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Growth curves of “normal” serum total IgE levels throughout childhood: a quantile analysis in a birth cohort.

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Abstract

Background: Previous studies of serum total IgE (t-IgE) were not able to discriminate well enough atopic from non-atopic subjects, *i.e.* with or without serum-specific IgE antibodies to allergens.

Objectives: To model growth curves of the total IgE levels in children without atopic sensitization (hereafter defined as “normal” t-IgE levels) and to test their usefulness in predicting atopic sensitization.

Methods: The German Multicentre Allergy Study (MAS), a birth cohort with 1314 recruited newborns, began in 1990 and examined the participants until age 20y. Total and specific IgE (t-IgE, s-IgE) were analysed with a fluorescent enzyme immunoassay ImmunoCAP (TFS, Sweden) at ages 1,2,3,5,6,7,10,13 and 20 years. Participants were classified as “never atopic” if all their available serum samples had negative response (cut-off: $<0.35 \text{ kU}_A/\text{l}$) for s-IgE to the 9 common foodborne and airborne allergenic extracts (milk, egg, soy, wheat, house dust mite, cat, dog, birch and grass) tested in the MAS birth cohort. By contrast, participants were defined as atopic if they had, for at least at one available serum sample, $\text{s-IgE} \geq 0.35 \text{ kU}_A/\text{l}$ to at least one allergenic extract tested. The evolution of t-IgE levels in the “never atopic” children was described by growth curves, estimated by exploiting a quantile regression model. A “reference” percentile, based on the t-IgE value measured at age 5y, was assigned to each child with no IgE sensitization at that age. Upward deviations from the own “reference” quantile of t-IgE in atopic and “never atopic” children were calculated and a ROC analysis was used to identify the best cut-off point for predicting atopic sensitization.

Results: Overall, 1113 of 1314 children were included in this analysis. Of these, 469 were “never atopic” and 644 atopic. Quantile trajectories of t-IgE levels in “never atopic” subjects were stable from 5y of age, increased to a plateau at age 10-13y, and decreased slightly afterwards. The onset of atopic s-IgE responses was characterized by an upward deviation of serum t-IgE levels from their “reference” trajectory. T-IgE quantiles predicted the onset of atopy with high efficiency (AUC >80%). ROC analysis showed that deviations from the t-IgE level “reference” quantile above 0.32, 0.41, 0.42, 0.30 and 0.58 kU/l (log-units) at 6,7,10,13 and 20 years of age, respectively, predicted an atopic sensitization.

Conclusions: The growth curves of “normal” serum t-IgE concentrations were estimated in “never atopic” children; for each individual who was non-atopic at 5y of age a “reference” quantile was identified that represented the individual’s “normal” level of t-IgE production. Upward deviations of observed t-IgE levels from the own “reference” quantile, from 6ys to 20ys of age, predicted at each year the occurrence of atopic sensitization.

Clinical implications: The trajectory of T-IgE levels can be elaborated since age 5y in non-atopic children. A child whose t-IgE levels are consistently higher than those predicted by his/her growth curve may have developed atopic sensitization.

Capsule summary: In 466 “*never atopic*” participants in the German MAS birth cohort, the individual trajectory of serum t-IgE concentrations can be predicted from the age of 5y until age 20y. A t-IgE value deviating upwards from the individual trajectory predicts atopic sensitization.

Author Keywords

Allergy; atopy; birth cohort; children; diagnosis; normal levels; quantile trajectories; sensitization, specific IgE; total IgE.

Abbreviations

AUC Area under the curve

CI Confidence interval

IgE Immunoglobulin E

IQR Interquartile range

kU_A/L Kilounits/liter

MAS Multicenter Allergy Study

Log Natural logarithm

ROC Receiver Operating Characteristic

SD Standard Deviation

Se Sensitivity

s-IgE Allergen-specific IgE

Sp Specificity

t-IgE Total IgE

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Introduction

Immunoglobulin E was discovered in the late 1960s¹ and soon associated with allergies and helminth infections.² Assays to measure total IgE concentration in the serum were validated³ and thresholds were proposed to discriminate normal from pathologic levels in childhood.^{4,5} Nevertheless, the observation that the distribution curves of total IgE levels in serum of normal and allergic subjects overlapped widely^{6,7} prevented further use of this parameter in diagnosing allergies⁸⁻¹⁰ and no studies or guidelines have examined this subject to date.

Improving our comprehension of the intrinsic nature of total IgE levels may facilitate a better use of this parameter for diagnostic purposes. Previous studies in the “Multicentre Allergy Study” (MAS) birth cohort examined the total serum IgE percentiles during the first 6ys of life in atopic and not-atopic children, showing that IgE percentiles were markedly higher in atopic children than in non-atopic¹¹ and suggested that, in westernized and virtually “helminth-free” populations, t-IgE level largely results from two different pools: spontaneously produced IgE (hereafter defined as “normal” IgE) and IgE generated during atopic responses to environmental allergens (atopic IgE).¹² Therefore, in “normal” (not-atopic, not-allergic, helminth-free) children, total IgE levels coincide with “normal” IgE levels, which grow only very little and very slowly during the first decade of life.^{8,12} In contrast, the trajectory of t-IgE levels increases from the “normal” trajectory when an allergen-specific IgE response initiates.¹²

The above reported study suggested that serial and quantitative determination of total IgE levels may provide more useful information than one determination at a single time point. In other words, the analysis of *variations* in total IgE levels may be more informative than the determination of their absolute levels.¹² However, “normal” IgE levels range widely both in children and in adults (<2 to >200 kU/L)⁶⁻¹⁰; hence, a careful analysis of the trajectories of “normal” IgE levels in not-allergic, not-atopic children is necessary before a clinical study is addressed.

The MAS birth cohort scheduled several peripheral blood drawings up to age 20y, providing us with a unique opportunity to investigate serum IgE responses in childhood and adolescence.¹²⁻¹⁵ We aimed this new study on the MAS cohort to model the growth curves of “normal” t-IgE levels in children never developing atopic sensitization from birth to age 20y (*i.e.*, “never atopic”). We also evaluated whether the onset of atopic sensitization can be predicted when a child’s t-IgE levels are significantly higher than those predicted by his/her own t-IgE growth curve.

Methods

Study design and population

The MAS, a prospective observational birth cohort, recruited a selection of 1314 out of 7609 infants born in 1990 on six delivery wards in five German cities (Berlin, Dusseldorf, Mainz, Freiburg, and Munich). A detailed description of the stratified sampling scheme and participants is given elsewhere.¹⁶ The study was approved by the local ethics committees. Each parent gave a written informed consent at the time of enrolment. In this analysis, we included subjects with at least one serum sample examined for total IgE (t-IgE).

IgE assays

Blood samples were collected at 1, 2, 3, 5, 6, 7, 10, 13, 20 years of age. t-IgE was examined with a fluorescent enzyme immunoassay (ImmunoCAP FEIA, Phadia, Freiburg, Germany) and expressed in kU/l. The detection range of t-IgE was 2–2000 kU/l. Sera, with a concentration approaching or passing the detection limit (>1600 kU/l), were diluted 1:10 to obtain a precise determination, while sera with undetectable t-IgE were assigned to an arbitrary value of 1 kU/l. A full set of IgE testing against five airborne allergens (house dust mite, cat and dog dander, mixed grass and birch pollen) and four food allergens (cow's milk, hen's egg, wheat and soy) was performed with ImmunoCAP FEIA (Phadia). Results were expressed in kU_A/l (detection range 0.35–100 kU_A/l). A participant was defined as “never atopic” if s-IgE level was <0.35 kU_A/l for all available sera and for all allergenic extracts tested. By contrast, a participant was defined as atopic if, for at least one allergenic extract tested, s-IgE level was $\geq 0.35 kU_A/l$ (or $\geq 1.00 kU_A/l$ in some analyses, see results) for at least one available serum. Sera of “never atopic” subjects suspected of “hidden” IgE antibodies (due to high variation on t-IgE levels) were tested with a microarray assay (Immuno Solid-phase Allergen Chip [ISAC-112™; ThermoFisher Scientific, Vienna, Austria] to determine whether they contained IgE to allergen molecules not included in the “routine tests”.

Statistical analysis

Characteristics of study population - The frequency of general characteristics between participants included and excluded from the present analysis and between “never atopic” and atopic subjects were compared.

Trajectories of absolute total IgE levels and their growth rate in “never atopic” subjects - A natural logarithmic (log) transformation was applied to t-IgE levels to reduce the asymmetry of data. For each subject, the levels and the variations of t-IgE between two consecutive follow-ups, i.e.

