Early appearance of thyroid autoimmunity in children followed from birth for type 1 diabetes risk

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Disclosure summary: The authors have nothing to disclose

Keywords: Thyroid autoimmunity, children, autoimmune thyroid disease

Abstract

Purpose – Autoantibodies to thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) define pre-clinical autoimmune thyroid disease (AITD) which can progress to either clinical hypo- or hyperthyroidism. We determined the age at seroconversion in children genetically at risk for type 1 diabetes.

Methods – TPOAb and TgAb seropositivity were determined in 5066 healthy children with HLA DR3 or DR4 containing haplogenotypes from The Environmental Determinants of Diabetes in the Young (TEDDY) Study. Children seropositive on the cross-sectional initial screen at 8-13 years of age had longitudinally collected samples (from 3.5 months of age) screened retrospectively and prospectively for thyroid autoantibodies to identify the age at seroconversion. First-appearing autoantibody was related to sex, HLA genotype, family history of AITD, and subsequent thyroid dysfunction and disease.

Results – The youngest appearance of TPOAb and TgAb was 10 and 15 months of age, respectively. Girls had higher incidence rates of both autoantibodies. Family history of AITD was associated with a higher risk of TPOAb hazard ratio [HR] 1.90, 95% confidence interval [CI] 1.17, 3.08; and TgAb HR 2.55, 95% CI 1.91, 3.41. The risk of progressing to hypo- or hyperthyroidism was not different between TgAb and TPOAb, but children with both autoantibodies appearing at the same visit had a higher risk compared to TPOAb appearing first (HR 6.34, 95% CI 2.72, 14.76).

Main conclusion – Thyroid autoantibodies may appear during the first years of life, especially in girls, and in children with a family history of AITD. Simultaneous appearance of both autoantibodies increases the risk for hypo- or hyperthyroidism.

Background

Autoantibodies to thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) define pre-clinical autoimmune thyroid disease (AITD), a common endocrine disease characterized by T and B lymphocyte infiltration of the thyroid gland. Over time, many patients with AITD progress to develop hypo- or hyperthyroidism, which we define as clinical thyroid disease (1, 2). Either autoantibody may be present independently of the other, not all patients with thyroid disease are autoantibody positive and not all autoantibody positive individuals develop hypo- or hyperthyroidism (3, 4). The immune-mediated destruction of the thyroid is likely initiated by environmental factors in individuals who are genetically susceptible to autoimmunity (5). AITD is thus more common in patients with other autoimmune diseases or with family history of autoimmune disease (6). Furthermore, AITD is diagnosed five to ten times more often in adult females compared to males, with a slightly lower female-to-male ratio, 4:1, in children and adolescents (7). Although hypothyroidism is the most common clinical presentation of individuals with AITD, hyperthyroidism can occur in patients with TPOAb and TgAb. Therefore, we include both hypo and hyperthyroidism in our definition of individuals with clinical thyroid disease.

The Environmental Determinants of Diabetes in the Young (TEDDY) study follows children from the general population at increased genetic risk for type 1 diabetes, tested at birth or in the neonatal period for HLA genotypes (8). The co-occurrence of type 1 diabetes and AITD is well documented, but less is known about the timing of the first appearance of thyroid autoantibodies in healthy children with increased genetic risk for type 1 diabetes. The aims of this study were to examine the TEDDY population for 1) the incidence of TPOAb and TgAb by age, 2) associations of key demographic factors with risk of thyroid autoimmunity, 3) the co-occurrence of TPOAb and TgAb and progression from single to double autoantibody positivity, and 4) the relation of autoimmunity to abnormal thyroid stimulating hormone (TSH) and risk of clinical thyroid disease. The primary outcome in this study was thyroid autoimmunity,

while the two secondary outcomes were abnormal TSH and clinically diagnosed thyroid disease, defined by documentation of hypo/hyperthyroidism by ICD-10 codes and/or use of thyroid medication.

Methods

Study design

TEDDY is a multinational prospective study with three centres in the US (Washington state, Colorado, and Georgia) and three centres in Europe (Finland, Germany, and Sweden). The aim is to identify environmental factors that initiate or protect against not only islet autoimmunity and type 1 diabetes, but also concomitant coeliac (9) and thyroid autoimmunity and disease in children at increased genetic risk for type 1 diabetes based on their HLA-DR-DQ genotype (8, 10). Enrolled children were followed from birth to 15 years of age or until type 1 diabetes diagnosis. Clinical visits and serum sample collection occurred quarterly until age four years, and then every six months until age 15 years. Children positive for islet autoantibodies continued follow-up every three months regardless of age (11). Samples were collected locally, kept at -80 C in aliquots, and sent every second week on dry ice to the TEDDY Repository managed by Fisher Biosciences (12). An aliquot was sent on dry ice from the TEDDY Repository directly to the Gainesville laboratory without thawing the samples.

In 2004-2010, TEDDY screened 424,788 newborns, and enrolled 8,676 children (8). The TEDDY study was approved by local Institutional Review Boards or European Ethics Committees. Written informed consent was obtained for all study participants from a parent or primary caretaker, separately, for genetic screening and enrollment to participate in the study. The primary outcome in the present analysis was thyroid autoimmunity, defined by the presence of TPOAb or TgAb. Beginning in March 2016, children were initially screened for TPOAb and TgAb at eight years of age or older (up to 13 years). Younger, actively enrolled children were tested when they reached age eight, which occurred as late as 2018, making up a total of 5066 children. This visit when thyroid autoantibody screening was first conducted is

hereafter referred to as the screening visit. The screening results were used as a base to perform further testing of samples and construct a retrospective analytical cohort at enrollment (age 3 months). Children who were negative for both autoantibodies at the screening visit were assumed to be negative at all previous time points back to study enrollment. If a child tested positive for either TPOAb or TgAb at the screening visit, both autoantibodies were measured again at the next scheduled visit to confirm the positive test, and a recursive alogrithm was applied to retrospective samples to identify the earliest age of thyroid autoantibody positivity (Figure 1).

