalities (17). Albeit less studied, advanced paternal age has been so far associated with single gene mutations birth defects, chromosomal abnormalities and neurodevelopmental disorders in the offspring (18). Thus, genomic sequencing studies have shown higher numbers of *de novo* mutations in the offspring of older parents (19, 20) and decreased DNA methylation patterns (21), potentially increasing offspring vulnerability to carcinogenesis (21, 22).

Advanced maternal but also paternal age at birth of the offspring has been associated with increased childhood ALL risk in the meta-analysis of numerous case-control and cohort studies;

marginally increased associations with young maternal and paternal age were also observed (23). Registry-based, record-linkage studies from California and Denmark using nested case-control designs have also found an increased ALL risk with advanced maternal age and marginally positive associations with older paternal age (24-26). Inherent methodological concerns, especially when synthesizing published data from individual studies to explore the parental age associations with the disease include inadequate control for confounding in underpowered studies, tentatively suboptimal use of continuous instead of categorical analysis or arbitrary determination of cut-off points for the two variables of interest, notably paternal and maternal age. Most importantly, collinearity between maternal and paternal age precludes clear attribution of the effect to sole maternal, sole paternal or dual parental impact. An additional limitation in meta-analysis of case-control studies is the invoked participation bias when control recruitment is conducted via interviews (27).

In this study, we used primary data from 15 case-control or nested case-control studies within CLIC conducted in 12 countries worldwide aiming to overcome, to the extent possible, several of the aforementioned shortcomings encountered in meta-analysis of published data (4). Specifically, we opted to compare the results from case-control studies taking into account their nationwide coverage and control representativity with those of nested case-control studies using cancer registration data for the case series and record-linkage for control selection and maximally adjust for potential confounders in pooled analyses, in meta-analyses or in sub-analyses and sensitivity analyses, as appropriate. To understand any differential effect of each of the two main variables of interest, we explored the individual and joint impact of parental age in multivariate models; furthermore, we assessed the effect in combinations of concordant and discordant parental age pairs with childhood ALL in the largest ever dataset of primary data on the topic.

METHODS

Study designs and availability of data

Individual-level (primary) data contributed by 15 studies participating in CLIC were used. Among them, four were population-based record-linkage nested case-control studies (Canada, Denmark, Finland, Washington). In addition to the primary data provided by these studies, summary effects were provided from the record-linkage population-based nested case-control study based on data for pediatric leukemia from the California Cancer Register (CCRLP), which were by necessity included only in meta-analyses.

Characteristics of the participating studies, along with the contributed number of cases and number of controls per case, and basic information on case and control recruitment (provided by the individual study principal investigator), are presented in Supplementary Table 1. All study subjects, namely cases with ALL and their controls were aged <15 years at diagnosis/recruitment. Children with a diagnosis of Down syndrome were excluded to avoid a major confounder.

Data collection and harmonization

According to the study protocol, CLIC studies contributed primary data on a series of *a priori* selected variables; the data were subsequently reviewed and harmonized by the Nationwide Registry for Childhood Hematological Malignancies and Solid Tumors (NARECHEM-ST) team. In the interview-based studies, the main exposure variables, namely maternal and paternal age at birth, were self-reported via face-to-face or telephone interviews usually conducted with the child's mother; in record-linkage studies, the variables were extracted from the registry data. Data on index child's age at diagnosis or recruitment, sex, ethnicity, birth weight, gestational age, maternal educational level, multiplicity, birth order, maternal smoking during pregnancy, as well as diagnosis of maternal diabetes during pregnancy and alcohol consumption in pregnancy had been also requested. The availability of each variable per study is presented in Supplementary Table 2. In the present analysis, maternal educational level was used as a proxy for socioeconomic

status and was harmonized as low (below completion of secondary education), intermediate (completion of secondary education) or high (college, university or higher degree) depending on individual study definition. Following an all-studies-inclusive policy in the analysis, a combined variable was constructed, defining pre-term birth as gestational age <37 weeks to account for the exact gestational age missing values in the study of Costa Rica. Likewise, regarding ethnicity (coded as Caucasian/non-Caucasian), as ~99% of the Danish population are of Caucasian origin and the ethnicity variable was missing, all subjects in the Danish dataset were considered Caucasians.

Statistical analysis

For the purposes of the pooled analyses, a maximum of randomly chosen 3-4 controls per case based on age (<1, 1-4, 5-9, 10-14 years) frequency matching and sex were used in the nested case-control studies.

Pooled multivariate logistic regression using the individual-level data was used to explore the role of maternal and paternal age at birth on childhood ALL risk. 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) and the availability of variables. The covariates were child's ethnicity (Caucasian vs. non-Caucasian), birth weight (continuous; 500 g increments), maternal education (categorical; low, intermediate [reference], high), maternal smoking during pregnancy (yes vs. no), pre-term birth (yes vs. no), multiple pregnancy (yes vs. no), birth order (continous; 1, 2, or ≥3), maternal diabetes during pregnancy (yes vs. no) and alcohol consumption during pregnancy (yes vs. no). All models were additionally adjusted for the matching variables of child's age (categorical; <1, 1-4 [reference], 5-9, 10-14 years), sex (male vs. female) and time period at diagnosis/recruitment (categorical; 1968-1993, 1994-2003 [reference], 2004-2015). Only six of the 16 studies collected data on maternal diabetes and alcohol consumption during

pregnancy (covering 28% and 32% of the total dataset, respectively); hence, these two variables were excluded from subsequent analyses. Based on the availability of the remaining variables across the individual studies, two different models were designed; a partially adjusted model, including child's age, sex, ethnicity, time period at diagnosis/recruitment, birth weight and maternal education, as well as a maximally adjusted model further controlling for maternal smoking during pregnancy, pre-term birth, birth order and multiple pregnancy.