$Growth\ rate\ (tIgE)_t = \frac{\log(tIgE)_t - \log(tIgE)_{t'}}{t - t'}$ where t and t' denoted the years of age, $t = 2, 3, 5, 6, 7, 10, 13, 20$ and $t' = 1, 2, 3, 5, 6, 7, 10, 13$, were considered to provide a deep characterization of the behavior of t-IgE over time. A quantile regression model for longitudinal data¹⁷ was adopted to estimate the quantile trajectories of absolute “normal” t-IgE levels and the growth rate by age considering 13 quantile levels: $\tau =$

{0.03, 0.10, 0.20, 0.25, 0.30, 0.40, 0.50, 0.60, 0.70, 0.75, 0.80, 0.90, 0.97}. The model proposed to estimate the curves for each quantile and each patient, including both linear and quadratic effects for age on t-IgE levels, whereas the growth rates of t-IgE levels could be described only by using a linear age effect.

Cut-off points of t-IgE levels. – The key idea of this study is that an upward deviation of t-IgE from the growth trajectory can be an index of atopic disease. The t-test, when applicable, (or Mann Whitney U test) was applied to determine whether the t-IgE growth rate between two consecutive follow-ups in “never atopics” could have been generated by a process with a mean (or median) equal to zero. The first three years of life show a high variability of t-IgE levels in comparison to a stabilization of t-IgE production from 5 years of age. For this reason, to classify a single individual as atopic or non-atopic at different time points in school age and adolescence t^* , $t^* = \{6, 7, 10, 13, 20\}$; first of all, a “reference” quantile specific for each participant was identified on the basis of the t-IgE value observed at 5 years of age in absence of atopic sensitization. Thus, we defined the “reference” or “best” quantile for each i -th subject as the minimum of the absolute value of the deviation between the observed value at 5 years of age, y_{5i} , and the predicted value of t-IgE, $\hat{y}_{5i}(\tau)$, for the quantile τ at 5 years of age:

$$\tau_i^{best}: \underset{\tau \in \{1, \dots, 13\}}{\operatorname{argmin}} |y_{5i} - \hat{y}_{5i}(\tau)|$$

In other words, τ_i^{best} is the quantile that minimizes the distance between the observed t-IgE level at 5 years of age and the corresponding predicted levels at that quantile.¹⁸ We can suppose that in absence of an atopic response, the deviation from the reference quantile τ_i^{best} is smaller than in the case of atopic sensitization. For this reason, once τ_i^{best} is fixed for every subject, we computed for each following year, the difference between the observed t-IgE level at t^* years of age, denoted by y_{t^*i} , and the predicted t-IgE value associated to τ_i^{best} at t^* years of age, $\hat{y}_{t^*i}(\tau_i^{best})$, in the following way:

$$d_{t^*i} = y_{t^*i} - \hat{y}_{t^*i}(\tau_i^{best})$$

for $t^* = \{6, 7, 10, 13, 20\}$ and $i = \{1, \dots, n\}$ [Figure E1].

ROC analysis - Since the proposed aim was a binary supervised classification problem (atopic vs non-atopic) and d_{t^*} is a continuous variable, ROC (Receiver Operating Characteristic) curves were constructed with the aim of identifying the best threshold $d_{t^*}^*$ to classify an individual as atopic: when $d_{t^*} > d_{t^*}^*$, and non-atopic if otherwise. The area under the curve (AUC) was used as an effective measure of the diagnostic test accuracy. For each analysis, we examined results choosing the best threshold with three different statistical criteria:

- Sensitivity (Se), Specificity (Sp) equality criterion: it identifies the cut-off point that minimizes the difference between $Sp(d_{t^*})$ and $Se(d_{t^*})$ values;
- Youden Index (I_Y) criterion: this index maximizes the difference between true positive and false positive rates and can be defined as $I_Y = \max_{d_{t^*}} \{Se(d_{t^*}) + Sp(d_{t^*}) - 1\}$ and ranges between 0 and 1;
- Closer top left criterion: it minimizes the distance from the “perfect” point at the upper-left corner of the ROC plot where both sensitivity and specificity are equal to 100%.

Missing values – Missing values were not considered for statistical analyses. A p-value <0.05 was considered statistically significant. Statistical analyses were performed using the R statistical software, R Core Team (2014), version 3.2.3.

Results

Study population - From 1314 subjects recruited in the MAS cohort, 1113 met the inclusion criteria [Figure 1]. The average number of follow-ups with t-IgE measures per subject was 5.21 ± 0.14 (95 % CI). Overall 85/1113 (7.6%) subjects had sera collected at all nine follow-ups. A male gender, a parental history of allergy, German nationality, a higher parental education, longer breast-feeding and a mother non-smoking status during pregnancy were more common in the group of included participants [Table 1]. Within the study population, 469 “never atopic” subjects (466 excluding the outliers, subjects with $t\text{-IgE} \leq 0.01$ kU/l for all available sera) and 644 atopic subjects (≥ 1 serum with $s\text{-IgE} \geq 0.35$ kU_A/l) were identified [Figure 1]. Among general characteristics examined for the study population, “never atopic” subjects had a lower frequency of males, a lower percentage of elevated cord blood IgE and shorter breast-feeding than atopic participants [Table 1]. Furthermore, within the group of “never atopic” children in the MAS cohort, males showed higher levels of t-IgE than females [Table E1]. Mann Whitney U tests assessed that the median differences were not statistically significant in correspondence of all ages [Table E1]; therefore, the “gender” variable was not included in the model as a risk factor.

Quantile trajectories of absolute total IgE levels and their growth rate in “never atopic” subjects – Quantile trajectories of predicted absolute t-IgE levels increased until 10 years of age, plateaued between 10 and 13 years of age, and slightly decreased afterwards [Figure 2a]. The growth of t-IgE levels in the first decade of life was stronger between the 3rd ($\tau = 0.03$) and the 75th percentile ($\tau = 0.75$), as confirmed by the coefficient values of the linear effect of age [Table E2], in comparison to more extreme percentiles ($\tau = 0.80, 0.90, 0.97$). The estimated value of the natural logarithm of t-IgE at 1 year for the 3rd percentile is 0.45 kU/l (log-units). Therefore, 3% of “never atopic” children had a value of t-IgE lower than 1.53 kU/l, while the estimated value of the natural logarithm of t-IgE at 1 year for the 90th percentile is 3.00 kU/l (log-units). In over 90% of the “never atopic” participants, t-IgE levels never exceeded 20.08 kU/l. Moreover, in 70% of the “never atopic” participants t-IgE levels never exceeded 12.13 kU/l at 1 year of age. The trajectories of t-IgE of individual “never atopic” participants are reported [Figure E2]. Quantile trajectories of predicted t-IgE growth rate showed a tendency toward zero over time [Figure 2b]. As confirmed by the coefficient values of the model [Table E3], the estimated trajectories of t-IgE growth rate decreased over time from the 97th to the 40th percentile, were almost parallel to the x-axis for 30th and 25th percentiles, and increased below the 25th percentile (20th, 10th and 3rd). As shown by the trend of t-IgE level growth-rate trajectories, t-IgE levels tended towards a plateau after 13 years of age. The trajectories of t-IgE growth rate of individual “never atopic” participants are reported [Figure E3].

T-IgE levels of atopic subjects matched against the estimated quantile trajectories – In the early years of life (1-3 years) t-IgE levels were not stable, as confirmed by the value of standard deviation (SD) and interquartile range (IQR) of t-IgE growth rate between 2 and 1 year and between 3 and 2

years [Table E4]. After 5 years of age, differences in t-IgE between two consecutive follow-ups tended to be close to 0; between 5 and 6 years of age, 50% of “never atopic” subjects have a variation of t-IgE next to zero and the same in the following years [Table E4]. For this reason, for further analysis, all subjects were considered from 5 year of age and up. Within “never atopic”, 202 subjects had t-IgE serum tested at 5 years of age [Figure 1]. Among atopic subjects, a sub-sample of 505 participants was selected considering a cut-off of sensitization $\geq 1.00 \text{ kU}_A/\text{l}$ and subsequently, only non-atopic subjects at 5 years of age were considered ($n=131$) [Figure 1]. The average level of t-IgE of participants with a constant condition of sensitization to s-IgE (kU_A/l) after their onset were matched against the estimated quantile trajectories, grouping them according to their first age of sensitization [Figure 3]. The average curves showed an increase of t-IgE in correspondence of the year of onset and a constant higher level in the following year.