Children who were diagnosed with type 1 diabetes before thyroid autoantibody screening commenced (n=426) were similarly tested retrospectively starting at the last sample prior to type 1 diabetes diagnosis.

Secondary outcomes were 1) thyroid dysfunction, defined as abnormal TSH based on measurements of screening samples positive for TPOAb or TgAb and 2) clinical thyroid disease. Diagnosis of clinical thyroid disease was carried out outside the purview of the TEDDY study, however, per the study protocol, diagnoses were recorded in a diary book and subsequently reported to TEDDY study staff at the next clinic visit. Study nurses translated reported diagnoses according to the International Classification of Diseases, Tenth Revision (ICD-10). Families similarly reported all medications used by the child. In the present analysis, hypothyroidism cases included children with a reported diagnosis of hypothyroidism (ICD-10 code E03.9 or E06.3) or use of hypothyroidism medication (thyroxine or triiodothyronine), excluding children with congenital hypothyroidism. Likewise, hyperthyroidism cases included children with a reported diagnosis of hyperthyroidism (ICD-10 codes E05.0, E05.8, or E05.9) or use of hyperthyroidism medication (methimazole, carbimazole, dipyrone, radioactive iodine, or propylthiouracil). Clinical thyroid disease cases included both hypothyroidism and hyperthyroidism.

Measurements of thyroid autoantibodies and TSH

Serum TPOAb (KRONUS Cat# KR6210, RRID:AB_3095086) and TgAb (KRONUS Cat# KR6270, RRID:AB_3095085) were measured by radioimmunoassay at the Pathology Laboratories, University of Florida, US. Final quantitation was calculated using a 5-point cubic spline, log/linear fit algorithm. Values of >1.0 U/mL were reported as positive. The cut-off was established by the kit vendor (Kronus, Star, Indiana). Most screening visit samples were tested within 30 days of collection, while retrospective samples were retrieved from long-term storage. Prior studies have shown TPOAb and TgAb to be stable for upwards of 12 years in storage (13, 14).

TSH was measured at the same lab using a *Siemens Immulite 2000Xpi* analyser, a solid-phase, two-sited chemiluminescent immunometric assay (Siemens Cat# L2KTS2, RRID:AB_3095056). Values of >4.0 uIU/mL were considered abnormal high, and values of <0.4 uIU/mL were considered abnormal low. Coefficient of variation summaries for the TPOAb, TgAb, and TSH assays are shown in Table 1.

Statistical analysis

Primary analyses included all children tested at the screening visit. Persistent positive status was defined as being positive for the same autoantibody at two consecutive visits, and the date of persistent positivity was the draw date of first of the two positives. Children positive for TPOAb or TgAb prior to the visit at 12 months of age who did not have a negative result before the first positive were investigated for potential transient positivity due to maternal autoantibodies. If maternal AITD was reported in the family history questionnaire, TPOAb and TgAb results were considered missing at visits prior to the first negative or 12 months, whichever was earlier. Defined by which autoantibody was present when the child first became persistent positive, children were classified as TPOAb-first, TgAb-first, both-first, or thyroid autoantibody negative.

Cox proportional hazards regression models assessed associations between autoantibodies and sex, HLA genotype, and family history. The baseline hazard was stratified by country in all models, and the

proportional hazards assumption was assessed with Schoenfeld residuals. Children were followed from birth to first thyroid autoantibody appearance or the screening visit. Sensitivity analyses accounting for the interval-censored data generating process were compared to the Cox regression models. Modified Poisson regression (15) was used to examine factors associated with risk of progressing from one to two thyroid autoantibodies. Associations of TSH with first-appearing autoantibody and with autoantibody positivity at the screening visit were also assessed with modified Poisson regression models, excluding children with AITD diagnosed prior to the screening visit. Hazard ratios were calculated to assess the association of first-appearing autoantibody with risk of progressing from AITD to clinical thyroid disease and a sensitivity analysis was conducted in which clinical thyroid disease was defined only by reported medication use and not by ICD-10 codes. Age-specific and cumulative incidence were determined in the primary cohort as well as among children who were tested retrospectively at or before type 1 diabetes diagnosis. Person-years for age-specific incidence was determined based on the number of children (risk-set) with a sample available within the specified age range.

A P-value of less than 0.05 was considered to indicate statistical significance, without adjustment for multiple testing. Data wrangling was done in SAS version 9.4. Analyses were performed with R version 4.3.2 using packages survival (16)version 3.5-7, icenReg (17) version 2.0.15 and survminer version 0.4.9.

Results

Through October 31, 2022, of 5,066 children screened for thyroid autoantibodies at eight to 13 years of age (2,492 girls and 2,574 boys; Table 2), 385 (7.6%) were positive for either TPOAb or TgAb, with a median age at first appearance of 6.1 years. Sequential retrospective analysis of these children demonstrated that the earliest appearance of TPOAb-first was at 10 months, with cumulative incidence of 0.3% at two years, and 1.0% at six years of age. Amongst those with TgAb-first, the earliest appearance

was at 15 months, with cumulative incidence of 0.2% at two and 2.2% at six years of age. Already at two years of age, 23 (0.5%) children were positive for either autoantibody.