To examine the relationship between paternal and maternal age and risk of childhood ALL, fractional polynomials ascertained the best-fitting curves across the pooled dataset. 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 maternal and paternal age in our analyses as 5-year age groups treated as continuous variables. To further assess the risk by specific parental age categories, analyses classifying maternal and paternal age in 3 groups (<25 [reference], 25-34, ≥35 years) were also peformed. On account of the collinearity between maternal and paternal age, the two variables were included in the model as paternal only, maternal only or concurrently paternal and maternal age.

Meta-analyses of study-specific risk estimates were thereafter undertaken, by pooling risk estimates calculated from multiple logistic regression models for each study. In the study-specific analyses, the maximally adjusted model based on the aforementioned covariates was preferred, as to thoroughly control for confounding; variables with >20% missing values within the individual studies were excluded from the study-specific multivariate model. Based on the individual study design, conditional or unconditional logistic regression analyses were performed. Maternal and paternal age were concurrently included in the model in all analyses. For the meta-analysis, random-effects models were implemented and heterogeneity across studies was evaluated with the Cochran Q and I^2 statistics. A p-value <0.10, as derived from the Cochran Q test was considered to indicate statistically significant presence of heterogeneity.

To evaluate potential bias due to method of parental age assessment, we conducted subgroup meta-analyses and sensitivity analyses of the available nested case-control studies (Canada-Quebec, Denmark, Finland, Washington State and California State, CCRLP) with medical record linkage. Subsequently, a variable comprising all 9 pairs of maternal (young, intermediate, advanced) X paternal (young, intermediate, advanced) age combinations was created, aiming to disentangle the overall collinear effect of the two exposure variables and was used in a meta-analysis of the five nested case control studies. Subgroup meta-analyses by child's age group (<1, 1-5, 6-14 years), sex, time period of diagnosis/recruitment and child's ethnicity were additionally conducted to assess effect modification.

Lastly, to assess the effect of potentially unmeasured confounding on our results, the E-value was estimated (28), based on maximally adjusted effect estimates for categories of maternal and paternal age on the risk for childhood ALL among the record-linkage population-based studies. E-values indicate the size of the effect estimate that potentially unmeasured or uncontrolled confounding should have to totally attenuate the observed associations.

Statistical analyses were conducted with SAS 9.4 version and STATA 14.1 version.

RESULTS

Distribution of the study variables

The 15 participating studies provided data for a total of 11,799 ALL cases and 25,900 controls, with maternal age being available for 11,696 cases and 25,702 controls and paternal age for 11,276 cases and 24,638 controls (Table 1). The time period of diagnosis or recruitment ranged from 1968 to 2016, and the majority of the participants (83% of cases and 87% of controls) were of Caucasian origin.

The distribution of maternal and paternal age at birth was highly variable across studies (Figure 1). The lowest frequency of maternal age at birth <25 years (12%) among controls was reported in the Italian study, whereas the respective proportions were 46% in Costa Rica and 54% in Brazil. More than 15% of participating cases and controls had maternal age at birth ≥35 years in California-CCLS, Finland, Greece, and Italy, as opposed to the lowest frequencies reported in Canada-Quebec (6%) and the E15-COG study (8%). Proportions of controls with paternal age at birth ≥35 years ranged from <20% in Canada-Quebec and the E15-COG study to >35% in Egypt, Greece and Italy; paternal age <25 years ranged from 3% (Italy) and 7% (Greece) to 32% (Texas) and 34% (Brazil).

Maternal and paternal age associations with childhood ALL

1. Pooled analyses and meta-analyses of 15 studies

Data from pooled analyses of all 15 studies (Table 2) show that increasing maternal age at birth was associated with a decreased risk for childhood ALL (fully adjusted OR: 0.92 per 5-year increment, 95% CI: 0.89-0.96); by contrast an increased risk with increasing paternal age (5-year increment; fully adjusted OR: 1.08, 95% CI: 1.04-1.11) was found. The same trends were noted in the categorical fully adjusted analyses, notably the ORs for maternal age ≥35 was 0.84 (95% CI:

0.74-0.95) and for 25-34 years 0.90 (95% CI: 0.83-0.98), compared to <25 years . Paternal age ≥35 years was associated with a 17% increased odds (OR: 1.17, 95% CI: 1.04-1.32) for childhood ALL. Practically identical findings were obtained in partially adjusted models with larger number of cases and controls. Further adjustment for study site, as well as alternative introduction of the maternal or paternal age variable in the models, did not essentially change the results (data not shown).