ROC analysis to identify cut-off points of t-IgE levels – ROC analysis results showed a performance similar to diagnostic tests for each year with all AUC values above 80% [Table 2]. Sensitivity and specificity changed according to statistical methodology used to define the best cut-off for discriminating atopic from non-atopic subjects. To obtain comparable values of sensitivity and specificity for each year, only solutions with the best cut-off, chosen by sensitivity equal to specificity criterion, were analysed [Figure 4]. Deviations between the observed t-IgE level and the predicted t-IgE level, identified by the reference quantile for each subject (see statistical methods for definition of d_t^*), above 0.32, 0.41, 0.42, 0.30 and 0.58 kU/l (log-units) predicted atopy at 6, 7, 10, 13 and 20 years of age respectively [Table 2, Figure 4]. The deviations from the predicted t-IgE level of the reference quantile in atopic subjects (d_t^* , see above method for definition) were statistically higher than non-atopic participants for each year ($p<0.001$) [Figure 4], but the two distributions were not completely divided by the cut-off line, and for this reason a few subjects were not well-identified by this procedure. Actually, most trajectories of t-IgE level confirmed that t-IgE levels of “never atopic” subjects tend to stabilize around the t-IgE trajectories of the reference quantile [Figure E2], while t-IgE levels of atopic subjects tend to increase in correspondence to sensitization episodes [Figure E4]. Nevertheless, the t-IgE trajectory of some “never atopic” subjects followed a different pattern, showing a considerable deviation from the reference quantile. Indeed, based on the estimated optimal cut-off, at several ages the subjects are classified as atopic although they don’t show an atopic response for the “common” allergens [Figure E5]. To validate the presence of atopy response for these subjects, an allergen microarray including testing for 112 allergen molecules was used to detect “hidden” atopic s-IgE in 6/14 of their sera [Table 3].

Discussion

Major findings - We modelled quantile growth curves of “normal” t-IgE levels by applying quantile regression to the data from 466 German “never atopic” children, i.e. children who never had s-IgE responses to a panel of nine locally common airborne and foodborne allergen extracts. We then used these quantile curves to compare observed t-IgE values of all children against their own “reference” t-IgE. We also used ROC analysis to identify empirical cut-off points that – in this specific population – best discriminate sera with from sera without s-IgE sensitization to common allergens. Lastly, we found specific IgE responses to “uncommon” allergens in 6/14 of the children with an upward deviation of t-IgE not justified by s-IgE to the nine “common” allergen extracts.

Growth curves of “normal” serum total IgE levels – Among the “never atopic” participants of the MAS cohort, we found serum t-IgE levels increased progressively in the first decade of life, plateaued at age 10-13y and slightly declined afterwards. An early study in a Swedish population showed that serum IgE concentration reaches adult levels already between 5 and 7 years of age.⁴ In addition, the levels of serum t-IgE are very unstable – even in “never atopic” children - during the first three years of life.^{11,19} Accordingly, the kinetics of post-natal development of cytokine functions and the maturation of the T-cell responses in normal children is heterogeneous and is not complete until 3-4 years after birth.^{20,21} Therefore, we need to wait until 5 years of age so that t-IgE levels become stable enough, so that for each child who is non-atopic at 5y of age, we can assign a specific quantile of the predicted “normal” growth curves, estimated by quantile regression. Therefore, non-atopic children with very low or very high levels of t-IgE at 5 years of age will continue, until adulthood, to keep their serum t-IgE at very low or very high levels, respectively. In other words, by measuring serum t-IgE at 5 years of age in a non-atopic child, we can predict his/her “normal” serum t-IgE levels until age 20y. Interestingly, the frequency distribution of IgE concentrations in non-atopic children at 5 years of age was extremely broad, with the 3rd and 97th percentile at 2.17 kU/l and 223.82 kU/l, respectively. This broad range confirms previous observations in other populations of non-atopic children examined with a cross-sectional approach.^{4,22}

Disclosing s-IgE responses by variations in t-IgE quantiles – We found that the trajectory of serum t-IgE levels of children starting a persistent atopic sensitization at a given age, undergoes at that precise age an abnormal upward deviation from its own quantile. Accordingly, at the individual level, any upward significant deviation from the own “reference” quantile implies a new IgE response that, in westernized and helminth-free children, is most frequently linked to a new atopic sensitization. The ROC analysis defined cut-off points (i.e. increase of percentile) useful to predict with AUC (>80%) where an upward variation implies the onset of an allergen-specific IgE response. Interestingly, these cut-off points were useful to identify the new onset of “hidden” IgE responses, *i.e.* those IgE responses directed against allergens not contained in the nine locally relevant common foodborne and airborne allergen extracts. (This procedure has not been implemented to replace the classic method to define atopic sensitization through specific IgE but can be used under special conditions.) In other words, our data suggest that a significant upward deviation of serum t-IgE may be useful to suspect an allergic probability, even when IgE screening or routine tests are negative.

Limitations - We should acknowledge a number of potential limitations. First, our “normal” growth curves computed for the included participants do not represent the whole general German population. Second, helminths and other parasitic infections strongly affect t-IgE levels, so the conclusions reached in our study should be limited to helminth-free children/populations. Third, t-IgE levels were measured over almost two decades, so that assay reproducibility over such a long time might be questioned. However, we used always the same test, whose analytical precision and batch-to-batch reproducibility is controlled by the manufacturer and by standardization against the standard from WHO/IUIS. Finally, we acknowledge that all participants were not followed at all given time points; however the number of available sera for each year of age was enough for the present study.

Conclusions - In conclusion, we estimated growth curve trajectories of serum t-IgE levels in children never developing specific IgE against common allergens from birth until age 20y. On this basis, we elaborated a novel and simple chart of “normal” values that may be useful to monitor t-IgE levels and identify the onset of IgE sensitization, even when routine tests for s-IgE antibodies to common allergens are negative. Indeed, for each individual, who is non-atopic at 5 years of age, we identified an own “reference” quantile. Upward deviations of observed t-IgE levels from it, from 6ys to 20ys of age, predict at each year the occurrence of atopic sensitization. The practical usefulness of this novel interpretation of t-IgE assays deserves to be tested in a clinical context.

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Legend to figures

Figure 1 - Flow diagram showing the selection of the population subsets used for analysis.

Figure 2 - Quantile trajectories of predicted log-transformed t-IgE levels (kU/l) by age estimated in the population of “never atopic” subjects (n=466) after the evaluation of quantile regression model for longitudinal data applied considering 13 quantiles (0.03, 0.10, 0.20, 0.25, 0.30, 0.40, 0.50, 0.60, 0.70, 0.75, 0.80, 0.90, 0.97). Quantile trajectories of absolute t-IgE levels (log (kU/l)) and quantile trajectories of the t-IgE growth rate levels (log (kU/l)) between two consecutive follow-ups are shown in panel **a)** and **b)** respectively, where the values -2, 0, 1, 2, 3, 4, 5, 6 of t-IgE levels in log-units (kU/l) correspond to 0.14, 1.00, 2.72, 7.39, 20.09, 54.60, 148.41, 403.43 kU/l.

Figure 3 - Quantile trajectories of log-transformed t-IgE levels (kU/l) by age estimated in the population of “never atopic” participants (n=466) matched against the average t-IgE levels (log (kU/l)) of non-atopic at 5 years of age subjects that became atopic at: **a)** 6 years of age (n=9), **b)** 10 years of age (n=25) and **c)** 13 years of age (n=21). Only participants with a constant condition of sensitization to s-IgE (kU_A/l) after their onset were considered for this analysis. The t-IgE levels values 0, 2, 4, 6 in log-units (kU/l) are equivalent to 1.00, 7.39, 54.60 and 403.4 kU/l, respectively.

Figure 4 – a-e) ROC analysis performances by age. ROC curves show the cut-offs to discriminate atopic vs non-atopic sera chosen by sensitivity equals to specificity criterion. **f)** Mean differences of d_{t^*} expressed in log (kU/l) for each year (see method for definition) between non-atopic and atopic subjects are represented by boxplots. The t-IgE levels values -6, -4, -3, -2, -1, 0, 1, 2, 3, 4, in log-units (kU/l) are equivalent to 0.00, 0.02, 0.05, 0.14, 0.37, 1.00, 2.72, 7.39, 20.09 and 54.60 kU/l, respectively. *Mann Whitney U test was used to compare not normally distributed variables between groups (Shapiro-Wilk test was used to assess normality of data).

Table 1**Table 1** - Characteristics of the study populations.

	Study population		Excluded		<i>Never atopics</i>		<i>Atopics</i>		p-value *
	n=1113 ¹		n=201 ¹		n=469 ¹		n=644 ¹		
	n	%	n	%	n	%	n	%	
Male gender	587	52.7	97	48.3	227	48.4	360	55.9	0.016
Elevated cord blood IgE (>0.9 kU/l)	205	19.2	36	18.6	69	15.2	136	22.2	0.005
Parental history of allergy	588	53.1	92	46.9	209	44.8	379	59.1	0.129
German nationality	1028	94.8	171	89.5	424	93.6	604	95.7	0.156
Older siblings	460	41.3	80	39.8	201	42.9	259	40.2	0.743
Parental education (> 12 y)	583	53.8	189	94.0	239	52.4	344	54.9	0.461
Breast-feeding (≥ 1 month)	805	73.2	88	45.6	315	68.3	490	76.8	0.002
Mother smoking in pregnancy	271	24.4	73	36.9	119	25.4	152	23.7	0.549

Data were summarized as numbers (n) and frequencies (%).