Incidence rates (per 1000 person-years) of TPOAb-first and TgAb-first at age two years were 2.5 and 6.2, respectively; and at age six years, 2.8 and 8.5, respectively (Figure2).

Overlap and order of appearance of thyroid autoantibodies

Among 102 children who developed TPOAb-first, 54 (53%) later tested persistent positive for TgAb. Similarly, among 251 children with TgAb-first, 120 (48%) later tested persistent positive for TPOAb. In 353 children initially persistent positive for only one autoantibody, there was no indication that progression to persistent positivity for the second autoantibody was associated with sex, HLA genotype, family history, or type of first-appearing autoantibody.

Risk factors for thyroid autoimmunity

Incidence was higher among girls (Figure 3 panels A-C; $p < 0.001$ for TPOAb-first and TgAb-first), a trend that was seen even at young ages: the age-specific incidence rate (per 1000 person-years) of TgAb-first at three years was 7.7 (95% CI 4.5, 11.7) among girls, and 4.2 (95% CI 2.0, 7.2) among boys. Risks of TPOAb-first (HR 2.55, 95% CI 1.67, 3.91, $p<0.001$) and TgAb-first (HR 2.18, 95% CI 1.68, 2.84, $p<0.001$) were more than two-fold higher for girls compared to boys (Figure 4).

Risk of TPOAb-first was higher among both DR4/4 and DR3/3 homozygous children compared to those with a heterozygous HLA genotype (HR 2.17, 95% CI 1.37, 3.45, $p<0.001$, and HR 1.78, 95% CI 1.10, 2.87, $p=0.018$, respectively). In contrast, risk of TgAb-first was lower for DR4/4 (HR 0.71, 95% CI 0.50, 1.00, $p=0.0497$) and DR3/3 (HR 0.68, 95% CI 0.49, 0.96, $p=0.030$) compared to HLA heterozygous children. A total of 12% of the children had a first-degree relative with AITD (Table 2), while the frequency of family history was over 20% among persistent positive children (Table 3). Family history was associated with a

higher risk of both TPOAb-first (HR 1.90, 95% CI 1.17, 3.08, p=0.010) and TgAb-first (HR 2.55, 95% CI 1.91, 3.41, p<0.001; Figure 4). Furthermore, the risks of TPOAb-first (HR 3.78, 95% CI 1.65, 8·66, p=0.002) and TgAb-first (HR 3.34, 95% CI 1.94, 5.76, p<0.001) were notably higher if the father had AITD, compared to fathers without AITD. The risk if the mother had AITD was less pronounced (TPOAb-first HR 1.74, 95% CI 1.03, 2.93, p=0.04; TgAb-first HR 2.19, 95% CI 1.60, 3.01, p<0.001).

Trends in cumulative incidence by first-appearing autoantibody are shown in Figure 3 and Figures 5-6 (panels A-C). Across all analysed potential risk factors, there were no notable differences in the estimates or their precision from models accounting for the interval-censored data generating process.

TSH and clinical thyroid disease

Among 371 persistent positive children without a clinical thyroid disease diagnosis prior to the screening visit, 46 had thyroid dysfunction; 43 had high TSH (>4.0 uIU/mL) and three had low TSH (<0.4 uIU/mL). Among children positive for both TPOAb and TgAb at the screening visit, 17% had high TSH and 1% had low TSH (Table 4). We did not find evidence of a difference in the risk of high TSH between TPOAb-only and TgAb-only positivity at the screening visit (RR 0.95, 95% CI 0.28, 3.24, p=0.933), while both-positive compared to TPOAb-only increased the risk of high TSH (RR 3.29, 95% CI 1.17, 9.20, p=0.023). There was no evidence of an association between first-appearing autoantibody and the risk of high TSH.

Among the 88 children diagnosed with clinical thyroid disease (11 with hyperthyroidism, 76 with hypothyroidism and one with both hyper- and hypothyroidism), we examined the 73 who were identified as persistent positive for either TPOAb or TgAb prior to diagnosis. Risk of progressing from AITD to clinical thyroid disease was not different between TgAb-first and TPOAb-first (HR 1.56, 95% CI 0.80, 3.03, p=0.188). In contrast, children with both autoantibodies appearing at the same visit had an increased risk of diagnosis with clinical thyroid disease (vs TPOAb-first, HR 6.34, 95% CI 2.72, 14.76, p<0.001). As not all families reported medication with the ICD-10 diagnosis of hypo- or hyperthyroidism, a sensitivity analysis

was performed, only including the ones on medication (66/73 children). This did not change the results: the risk of progression from thyroid autoimmunity to treatment for hypo- or hyperthyroidism; TgAb-first vs TPOAb-first: HR 1.49 (95% CI 0.75, 2.99) and both-first vs TPOAb-first: HR 5.74 (95% CI 2.33, 14.13) and for progression to treatment for hypothyroidism only; TgAb-first vs TPOAb-first: HR 1.75 (95% CI 0.83, 3.71), both-first vs TPOAb-first: HR 5.15 (95% CI 1.88, 14.13).

TPOAb and TgAb prior to type 1 diabetes diagnosis

TPOAb and TgAb were analysed in 424 children at the time of type 1 diabetes diagnosis or the nearest visit prior to diagnosis. There were 191 girls with a median follow-up time of 5.1 years (IQR 2.1-9.3 years), and 233 boys with a median follow-up time of 6.3 years (IQR 2.4-9.9 years; Table 2). The cumulative incidence of TPOAb-first was 1.9% compared to 5.9% for TgAb-first at the diagnosis of type 1 diabetes (Figure 3 and Figures 5-6; panels D-F), and the median age at first appearance was 4.7 years (Table 2).