The meta-analysis (right panel of Table 2 and Supplementary Figure 2) confirmed the higher risk for childhood ALL imparted by increasing paternal age ($OR_{5\text{-year increment}}$: 1.05, 95% CI: 1.02-1.09; $OR_{\geq 35 \text{ vs. } <25 \text{ years}}$: 1.18, 95% CI: 1.03-1.36; no substantial heterogeneity), but not with increasing maternal age ($OR_{5\text{-year increment}}$: 1.00, 95% CI: 0.95-1.06; $OR_{\geq 35 \text{ vs. } <25 \text{ years}}$: 0.98, 95% CI: 0.84-1.15; statistically significant heterogeneity). After excluding one study at a time, the incremental effect of paternal age on the risk for childhood ALL remained statistically significant in all analyses and the effect of maternal age did not considerably vary (Supplementary Figure 3).

2. Meta-analysis by study design (5 population-based record-linkage studies vs. 11 case-control studies)

Following the statistically significant heterogeneity revealed for the maternal age meta-analysis and the non-converging results of the pooled analyses with those of the meta-analysis, we proceeded with subgroup meta-analyses by the method of control recruitment; thus, separate analyses of the 11 case-control studies (including the Californian CCLS) based on interview-based vs. record linkage case-control studies were performed (Figure 2); in addition, the Californian CCRLP-derived summary effect estimates were added to the 4 record linkage studies providing primary data, with five record linkage studies included in the meta-analysis.

Results derived for the maternal age from case-control studies ($OR_{5\text{-year-increment}}$: 0.99, 95% CI: 0.91-1.07; heterogeneity I^2: 64%, p=0.002) were in stark contrast with those derived from the

record-linkage ones; as expected, the latter demonstrated a statistically significant increased risk for childhood ALL with increasing maternal age ($OR_{5\text{-year-increment}}$: 1.05, 95% CI: 1.01-1.08; I^2: 0%, p=0.64). Of note, irrespective of control recruitment methodology, similar results were obtained regarding the association of increasing paternal age with childhood ALL risk, (non-record-linkage studies, $OR_{5\text{-year-increment}}$: 1.05, 95% CI: 1.00-1.11, I^2: 29%, p=0.17 vs. record-linkage studies: $OR_{5\text{-year-increment}}$: 1.04, 95% CI: 1.01-1.07; I^2: 0%, p=0.86). The categorical meta-analyses (Supplementary Figure 4) showed comparable results. Essentially similar results were also obtained when repeating the analyses introducing only the maternal or only the paternal age variable in the model (data not shown).

Collinearity of paternal with maternal age

All subsequent meta-analyses were conducted using the five population-based record-linkage studies. Table 3 shows the grid of concordant (both parents in the same age group) and discordant maternal and paternal age groups (each parent in different age groups), in an effort to assess the combined effect of maternal and paternal age at birth on childhood ALL risk. Overall, results of the concordant cells confirm the findings that the highest odds were noted for the combined category of both parents' age ≥35 years (OR: 1.16, 95% CI: 1.04-1.28), whereas when both parents were <25 years at birth, the child was at decreased risk for ALL (OR: 0.84, 95% CI: 0.77-0.91). These cells, however, are non-informative in disentangling collinearity. Although underpowered, cells with the informative discordant age categories, notably younger than 25 years maternal and ≥35 years paternal age (OR: 1.17; 95% CI: 0.77-1.77) and intermediate/old maternal age (≥25 years) and young paternal age (<25 years; OR: 0.88; 95% CI: 0.74-1.04) may be interpreted as showing a tentatively stronger effect of older paternal age on childhood ALL risk, without reaching however, statistical significance.

Subgroup analyses

Table 4 presents results of sub-analyses by child's age group, sex, ethnicity and time period at diagnosis/ recruitment. The association with both paternal and maternal age is confined to the age group 1-5 years; for advanced maternal age, similar positive association with ALL is evident for both genders and non-Caucasian ethnicity, as opposed to the positive association of the advanced paternal age with ALL risk confined to boys and Caucasian ethnicity.

Unmeasured confounding

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

DISCUSSION

This large size CLIC study including primary data from 11 case control (7919 cases; 12,942 controls) and 5 nested case control studies (8801 cases; 29,690 controls), yielded a statistically significant and linearly increasing risk for childhood ALL with advancing paternal age. In line with a recently published meta-analysis, the five record-linkage studies also showed an increasing risk with advancing maternal age; underlying reasons for the contradictory and heterogeneous results derived from few interview-based case-control CLIC studies, such as selectin bias, should be further explored. The results were practically unchanged when the two variables of interest were alternatively or jointly introduced in the multivariate models; compared to the non-informative concordant on parental age pairs, analysis of the fewer discordant parental age pairs, may be interpreted as pointing to paternal age as the main determinant of ALL risk compared to maternal age; disentangling collinearity in parental age should, however, be further explored.

In terms of effect directionality and magnitude, our results are in line both with those of the recently published meta-analysis of 77 studies showing a higher risk for ALL with advanced age of both mother and father (23), with the exception of the maternal age association estimates derived solely from register-based case-control studies within CLIC. Separate analyses by study design –cohort vs. case control – had also been undertaken in the 2015 meta-analysis (23), albeit no attempt was then employed to tackle collinearity between the two exposure variables of interest. Moreover, results in the large series of case-control studies conducted worldwide showing a positive association with maternal age were not mutually adjusted for paternal age. Likewise, the results from the two included population-based cohort studies were positive, notably one from the region of Piedmont in Italy (29) and the registry-based nationwide study of Denmark (30), as well as those of two recent population-based case-control studies in California and again Denmark, the latter confined only to the paternal associations (24-26).