*Chi squared test was used to evaluate the association of categorical data between never atopic and atopic subjects .

¹Sporadic missing values for each of the variable examined.

Table 2

Table 2 - ROC analysis performances by age evaluated to identify a best threshold of d_t^* to classify an individual as atopic and non-atopic with three different statistical criterions.

	6 years₁			7 years₁			10 years₁			13 years₁			20 years₁		
Methodology	Se=Sp †	Youden‡	Closest top left•	Se=Sp †	Youden‡	Closest top left•	Se=Sp †	Youden‡	Closest top left•	Se=Sp †	Youden‡	Closest top left•	Se=Sp †	Youden‡	Closest top left•
Cut-off	0.32	0.16	0.16	0.41	0.26	0.33	0.42	0.24	0.24	0.30	0.05	0.11	0.58	0.31	0.31
Sensitivity	0.69	0.92	0.92	0.72	0.90	0.86	0.73	0.81	0.81	0.71	0.85	0.81	0.71	0.80	0.80
Specificity	0.70	0.66	0.66	0.76	0.70	0.73	0.76	0.71	0.71	0.73	0.65	0.69	0.72	0.68	0.68
Accuracy	0.70	0.68	0.68	0.75	0.72	0.74	0.75	0.74	0.74	0.72	0.71	0.72	0.71	0.73	0.73
PPV	0.16	0.18	0.18	0.30	0.30	0.31	0.51	0.49	0.49	0.51	0.50	0.51	0.62	0.63	0.63
NPV	0.97	0.99	0.99	0.95	0.98	0.97	0.89	0.92	0.92	0.86	0.92	0.90	0.79	0.84	0.84
AUC	82.21%			81.94%			83.27%			81.01%			80.50%		

AUC: area under the ROC curve. Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV) and optimal cut-off point of d_t^* chosen to discriminate atopic vs non-atopic subjects are represented by age and according the statistical method used to choose the cut-off.

† Sensibility equals to specificity criterion (see methods for definition).

‡ Youden Index criterion (see methods for definition).

• Closer top left criterion (see methods for definition).

₁Atopy sample sizes by age: n=176 (6 ys); n=230 (7 ys); n=204 (10 ys); n=218 (13 ys); n=189 (20 ys).

Table 3

Table 3 - "Hidden" sIgE in sera of 8 "never atopic" children with "abnormal" upward deviation of tIgE from the predicted level of the reference quantile (τ^{best})

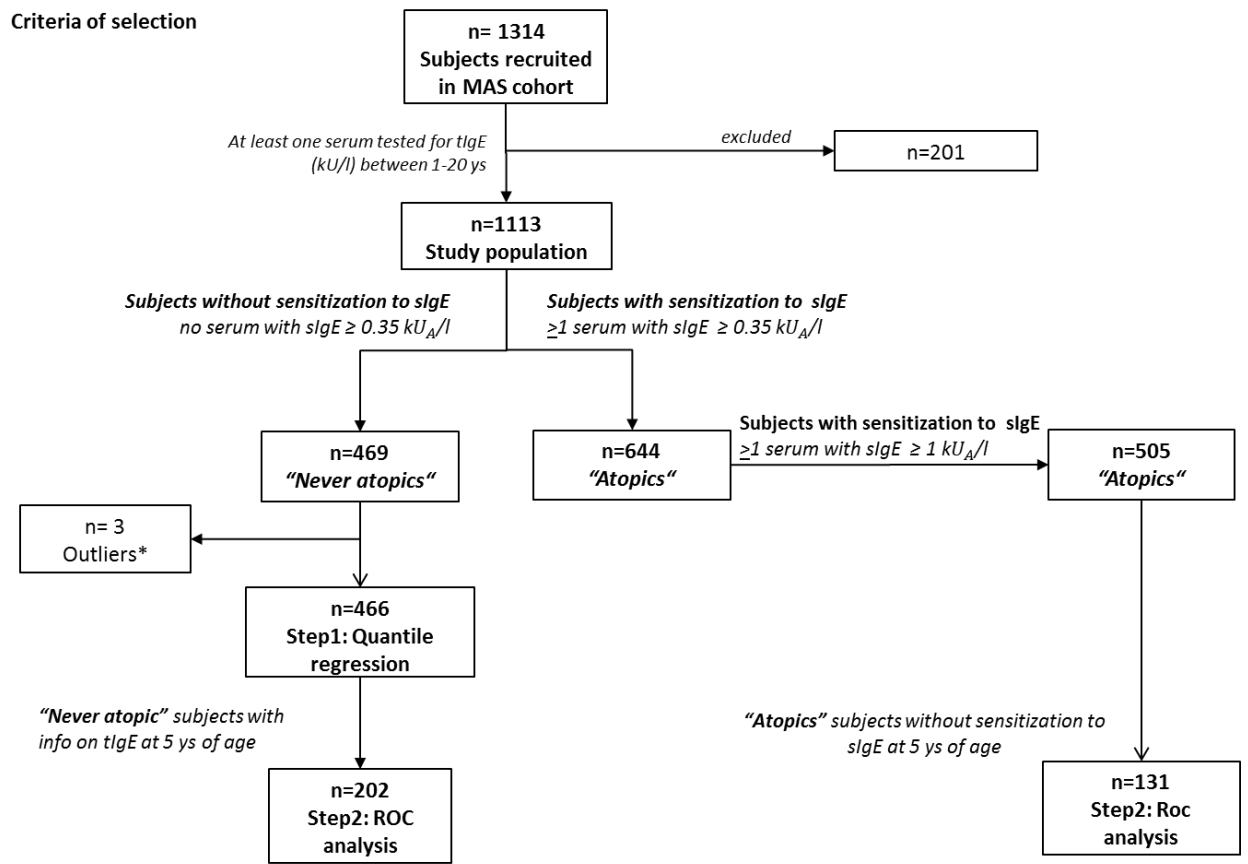
Case	age (t*)	Observed tIgE level (kU/l)		Percentile tIgE (τ) (%)			Specific IgE sensitization	
		<i>tIgE_s</i>	<i>tIgE_t</i> *	τ^{best} (%)	τ^{t*} (%)	$[\tau^{t*} - \tau^{best}]$ (%)	extracts°	molecules^‡
#1	7	11.6	33.9	40	60	20	neg	
#1	10	11.6	74.5	40	80	40	neg	
#1	13	11.6	138.0	40	90	50	neg	
#2	10	18.5	373.0	60	97	37	neg	
#2	13	18.5	863.0	60	97	37	neg	Cyn d 1, Jug r 2, Phl p 4, Pla a 2,
#3	20	38.0	85.8	75	90	15	neg	
#4	10	20.4	270.0	60	97	37	neg	
#5	13	6.8	122.0	25	90	65	neg	
#5	20	6.8	38.9	25	75	50	neg	Hev b 6,
#6	10	24.7	97.3	60	90	30	neg	Ara h 1, Hev b 1, Hev b 6,
#6	13	24.7	141.0	60	90	30	neg	
#7	10	19.0	111.0	60	90	30	neg	Phl p 1, Phl p 4
#7	20	19.0	29.2	60	70	10	neg	Aln g 1, Cyn d 1, Mal d 1, Pen m 2, Phl p 4
#8	13	23.1	94.5	60	90	30	neg	Phl p 4

° sIgE <0.35 kU/l to the extracts of *Dermatophagoides pteronyssinus*, cat, dog, grass and birch pollens, milk, egg, soy, wheat.

^ the following 112 molecules have been tested: Act d 1, Act d 2, Act d 5, Act d 8, Aln g 1, Alt a 1, Alt a 6, Amb a 1, Ana o 2, Ani s 1, Ani s 3, Api g 1, Api m 1, Api m 4, Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 8, Ara h 9, Art v 1, Art v 3, Asp f 1, Asp f 3, Asp f 6, Ber e 1, Bet v 1, Bet v 2, Bet v 4, Bla g 1, Bla g 2, Bla g 5, Bla g 7, Blo t 5, Bos d 4, Bos d 5, Bos d 6, Bos d 8, Bos d Lactoferrin, Can f 1, Can f 2, Can f 3, Can f 5, Che a 1, Cla h 8, Cor a 1.0101, Cor a 1.0401, Cor a 8, Cor a 9, Cry j 1, Cup a 1, Cyn d 1, Der f 1, Der f 2, Der p 1, Der p 10, Der p 2, Equ c 1, Equ c 3, Fag e 2, Fel d 1, Fel d 2, Fel d 4, Gad c 1, Gal d 1, Gal d 2, Gal d 3, Gal d 5, Gly m 4, Gly m 5, Gly m 6, Hev b 1, Hev b 3, Hev b 5, Hev b 6.01, Hev b 8, Jug r 1, Jug r 2, Jug r 3, Lep d 2, Mal d 1, Mer a 1, Mus m 1, MUXF3, Ole e 1, Ole e 7, Ole e 9, Par j 2, Pen m 1, Pen m 2, Pen m 4, Phl p 1, Phl p 2, Phl p 4, Phl p 5, Phl p 6, Phl p 7, Phl p 11, Phl p 12, Pla a 1, Pla a 2, Pla a 3, Pla l 1, Pol d 5, Pru p 1, Pru p 3, Sal k 1, Ses i 1, Tri a 14, Tri a 19.0101, Tri a aATl, Ves v 5.