Discussion

The incidence and risk factors of TPOAb and TgAb in a cohort of 5,066 healthy children from the genetically high-risk TEDDY study revealed increased risk among girls and those with family history of AITD. The association with HLA genotype varied depending on which of the thyroid autoantibodies was first appearing. A major finding was that seroconversion to a positive thyroid autoantibody started as early as 10 months of age for TPOAb-first and 15 months of age for TgAb-first. The consequences of developing TPOAb-first versus TgAb-first are unclear, as first appearing autoantibody (among children developing a single autoantibody first) did not alter the risk of either thyroid dysfunction, measured as abnormal TSH or later clinical diagnosis of thyroid disease. The observed upward trend in risk with age confirmed findings from previous prospective studies of children at increased genetic risk or born to parents with type 1 diabetes (18, 19). Although the aetiological factors that trigger either TPOAb-first or

TgAb-first remain to be determined, it is a major finding that about half of the children who developed a first autoantibody went on to develop a second by median age nine years.

Long-term follow-up studies in children born to parents with type 1 diabetes showed that cumulative risk of developing TPOAb by age eight years was 4.3% (18), and that 10.7 % had developed TPOAb by 20 years of age (19), while the prevalence of TPOAb by age 10 years among children at increased genetic risk for type 1 diabetes was 4.4% in Sweden and 5% in the US (20, 21). Our finding of 7.6% of children having either TPOAb or TgAb at the initial screening is in line with these prior reports of prevalence among children at increased risk for type 1 diabetes. Our study, however, adds to the literature by including children both with and without family history of type 1 diabetes, measuring two thyroid autoantibodies, and determining the age at first autoantibody appearance with a high level of precision.

The prevalence of thyroid autoimmunity in children after diagnosis of type 1 diabetes has been reported as 14.4% at a median age of 11.3 years or 12.1% at a median age of 12.2 years (22, 23). The lower proportion of children diagnosed with type 1 diabetes and positive for thyroid autoimmunity at or before diabetes diagnosis in our study may therefore be explained by the low median age of 5.9 years at the diagnosis of type 1 diabetes. Seroconversion at a young age is consistent with an increasing occurrence of co-existing autoimmune disorders in children newly diagnosed with type 1 diabetes (24).

Autoimmune endocrine disorders such as AITD, with the exception of type 1 diabetes, are predominately diagnosed in females. Our results, in this cohort of children with genetic risk of type 1 diabetes, demonstrate that the risk for girls was more than twice that of boys, even at an early age. This association between thyroid autoimmunity and sex in our young cohort is notable, as the sex difference for AITD was previously reported to be less apparent in prepubertal children (25).

The increased risk among children with a family history of AITD aligns with previous studies, and increased risk for thyroid autoimmunity has also been shown among children with family members with

type 1 diabetes or coeliac disease (19, 26, 27). Our finding of an increased risk of thyroid autoimmunity in children who had fathers with AITD is reminiscent of the well-known increased risk for type 1 diabetes in children if the father rather than the mother has type 1 diabetes (28, 29). Furthermore, the HLA DR3/4 genotype is known to confer the highest risk of type 1 diabetes. The increased risk of TgAb-first, but not TPOAb-first, among the HLA-heterozygous individuals raises the possibility that the co-occurrence of type 1 diabetes and clinical thyroid disease may be driven by a TgAb-first subtype, an observation which deserves further exploration.

The predictive value of TPOAb, TgAb, or both, for abnormal TSH and diagnosis of clinical thyroid disease in clinical practice has been a matter of debate. Interestingly, when we examined if the order of appearance of TPOAb versus TgAb was a risk for later disease, no difference was found between the first-appearing autoantibody and later clinical thyroid disease. In contrast, children with both autoantibodies appearing at the same visit had a 6-fold higher risk of developing clinical thyroid disease compared to children with TPOAb-first. Children with only TPOAb and those with only TgAb at the screening visit at 8-13 years of age did not differ in risk of thyroid dysfunction, i.e abnormal TSH, while the risk was higher for those with both autoantibodies. Therefore, since half of the children with thyroid autoimmunity had both TPOAb and TgAb at the time of the screening visit, a high overall burden of morbidity is expected in this population (30). Our investigation raises the possibility that measurement of both thyroid autoantibodies in children in clinical practice should be considered.

A diagnosis of clinical thyroid disease in infants and young children is rare and is likely to be missed as the diagnosis is more common in older children and adolescents (7). Our finding of thyroid autoimmunity in the very young, although in a selected cohort at risk for type 1 diabetes, stresses the importance of early awareness, to avoid a delay in treatment which may result in an increased risk for neurological damage and growth deficits (31, 32). Further studies of TPOAb and TgAb in children in the general population will

be needed to assess the ability of these autoantibody markers to predict thyroid dysfunction and clinical thyroid disease to prevent symptoms.

The HLA-restricted TEDDY population has a higher incidence of autoimmune disease than the general population, allowing us to identify a substantial number of children with thyroid autoimmunity. However, this limits generalization as the cohort is not representative of the general population in each of the respective countries. The TEDDY study is a uniquely valuable resource given its extensive longitudinal follow-up and standardized protocol, but there are constraints inherent to observational studies to consider including unmeasured confounding and sparse data bias (33). Furthermore, retrospective analyses were only done in children positive for a thyroid autoantibody at the screening visit, so we were unable to assess if children who were negative at the screening visit were transiently positive at a younger age. Therefore, a major limitation is that additional thyroid autoimmunity may have been missed, as subclinical hypothyroidism can spontaneously remit (34) and the precence of TPOAb, TgAb, or both, can be transient with or without normal TSH levels (35).