The paternal age association was consistent and of similar size across different study methodologies and robust in both the CLIC pooled analysis and meta-analysis, without evidence of heterogeneity. The findings, however, were not similar for maternal age; surprisingly, however, an inverse association of advanced maternal age with ALL risk was found in the pooled analysis entailing all study designs; a null effect in considerably heterogeneous random-effects meta-analysis; and a positive, non-heterogeneous one in the sensitivity analyses of the five population-based record-linkage studies less prone to biases. The sources of error leading to an inverse or null associations with advancement of maternal age, found in some classic CLIC case control studies, cannot be easily explored. This may be partly attributed to differential control recruitment in case-control studies, especially non-participation bias, as previously reported by the German study, also included in the present analysis of primary data, showing a deficit in the young age in maternal distribution among selected controls in comparison to that in the underlying population (27). Specifically, the majority of controls are derived following maternal consent in case-control studies of childhood cancer and this might possibly explain why these differences were evident only for maternal, and not for paternal age, distributions.

Population-based studies, in which both cases and the comparison group are derived from record linkage of cancer registry population data, are considered free from selection bias due to non-participation. In this CLIC study, researchers from California contributed primary data from an interview-based recruitment case-control study (CCLS), as well as effect estimates from the record linkage case-control study (CCRLP). The CCLS case-control study, comprising subjects not included in the larger and lengthier CCRLP study, yielded an inverse association of maternal age with ALL, whereas the latter clearly showed a higher risk with increasing maternal age. Of note, the mean maternal age of controls in CCLS was almost 2 years older compared to the CCRLP study (29.3 vs. 27.4 years); while no such difference was noted among leukemia cases (28.2 vs. 27.8 years). Not all CLIC case control studies, however, are subject to the same bias. For example, there seemed to be no difference in the age distribution of the Greek NARECHEM-ST control

series in maternal age at fist pregnancy compared with the national statistics. Similarly, the maternal age distribution among controls in the Italian SETIL study followed the population pattern. Of note, however, both the Greek and the Italian studies had parental age distributions heavily skewed towards older age categories, i.e. there was little variation in the exposure variables of interest, possibly contributing to the null associations for maternal age.

Unlike record-linkage studies, the classic interview-based case-control studies can also be subject to recall bias; it is considered unlikely, however, that there might be major reporting inaccuracies with regards to the age of parental age at index child's birth (31). Worth noting are the large differences across CLIC studies regarding the age distributions of the two exposure variables of interest; the between-study parental age differences probably reflect socioeconomic and cultural variations between populations. Age distributions show dramatic increases in maternal and paternal age in the recent decades (14, 15); their distribution across the studies essentially depends on the study period, which could span almost five decades (1968-2015). The case to control ratio in the nested case-control studies ranged from 1:1 to 1:100, the latter aiming to increase precision for rare exposures; in order to reduce the impact of the heavy imbalance on the effect estimates, especially in pooled analyses, the investigators randomly selected controls in a ratio of approximately 1:3 for studies with more than 1 controls per case.

In both the pooled and the study-specific analyses, we aimed to include the maximum number of available co-variates in the multiple regression models. Thus, we adjusted for most perinatal factors that have been implicated as potential confounders in the literature. Nevertheless, given the high proportion of missing values for some covariates or individual studies, it was not eventually possible to control for alcohol consumption (32), maternal diabetes during pregnancy (33), breastfeeding (34) or genetic markers. To further assess for the potential effect of unmeasured confounding, however, we assessed its magnitude by calculating the recently described E-value (28). In order to sufficiently explain the observed effect estimates for both maternal and paternal

age, as derived from population-based record-linkage studies, an unmeasured confounder should impact on 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 associations with the perinatal factors that have already been described in the literature.

The high collinearity between the two main variables of interest restricted our ability to disentangle whether advanced maternal, paternal or joint parental age contribute to ALL risk. To this end, we opted to include separately each variable in the models, whereas the two variables were simultaneously introduced in subsequent models; the results, remained practically the same. Lastly, the discordant maternal and paternal age pairs used in a joint analysis of the record-linkage studies showed some indication that advanced paternal age might be more important, albeit the findings were imprecise.

Several outcomes, including chromosomal abnormalities (17, 18), neurodevelopmental disorders (35, 36), psychiatric diseases (37, 38) and cancer (26) in the offspring have been associated with older parental age. Accumulation of *de novo* genetic mutations in the paternal germ cells (19, 20) that could increase the risk for childhood cancer (39, 40) is a plausible explanation for the association with advanced paternal age. Moreover, DNA methylation in the offspring related to advanced maternal age was correlated with cancer in an epigenome-wide association study (21). The well-established association of advanced maternal age with chromosomal abnormalities and birth defects (41, 42) as well as ALL (43-45) could possibly mediate the observed effect; of note, we excluded children with Down syndrome, who are more likely to develop the disease.

According to the findings from the record-linkage studies, the magnitude of the impact of advanced maternal and paternal age seemed to be rather small (5% and 4% per 5-year increment, respectively); the figure can be translated to a ~20% increase in the risk for a 20-year increase in parental age. Nevertheless, it may have substantial public health relevance, given the recent sharp increase of parental age at birth of first child worldwide (14, 15). Indeed, previous studies have

suggested that increasing maternal and paternal ages might contribute to the increasing trend in the rates of childhood ALL (1, 46-48) (26, 29).