‡ normal font-style: sIgE ≥ 0.3 ISU, *italic font-style* sIgE >0.1 ISU, <0.3 ISU, (ISAC-112)

Figure 1



* Subjects with all available sera with tlgE ≤ 0.01 kU/l

Figure 2

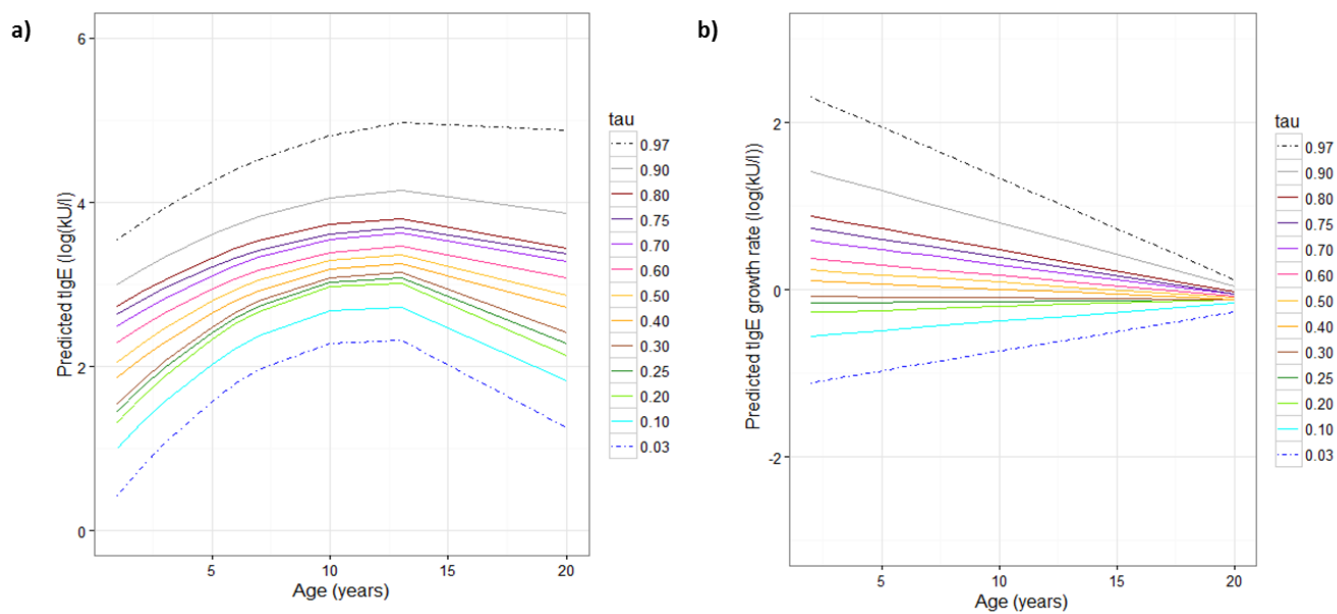


Figure 3

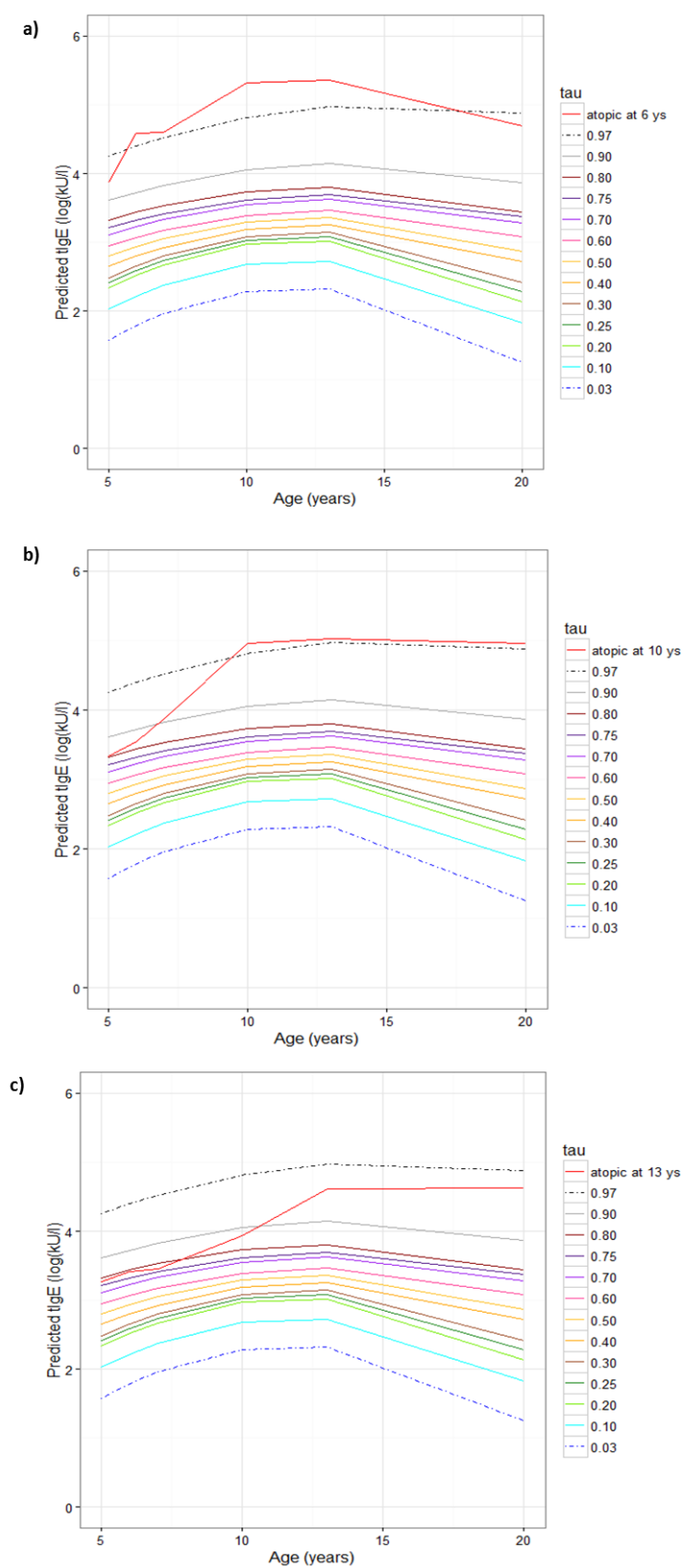
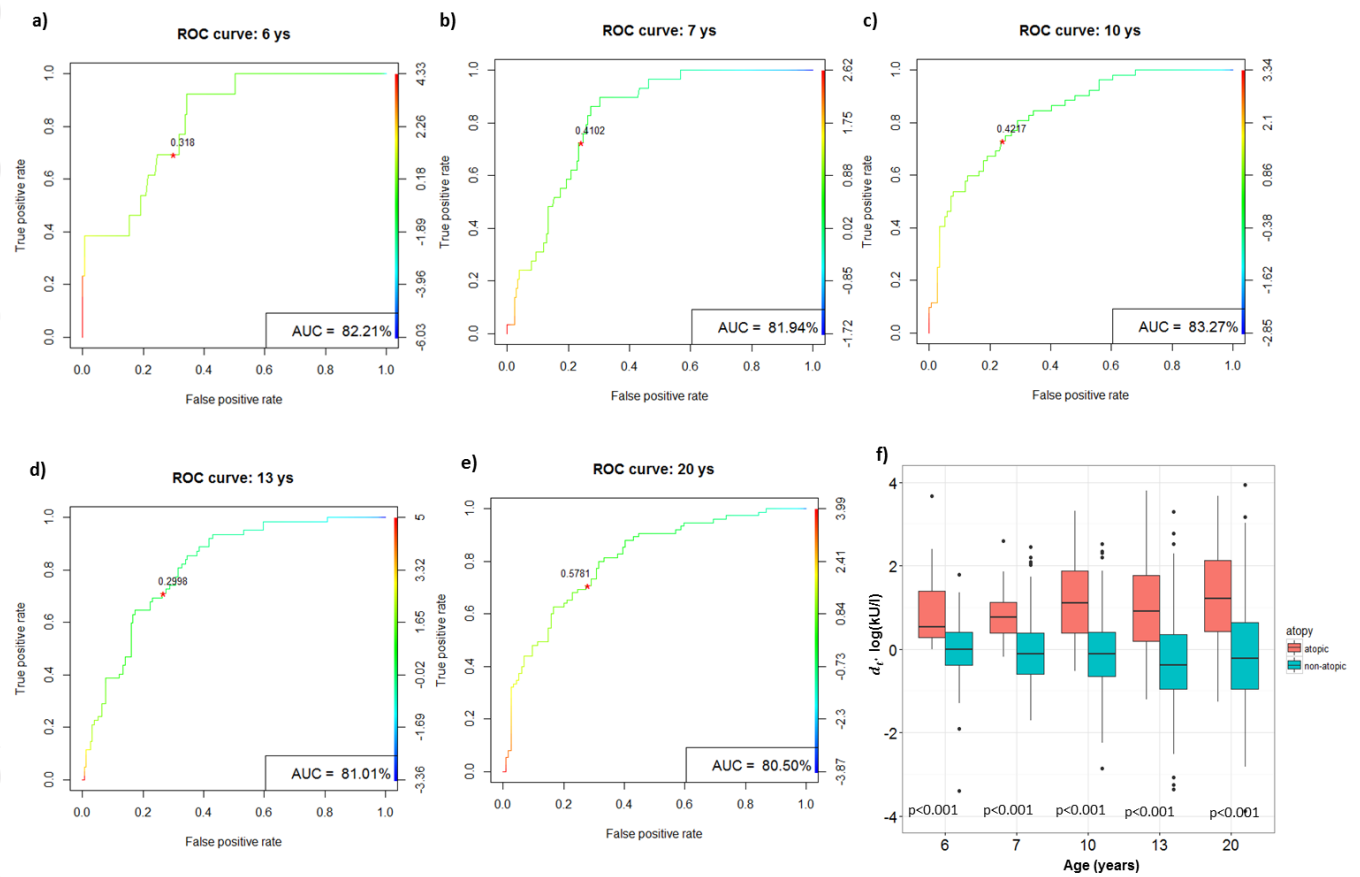