Since blood samples were drawn three months apart, there were 32 children in whom both autoantibodies appeared at the same visit, therefore we do not know the true order of appearance. It was nonetheless valuable to identify this group of children because they progressed rapidly from one to two autoantibodies and showed an increased risk for clinical thyroid disease. The low prevalence of Graves' disease in children (36) was, at planning of the study, considered reason for not testing for autoantibodies to the TSH receptor. However, three children had low TSH, and 12 had a diagnosis of hyperthyroidism, which suggests that such analyses should be considered in future studies of children at risk.

Taken together, our investigation revealed that thyroid autoimmunity may be triggered in younger children than has hitherto been recognized and that double positivity at seroconversion showed the

highest risk for progression to clinical thyroid disease, within an HLA-selected population. It is of interest to further explore the aetiological factors that trigger thyroid autoimmunity.

Acknowledgements

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Financial Support

The TEDDY Study is funded by U01 DK63829, U01 DK63861, U01 DK63821, U01 DK63865, U01 DK63863, U01 DK63836, U01 DK63790, UC4 DK63829, UC4 DK63861, UC4 DK63821, UC4 DK63865, UC4 DK63863, UC4 DK63836, UC4 DK95300, UC4 DK100238, UC4 DK106955, UC4 DK112243, UC4 DK117483, U01 DK124166, U01 DK128847, and Contract No. HHSN267200700014C from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institute of Allergy and Infectious Diseases (NIAID), Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institute of Environmental Health Sciences (NIEHS), Centers for Disease Control and Prevention (CDC), and JDRF. This work is supported in part by the NIH/NCATS Clinical and Translational Science Awards to the University of Florida (UL1 TR000064) and the University of Colorado (UL1 TR002535). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Data Avalibility Statment

Data from The Environmental Determinants of Diabetes in the Young (https://doi.org/10.58020/y3jk-x087) reported here will be made available for request at the NIDDK Central Repository (NIDDK-CR) website, Resources for Research (R4R), https://repository.niddk.nih.gov/.

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Tables

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Figures

Figure 1: The algorithm for sample selection for testing of TPOAb and TgAb in the TEDDY study. Children actively enrolled and 8 years of age or older in 2016 were screened at the next scheduled clinical visit, while younger children were screened when they reached age 8 years. All children diagnosed with type 1 diabetes were eligible for TPOAb and TgAb testing in the last sample collected at or before diagnosis.

Figure 2: Age-specific incidence rates and 95% confidence intervals for TPOAb-first (green), TgAb-first (orange), and both-first (purple) in one-year intervals among 5066 children in the TEDDY study. The age 1 interval includes 1.0 years to < 2 years, and likewise for subsequent intervals. Incidence rates (per 1000 person-years) of TPOAb-first and TgAb-first at age 2 were 2.5 (95% CI 1.3, 4.2) and 6.2 (95% CI 4.1, 8.6), respectively, and at age 6, 2.8 (95% CI 1.5, 4.5) and 8.5 (95% CI 6.1, 11.4), respectively. Ages 10-13 years are not shown because estimates are unstable due to small sample sizes at those ages.

Figure 3: Sex-stratified cumulative incidence and log-rank test p-value among 5066 children in the TEDDY study of A) TPOAb-first, B) TgAb-first, C) both-first, and among 424 children at or before type I diabetes diagnosis of D) TPOAb-first, E) TgAb-first, and F) both-first.

Figure 4: Forest plot of hazard ratios and 95% confidence intervals for associations of key covariates with risk of TPOAb-first (green), TgAb-first (orange), and both-first (purple) among 5025 children in the TEDDY study.

Figure 5: Cumulative incidence and log-rank test p-value among 5066 children in the TEDDY study of, A) TPOA-first, B) TgAb-first and C) Both first, and among 424 children at or before type I diabetes diagnosis of D) TPOA-first, and E) TgAb-first and F) Both first. Stratified by HLA genotype**.**

Figure 6: Cumulative incidence and log-rank test p-value among 5025 children in the TEDDY study of, A) TPOA-first, B) TgAb-first and C) Both first, and among 403 children at or before type I diabetes diagnosis of D) TPOA-first, and E) TgAb-first and F) Both first. Stratified by family history of autoimmune thyroid disease.

Table 1: Coefficients of variation at representative mean levels for the TPOAb, TgAb, and TSH assays.

Analyte	mean (µIU/mL)	CV%
TSH	0.42	7.1
TSH	4.89	5.5
TSH	31.64	8
Analyte	**mean (U/mL)**	**CV%**
TgAb	0.73	15.1
TgAb	2.52	6.3
TgAb	5.34	7.1

TPOAb	0.66	6.1
TPOAb	3.35	5.1
TPOAb	8.39	7.7

1

2 **Table 2**: Characteristics of children tested for thyroid autoantibodies in the TEDDY study.