The strengths of the present CLIC study include the large numbers of cases and controls that also allowed to explore robustness of the observed associations in several sub-analyses; the availability of two study designs for testing etiological hypotheses while assessing the potential effect of study design on the results. Besides the experience from conducting the 2015 meta-analysis that guided the current analyses, the results of the pooled analysis were contrasted to those derived from the meta-analysis of the individual study results. Among the limitations of the study are the divergent data collection methods for cases and controls, as well as divergent time periods and parental age distributions between studies, and minimal exposure variation in some studies; the high proportions of missing values in several essential covariates, including confounding factors, which led to considerable decrease of the sample size in the fully-adjusted analyses; the heterogeneity regarding the distribution of maternal and paternal age among studies; lastly, lack of ALL immune-phenotype data in the majority of the studies, especially the record linkage studies.

In conclusion, we used the largest set of primary data so far exploring the association of parental age at birth with childhood ALL and the results demonstrated an association of advanced parental with increased risk, which is consistent with earlier reports. *De novo* mutations in the fathers' germ cells and epigenetic alterations in the offspring born to older mothers could explain the observed associations. Methodological challenges related to methods of control selection and data collection, as well as unmeasured confounding should be further explored within CLIC. Longitudinal cohort studies with cytogenetic data are important to broaden our understanding on the mechanisms through which advanced maternal and paternal age affect leukemogenesis among children.

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

Variables	ALL Cases (N=11799)		Controls (N=25900)	
	N	%	N	%
Index child's age at diagnosis/recruitment (years)				
<1	444	3.8	1636	6.3
1-4	6392	54.2	12837	49.6
5-9	3367	28.5	7216	27.9
10-14	1596	13.5	4211	16.2
Index child's sex				
Male	6565	55.6	14057	54.3
Female	5234	44.4	11842	45.7
Time period of diagnosis/recruitment				
1968-1993	4536	38.4	9848	38.0
1994-2003	4281	36.3	9979	38.5
2004-2016	2982	25.3	6073	23.5
Index child's ethnicity				
Caucasian	9780	83.1	22378	86.7
Non-Caucasian	1990	16.9	3443	13.3
Missing	29	*0.3*	79	*0.3*
Birth weight (g)				
<2500	584	5.2	1391	5.7
2500-2999	1556	13.9	3612	14.8
3000-3499	3968	35.5	8690	35.6
3500-3999	3493	31.2	7521	30.9
≥4000	1590	14.2	3166	13.0
Missing	608	*5.2*	1520	*5.9*
Maternal education				
Low	2507	23.3	4882	22.1
Intermediate	5426	50.5	11081	50.2
High	2813	26.2	6104	27.7
Missing	1053	*8.9*	3833	*14.8*
Maternal smoking during pregnancy				
No	8305	78.5	17144	79.2
Yes	2272	21.5	4498	20.8
Missing	1222	*10.4*	4258	*16.4*
Pre-term birth[a]				
No	8900	92.8	19124	93.2
Yes	692	7.2	1393	6.8
Missing	2207	*18.7*	5383	*20.8*
Multiple pregnancy				
No	9854	97.5	20938	97.7
Yes	251	2.5	495	2.3

Missing	1694	*14.4*	4467	*17.3*
Birth order				
1	5066	44.9	10976	44.2
2	3867	34.3	8543	34.5
≥3	2348	20.8	5275	21.3
Missing	518	*4.4*	1106	*4.3*
Maternal age at birth (years)				
<20	687	5.9	1470	5.7
20-24	2699	23.1	5802	22.6
25-29	3958	33.8	8861	34.5
30-34	2970	25.4	6528	25.4
≥35	1382	11.8	3041	11.8
Missing	103	*0.9*	198	*0.8*
Mean ± SD	29.92 ± 5.48		29.96 ± 5.46	
Paternal age at birth (years)				
<25	1628	14.4	3587	14.6
25-29	3286	29.1	7471	30.3
30-34	3413	30.3	7505	30.4
35-39	1900	16.9	3979	16.2
≥40	1049	9.3	2096	8.5
Missing	523	*4.4*	1262	*4.9*
Mean ± SD	31.11 ± 6.41		30.90 ± 6.25	

Table 2. 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-year-old) acute lymphoblastic leukemia (ALL).

Variable	Pooled analysis (partially adjusted model)[a]			Pooled analysis (fully 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)			**0.93 (0.90-0.96)**			**0.92 (0.89-0.96)**			1.00 (0.95-1.06)	**58%, 0.002**
	9749	19803		7173	13054		10361	18667		
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 (continuous; 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 (continuous; 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 (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. Random-effects meta-analysis derived Odds Ratios (OR) and 95% Confidence Intervals (95% CI) from the 5 record-linkage studies (Canada, Quebec, QCLS; 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-year-old) acute lymphoblastic leukemia (ALL).

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).