Figure 4



ELECTRONIC REPOSITORY

Growth curves of “normal” serum total IgE throughout childhood: a quantile analysis in a birth cohort.

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Tables: 4

Figures: 5

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Legend to figures

Figure E1 - Example of calculation of d_{t^*} values for a specific subject. First step is: to evaluate the reference quantile τ_i^{best} , the quantile that minimizes the distances between the observed t-IgE level at 5 year of age, y_{5i} , and the predicted values from the quantile regressions at 5 years of age, $\hat{y}_{5i}(\tau)$, (with $\tau = \{0.03, 0.10, 0.20, 0.25, 0.30, 0.40, 0.50, 0.60, 0.70, 0.75, 0.80, 0.90, 0.97\}$). Once that τ_i^{best} is fixed, the second step is: to estimate d_{t^*} for each year, $t^* = \{6, 7, 10, 13, 20\}$. As previously explained d_{t^*} is the difference between the observed t-IgE level (log kU/l) at t^* years of age and the predicted basal value associated to τ_i^{best} at t^* years of age, thus having $d_6 = y_6 - \hat{y}_6(\tau^{best})$, $d_7 = y_7 - \hat{y}_7(\tau^{best})$, $d_{10} = y_{10} - \hat{y}_{10}(\tau^{best})$, $d_{13} = y_{13} - \hat{y}_{13}(\tau^{best})$ and $d_{20} = y_{20} - \hat{y}_{20}(\tau^{best})$. The t-IgE levels values -6, -4, -3, -2, -1, 0, 1, 2, 3, 4, in log-units (kU/l) are equivalent to 0.00, 0.02, 0.05, 0.14, 0.37, 1.00, 2.72, 7.39, 20.09 and 54.60 kU/l, respectively.

Figure E2 - Trajectories of absolute log-transformed t-IgE levels (kU/l) in four “never atopic” children with no missing values, matched against the quantile trajectories of “normal” t-IgE levels (log(kU/l)). The t-IgE levels values 0, 1, 2, 3, 4, 5, 6 in log-units (kU/l) are equivalent to 2.72, 7.39, 20.09, 54.60, 148.41, 403.43 kU/l, respectively.

Figure E3 - Trajectories of log-transformed t-IgE growth rate levels (kU/l) in four “never atopic” children with no missing values, matched against the quantile trajectories of “normal” t-IgE growth rate levels (log(kU/l)) between two consecutive follow-ups. The t-IgE levels values -3, -2, -1, 0, 1, 2, 3 in log-units (kU/l) are equivalent to 0.05, 0.14, 0.37, 1.00, 2.72, 7.39 and 20.09, respectively.

Figure E4 - Trajectories of log-transformed t-IgE levels (kU/l), matched against the quantile curves of “normal” t-IgE levels (kU/l), in three atopic participants with no missing values who were non-atopic at 5 years of age and became sensitized to s-IgE at: **a)** 6 years of age, **b)** 10 years of age, **c)** 13 years of age. The t-IgE levels values 0, 1, 2, 3, 4, 5, 6, 7 in log-units (kU/l) are equivalent to 2.72, 7.39, 20.09, 54.60, 148.41, 403.43, 1096.63 kU/l, respectively. Only participants with a persistent atopic sensitization were considered for this analysis.

Figure E5 - Trajectories of absolute log-transformed t-IgE levels (kU/l) in eight “never atopic” children with no missing values, matched against quantile trajectories of “normal” t-IgE levels (log(kU/l)), where values 0, 1, 2, 3, 4, 5, 6, 7 in log-units (kU/l) of t-IgE levels correspond to 2.72, 7.39, 20.09, 54.60, 148.41, 403.43, 1096.63 kU/l.

Table E1

Table E1 - Risk factor affecting total IgE levels (log (kU/l)) in "*never atopics*" subjects of MAS cohort (n=466).

Age	male (n=224)					female (n=242)					p-value*
	n†	Mean	SD	Median	IQR	n†	Mean	SD	Median	IQR	
1	157	2.0	1.2	1.8	(1.1-2.7)	159	1.5	1.1	1.3	(-0.9-2.2)	0.002
2	116	2.8	1.5	2.7	(1.6-3.8)	128	2.3	1.2	2.3	(-0.4-3.2)	0.023
3	102	2.8	1.4	2.8	(1.7-3.6)	100	2.7	1.2	2.4	(0.7-3.6)	0.706
5	95	3.1	1.2	3.2	(2.2-3.9)	107	3.0	1.1	3.1	(0.7-3.6)	0.513
6	74	3.1	1.2	3.1	(2.2-4.0)	96	2.9	1.2	2.8	(-1.6-3.7)	0.226
7	90	3.3	1.1	3.4	(2.6-4.1)	124	3.0	1.0	3.1	(0.7-3.7)	0.028
10	85	3.5	1.1	3.4	(2.9-4.1)	113	3.2	1.1	3.2	(0.7-3.9)	0.028
13	97	3.1	1.3	3.3	(2.6-3.9)	119	3.2	1.1	3.2	(0.0-3.9)	0.093
20	89	3.1	1.3	3.1	(2.3-3.7)	108	2.9	1.3	3.1	(0.0-3.7)	0.514

† number of participants whose sera were available for tIgE descriptive statistics.

* Mann Whitney U test was applied to compare quantitative variables between independent groups.

Table E2

Table E2 - Parameter estimates of quantile trajectories of log-transformed t-IgE (**Fig 2.a**) after quantile regression model for longitudinal data.

Quantile (τ)	Coefficient	Value [†]	Std. Error	p-value
0.03	age	0.386	0.036	<0.001
	age*age	-0.016	0.002	<0.001
0.10	age	0.343	0.02509	<0.001
	age*age	-0.014	0.00133	<0.001
0.20	age	0.339	0.02019	<0.001
	age*age	-0.014	0.00107	0.003
0.25	age	0.318	0.01904	<0.001
	age*age	-0.013	0.00102	0.001
0.30	age	0.309	0.01886	<0.001
	age*age	-0.013	0.00099	<0.001
0.40	age	0.258	0.0161	<0.001
	age*age	-0.010	0.0008	<0.001
0.50	age	0.241	0.01934	<0.001
	age*age	-0.009	0.0009	<0.001
0.60	age	0.211	0.01981	<0.001
	age*age	-0.008	0.00087	<0.001
0.70	age	0.200	0.02425	<0.001
	age*age	-0.008	0.00108	<0.001
0.75	age	0.185	0.02614	<0.001
	age*age	-0.007	0.00115	<0.001
0.80	age	0.193	0.02699	<0.001
	age*age	-0.007	0.00121	<0.001
0.90	age	0.197	0.03492	<0.001
	age*age	-0.007	0.00163	<0.001
0.97	age	0.217	0.04874	<0.001
	age*age	-0.007	0.00259	0.007

[†]Coefficient values, its standard error and its p-values were estimated by penalized quantile regression model. "age" and "age*age" represent the linear and the quadratic effects of age respectively.