	Tested at the screening visit			At or before type I diabetes diagnosis		
	Overall	Boys	Girls	Overall	Boys	Girls
n	5066	2574	2492	424	233	191
Follow-up time, years, median [IQR]	9.0 [8.1, 10.2]	9.0 [8.1, 10.3]	9.0 [8.1, 10.2]	5.9 [2.2, 9.8]	6.3 [2.4, 9.9]	5.1 [2.1, 9.3]
TPOAb+ or TgAb+, N (%)	385 (7.6)	118 (4.6)	267 (10.7)	41 (9.7)	15 (6.4)	26 (13.6)
TPOAb+ or TgAb+ youngest age at first appearance, years, median [IQR]	6.1 [3.7, 8.0]	6.0 [3.4, 8.7]	6.1 [3.8, 7.9]	4.7 [3.0, 7.2]	6.0 [3.6, 9.0]	4.3 [3.0, 6.5]
TPOAb-only first, N (%)	102 (2.0)	30 (1.2)	72 (2.9)	8 (1.9)	3 (1.3)	5 (2.6)
TgAb-only first, N (%)	251 (5.0)	82 (3.2)	169 (6.8)	25 (5.9)	10 (4.3)	15 (7.9)
TPOAb and TgAb first, N (%)	32 (0.6)	6 (0.2)	26 (1.0)	8 (1.9)	2 (0.9)	6 (3.1)
Country, N (%)						
USA	1961 (38.7)	1004 (39.0)	957 (38.4)	161 (38.0)	87 (37.3)	74 (38.7)
Finland	1161 (22.9)	577 (22.4)	584 (23.4)	109 (25.7)	60 (25.8)	49 (25.7)
Germany	275 (5.4)	152 (5.9)	123 (4.9)	41 (9.7)	20 (8.6)	21 (11.0)
Sweden	1669 (32.9)	841 (32.7)	828 (33.2)	113 (26.7)	66 (28.3)	47 (24.6)
HLA, N (%)						
Heterozygous	2983 (58.9)	1507 (58.5)	1476 (59.2)	305 (71.9)	170 (73.0)	135 (70.7)
DR4/4	993 (19.6)	482 (18.7)	511 (20.5)	79 (18.6)	41 (17.6)	38 (19.9)
DR3/3	1090 (21.5)	585 (22.7)	505 (20.3)	40 (9.4)	22 (9.4)	18 (9.4)
Family history of autoimmune thyroid disease, N (%)[1]	616 (12.3)	314 (12.3)	302 (12.2)	50 (12.4)	26 (11.6)	24 (13.4)
Clinical Thyroid Disease	88 (1.7)	30 (1.2)	58 (2.3)			

	Tested at the screening visit			At or before type I diabetes diagnosis		
	Overall	Boys	Girls	Overall	Boys	Girls

[1]n = 41 children at the initial visit and n = 21 children at or before type I diabetes were missing family history of autoimmune thyroid disease

1

2 **Table 3:** Demographic factors stratified by first appearing autoantibody

		TPOAb-first	TgAb-first	Both-first	Thyroid autoantibody negative
n		102	251	32	4681
Sex, N (%)	Male	30 (29.4)	82 (32.7)	6 (18.8)	2456 (52.5)
	Female	72 (70.6)	169 (67.3)	26 (81.2)	2225 (47.5)
HLA, N (%)	DR3/3	28 (27.5)	42 (16.7)	8 (25.0)	1012 (21.6)
	DR4/4	31 (30.4)	39 (15.5)	6 (18.8)	917 (19.6)
	DR3/4	31 (30.4)	124 (49.4)	9 (28.1)	1783 (38.1)
	DR4/8	10 (9.8)	38 (15.1)	7 (21.9)	806 (17.2)
	FDR specific	2 (2.0)	8 (3.2)	2 (6.2)	163 (3.5)
Family history of autoimmune thyroid disease	No	81 (79.4)	189 (75.3)	23 (71.9)	4116 (88.7)
	Yes	21 (20.6)	62 (24.7)	9 (28.1)	524 (11.3)
Country, N (%)	USA	49 (48.0)	104 (41.4)	15 (46.9)	1793 (38.3)
	Finland	20 (19.6)	69 (27.5)	9 (28.1)	1063 (22.7)
	Germany	5 (4.9)	5 (2.0)	1 (3.1)	264 (5.6)

		TPOAb-first	TgAb-first	Both-first	Thyroid autoantibody negative
	Sweden	28 (27.5)	73 (29.1)	7 (21.9)	1561 (33.3)

Table 4: TSH results by first-appearing thyroid autoantibody and by thyroid autoantibodies at the time of the TSH measurement among 371 children positive for at least one thyroid autoantibody and without an existing clinical thyroid disease diagnosis in the TEDDY study.

		High TSH	Low TSH	Normal TSH
	Total	43 (12%)	3 (1%)	325 (88%)
First appearing autoantibody	TPOAb-first	12 (12%)	2 (2%)	83 (86%)
	TgAb-first	26 (11%)	1 (0%)	219 (89%)
	Both-first	5 (18%)	0 (0%)	23 (82%)
Autoantibodies at the time of TSH draw	Only TPOAb positive	3 (7%)	0 (0%)	43 (93%)
	Only TgAb positive	6 (5%)	1 (1%)	120 (94%)
	Both positive	34 (17%)	2 (1%)	162 (82%)