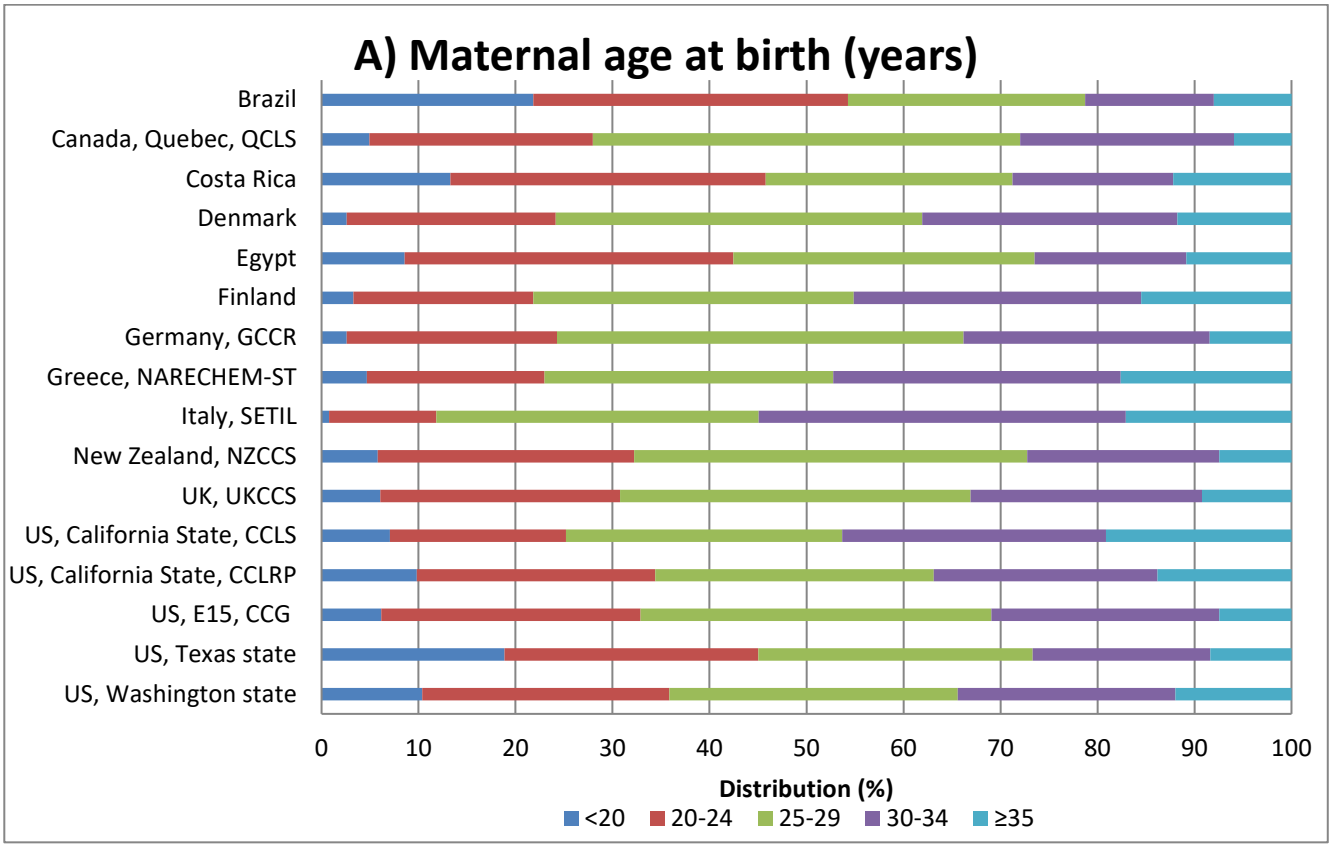
Table 4. Random-effects meta-analysis[a] derived Odds Ratios (OR) and 95% Confidence Intervals (95% CI) on the association of parental age at birth of the index child with childhood (0-14-year-old) acute lymphoblastic leukemia (ALL) in sub-analyses by index child's age group, sex, ethnicity, and time period of diagnosis/recruitment, as determined by the 5 record-linkage studies (Canada, Quebec, QCLS; Denmark; Finland; US, California State, CCLRP; US, Washington State).

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

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 1. Distribution of (A) maternal and (B) paternal age at index child's birth among controls across the participating studies**.**