Table E3

Table E3 - Parameter estimates of quantile trajectories of growth rate of log-transformed tlgE (**Fig 2.b**) after quantile regression model for longitudinal data.

Quantile (τ)	Value [†]	Std. Error	p-value
<i>0.03</i>	0.047	0.007	<0.001
<i>0.10</i>	0.022	0.004	<0.001
<i>0.20</i>	0.009	0.004	0.015
<i>0.25</i>	0.002	0.003	0.466
<i>0.30</i>	-0.002	0.003	0.539
<i>0.40</i>	-0.012	0.003	<0.001
<i>0.50</i>	-0.018	0.003	<0.001
<i>0.60</i>	-0.025	0.003	<0.001
<i>0.70</i>	-0.036	0.004	<0.001
<i>0.75</i>	-0.043	0.004	<0.001
<i>0.80</i>	-0.050	0.004	<0.001
<i>0.90</i>	-0.076	0.006	<0.001
<i>0.97</i>	-0.121	0.007	<0.001

[†] Coefficient value of linear effect of age, its standard error and its p-value, for each quantile (τ), were estimated by penalized quantile regression model.

Table E4

Table E4 -tIgE (log (kU/l)) growth rate levels between two consecutive follow-ups in "never atopic" subjects (n=466).

Growth rate (tIgE)	n†	Mean	SD	Median	IQR	p-value*
r _{2,1}	182	0.80	1.14	0.72	(0.08-1.52)	<0.001
r _{3,2}	153	0.19	1.16	0.14	(-0.38-0.72)	0.047
r _{5,3}	113	0.18	0.49	0.21	(-0.12-0.42)	<0.001
r _{6,5}	110	0.03	0.62	0.04	(-0.29-0.36)	0.612
r _{7,6}	136	0.08	0.69	0.07	(-0.32-0.40)	0.218
r _{10,7}	145	0.08	0.25	0.06	(-0.07-0.22)	<0.001
r _{13,10}	158	-0.03	0.25	-0.04	(-0.17-0.120)	0.053
r _{20,13}	136	-0.03	0.18	-0.03	(-0.13-0.06)	0.103

† Number of available pairs of consecutive follow-ups indicated by the index subscripts available to calculate descriptive statistics.

*One sample T-test, when condition were respected, or Mann Whitney U test was applied to to determine whether the t-IgE growth rate, for each pair of years, could have been generated by a process with a mean equals to zero.