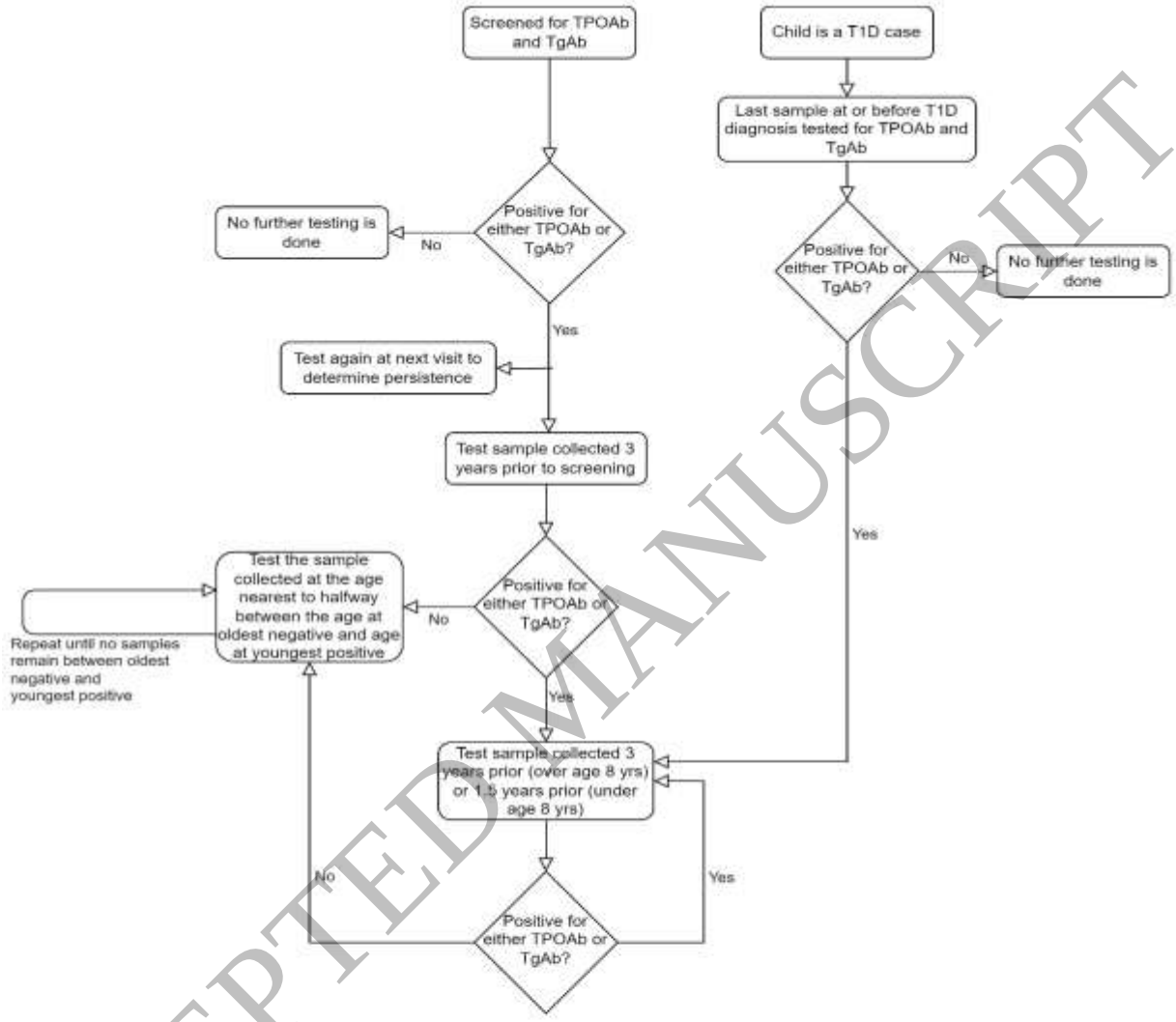

Figure 1
395x559 mm (x DPI)