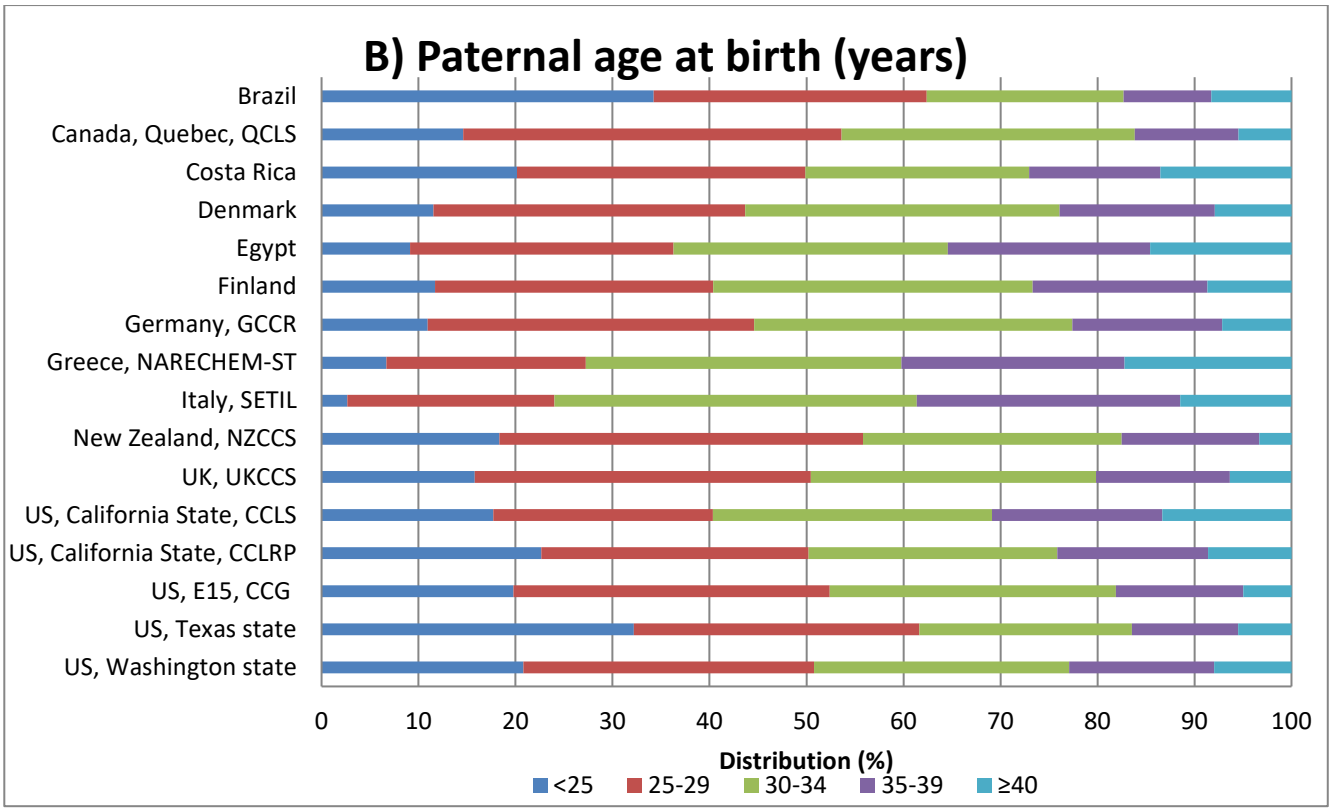
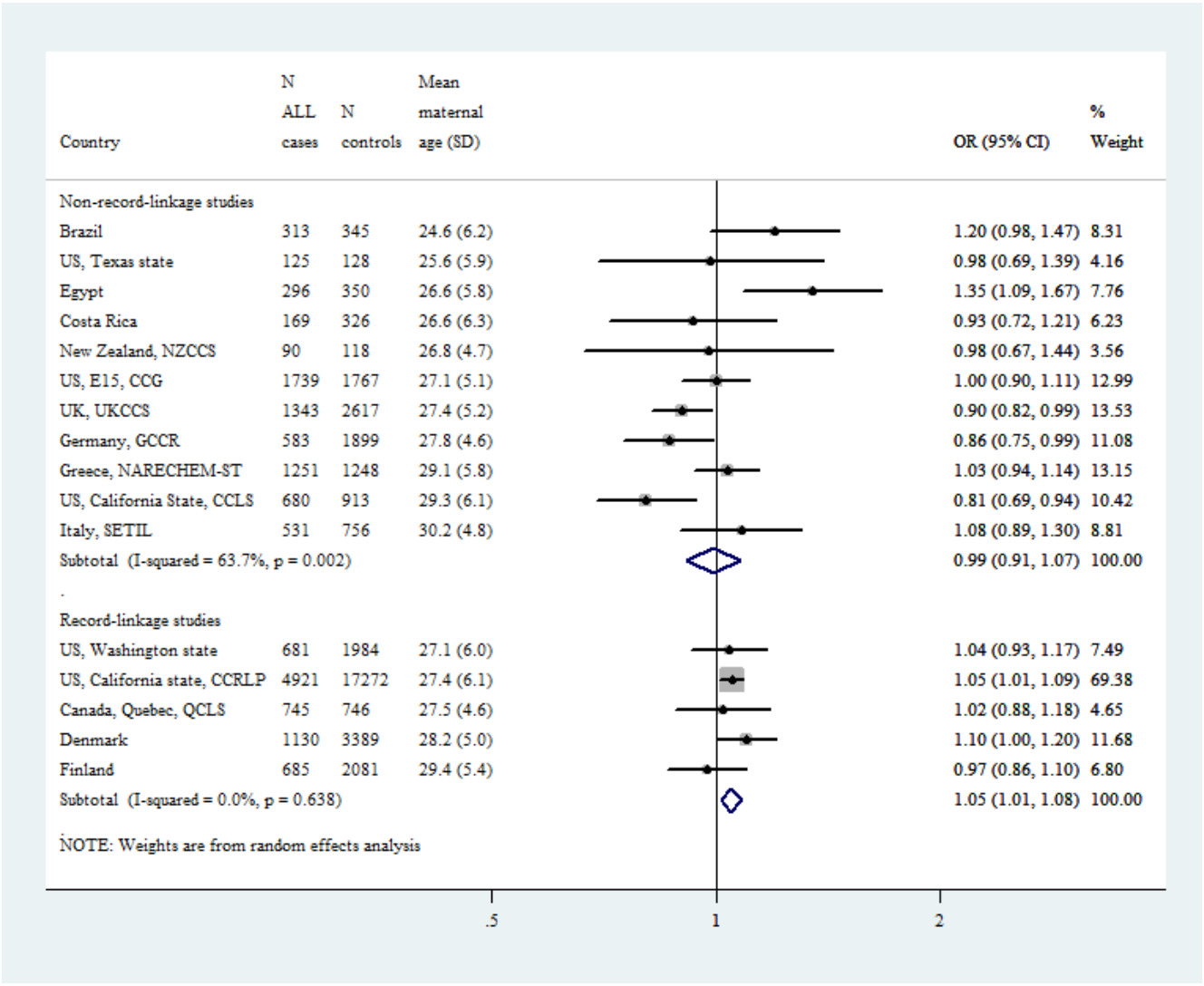
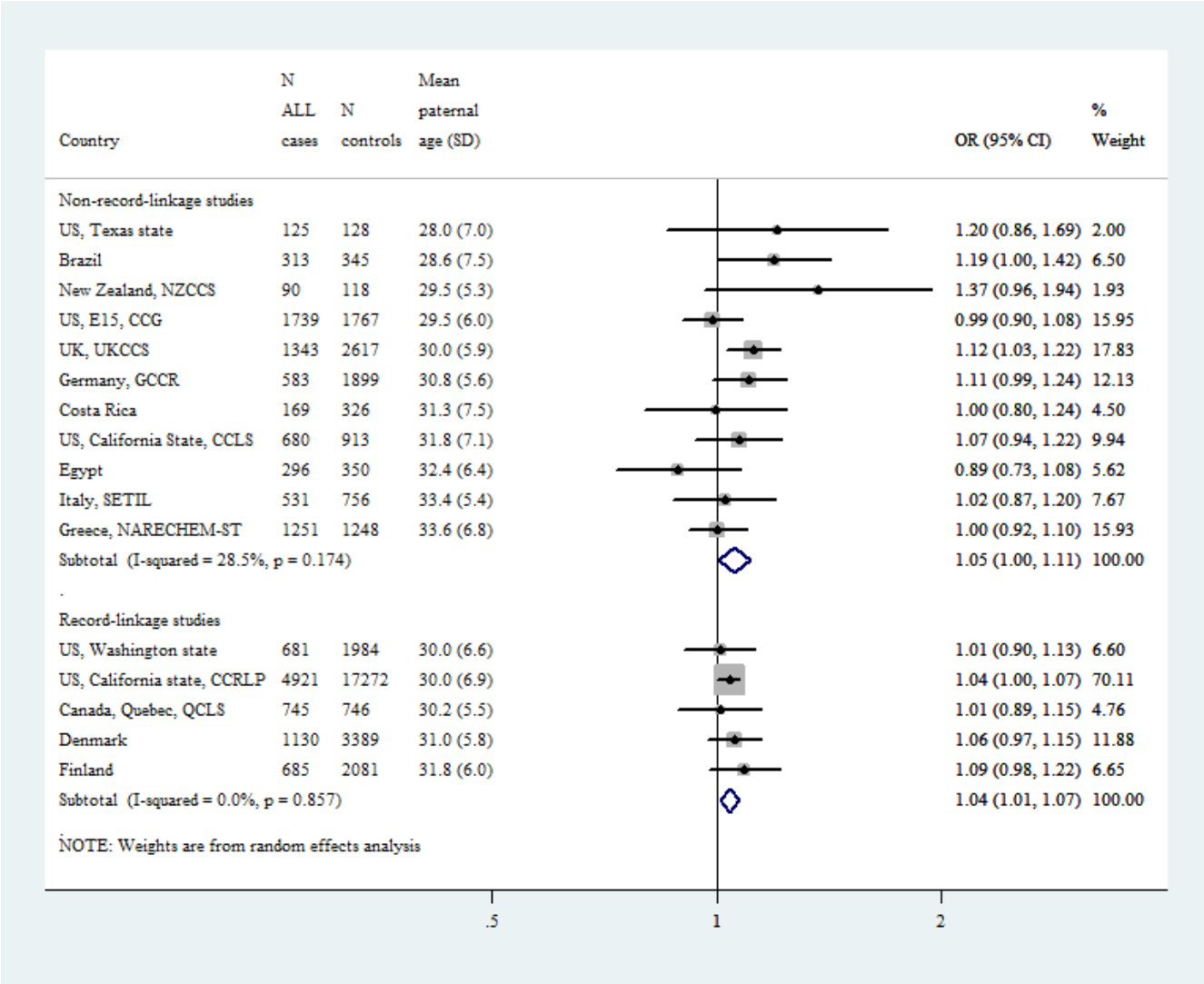


Figure 2. Forest plots from the meta-analyses of classic non-record linkage and nested record-linkage case-control studies on the association of (a) maternal and (b) paternal age (continuous in 5-year increments) with childhood (0-14-year-old) acute lymphoblastic leukemia (ALL).

a. Maternal age (5-year increment)



b. Paternal age (5-year increment)



Studies are presented in ascending order according to the mean maternal and paternal age.

Maternal and paternal age are simultaneously introduced in all models.

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).