Figure E1

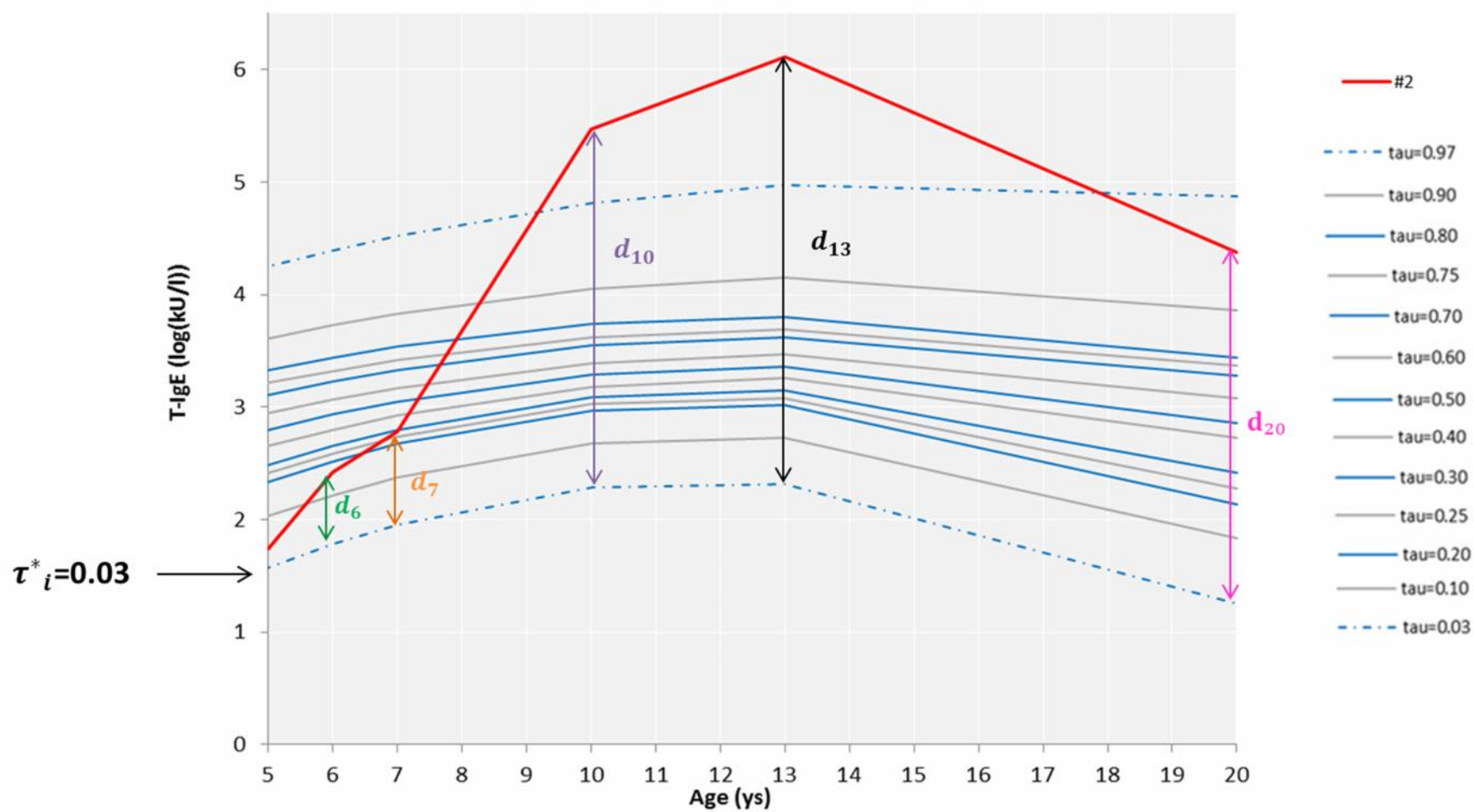


Figure E2

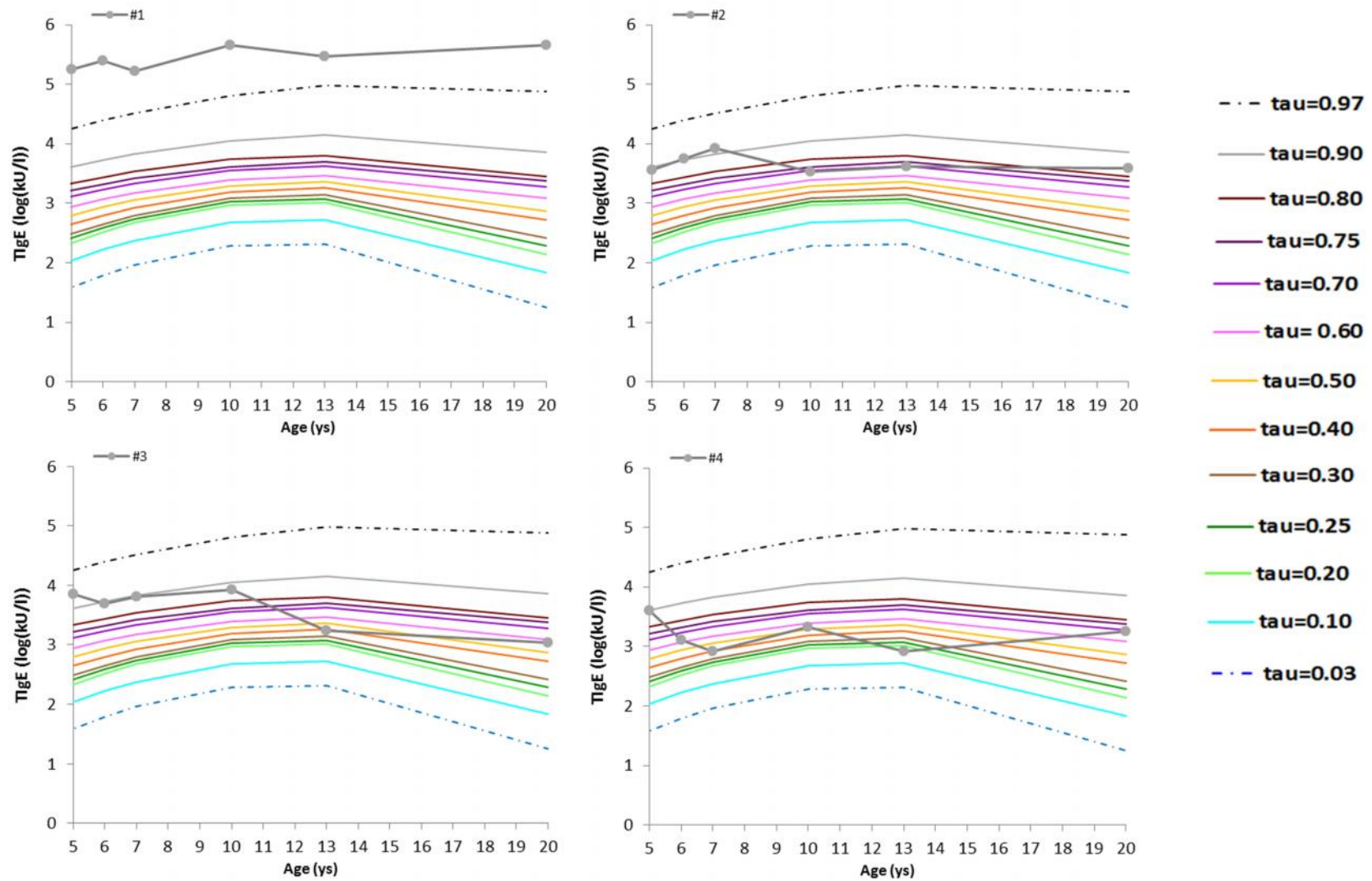


Figure E3

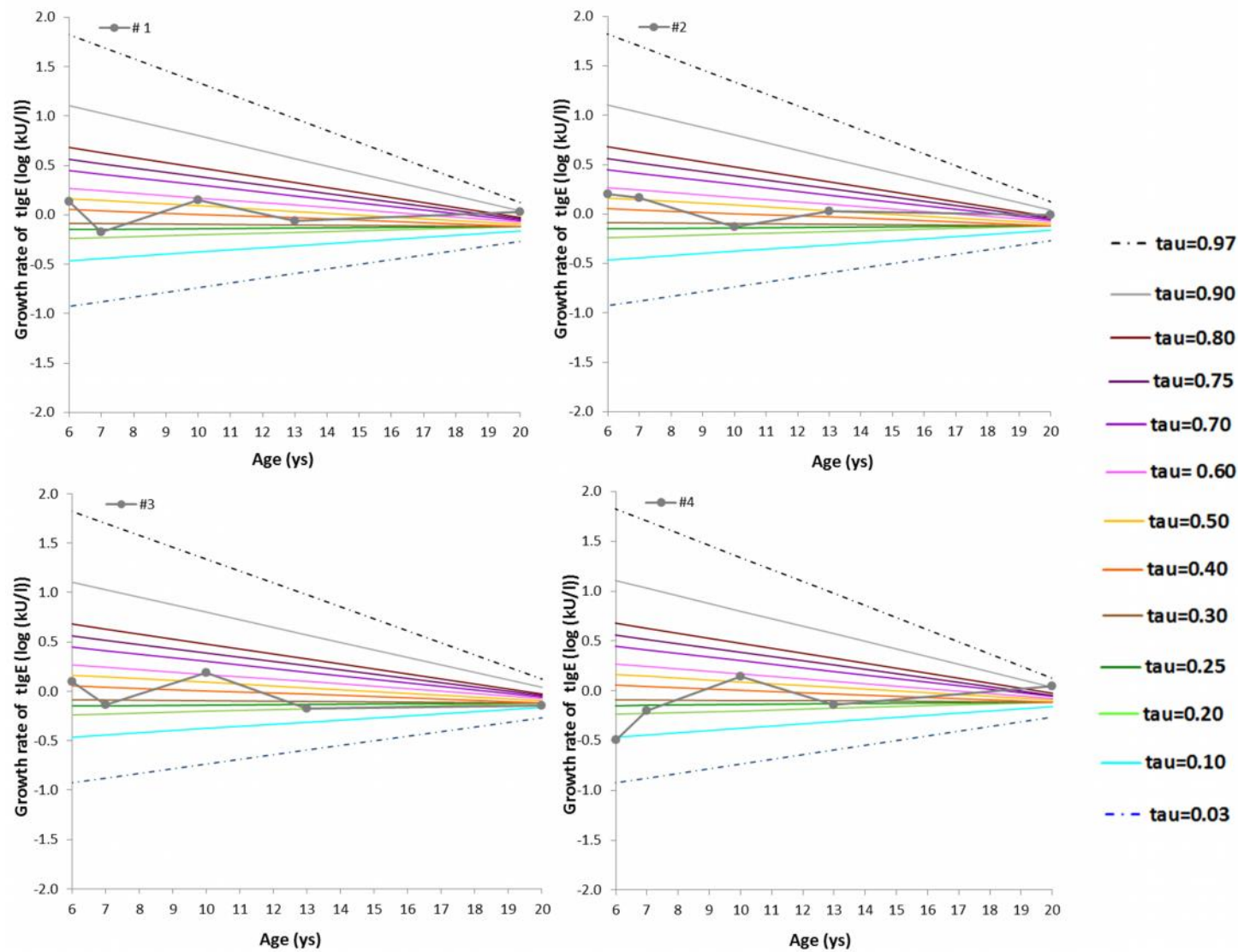


Figure E4

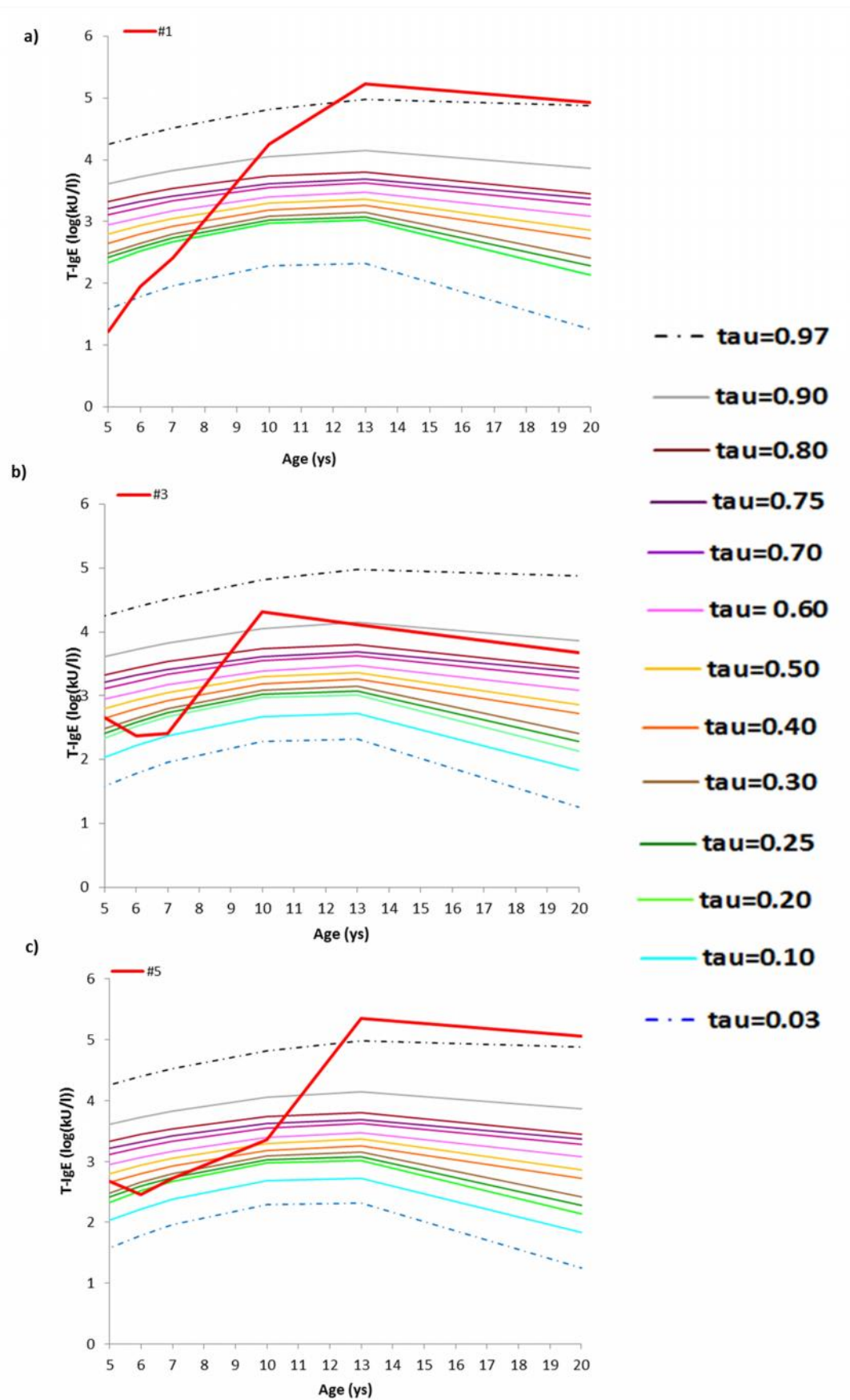


Figure E5