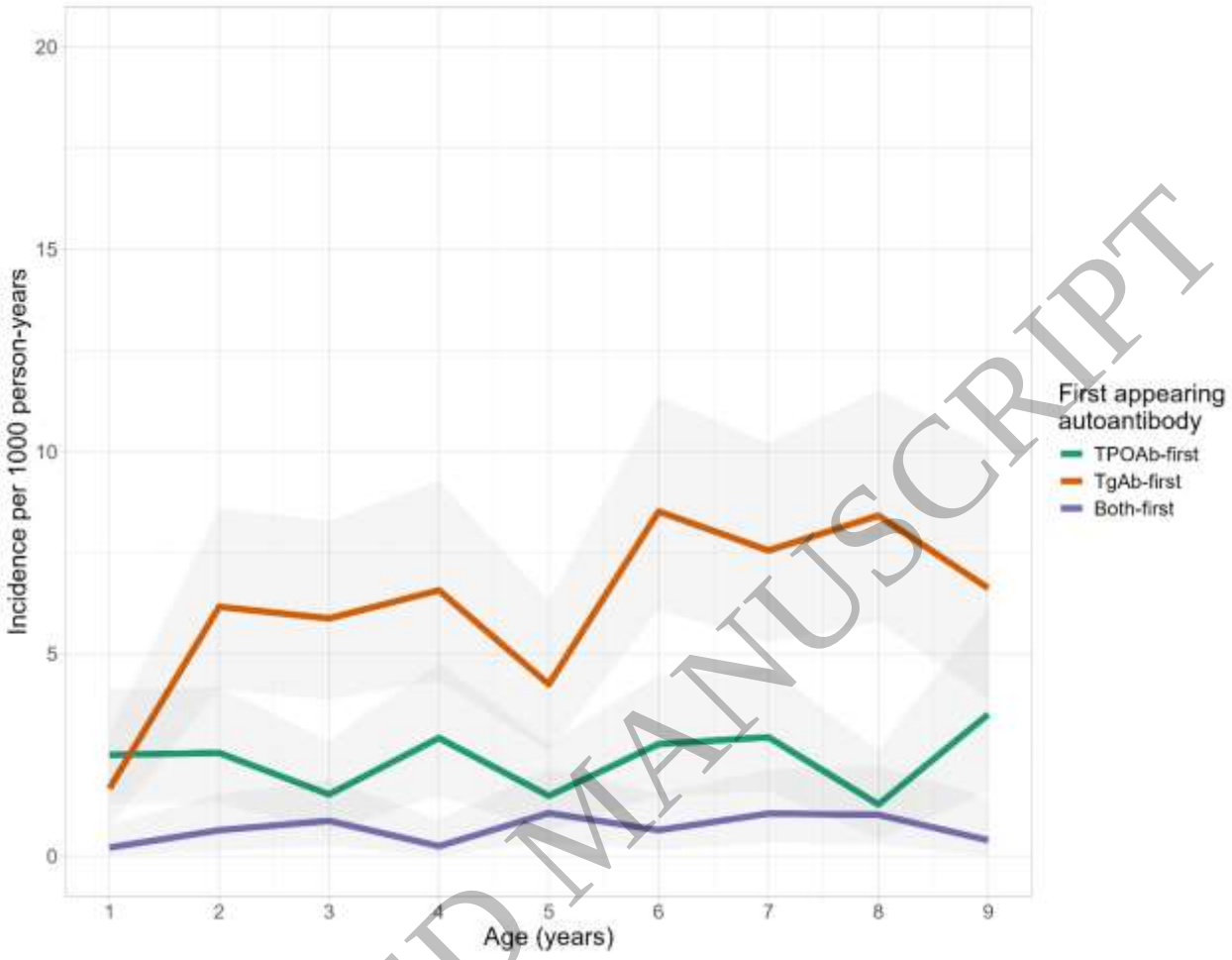

Figure 2
279x216 mm (x DPI)

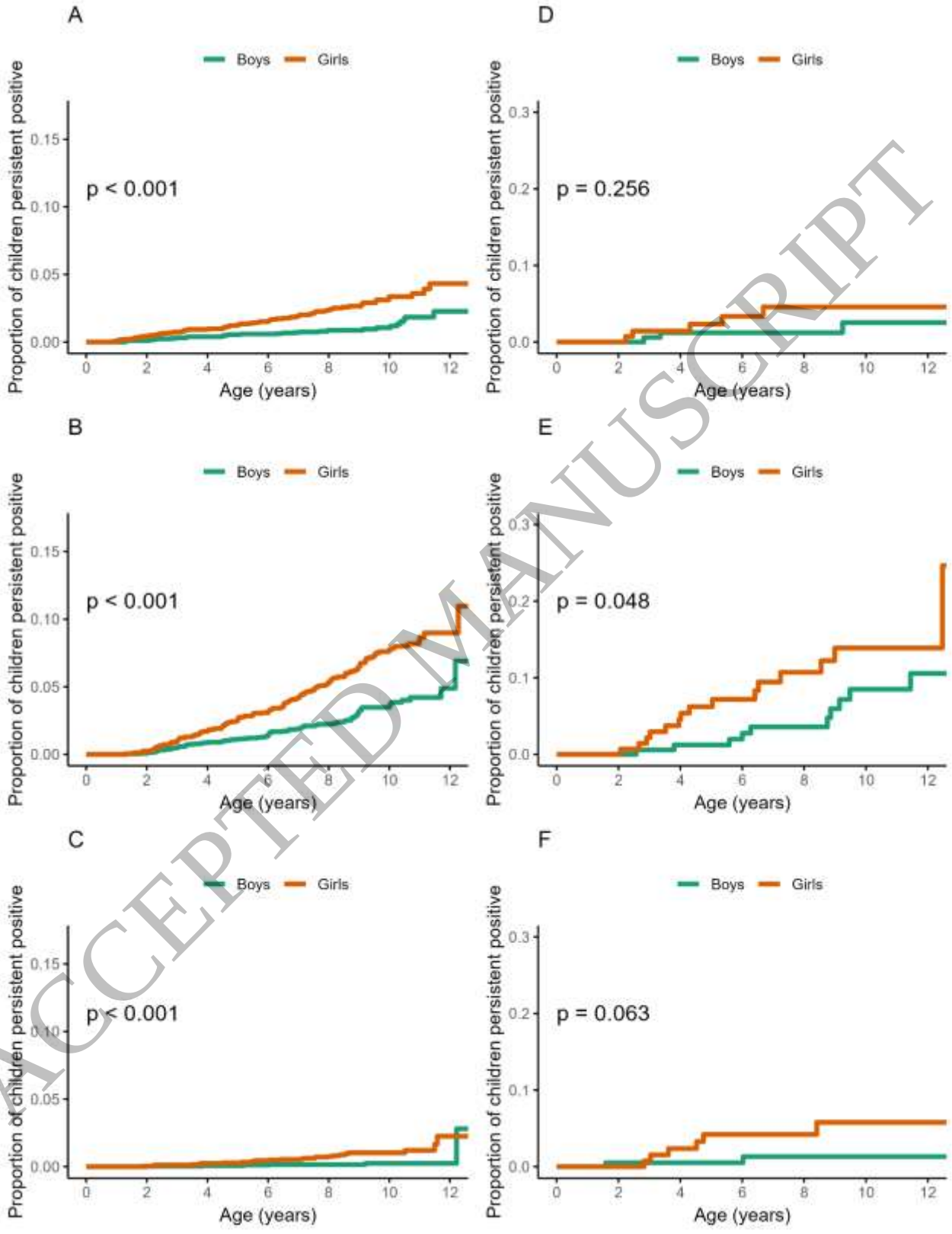

Figure 3
216x279 mm (x DPI)

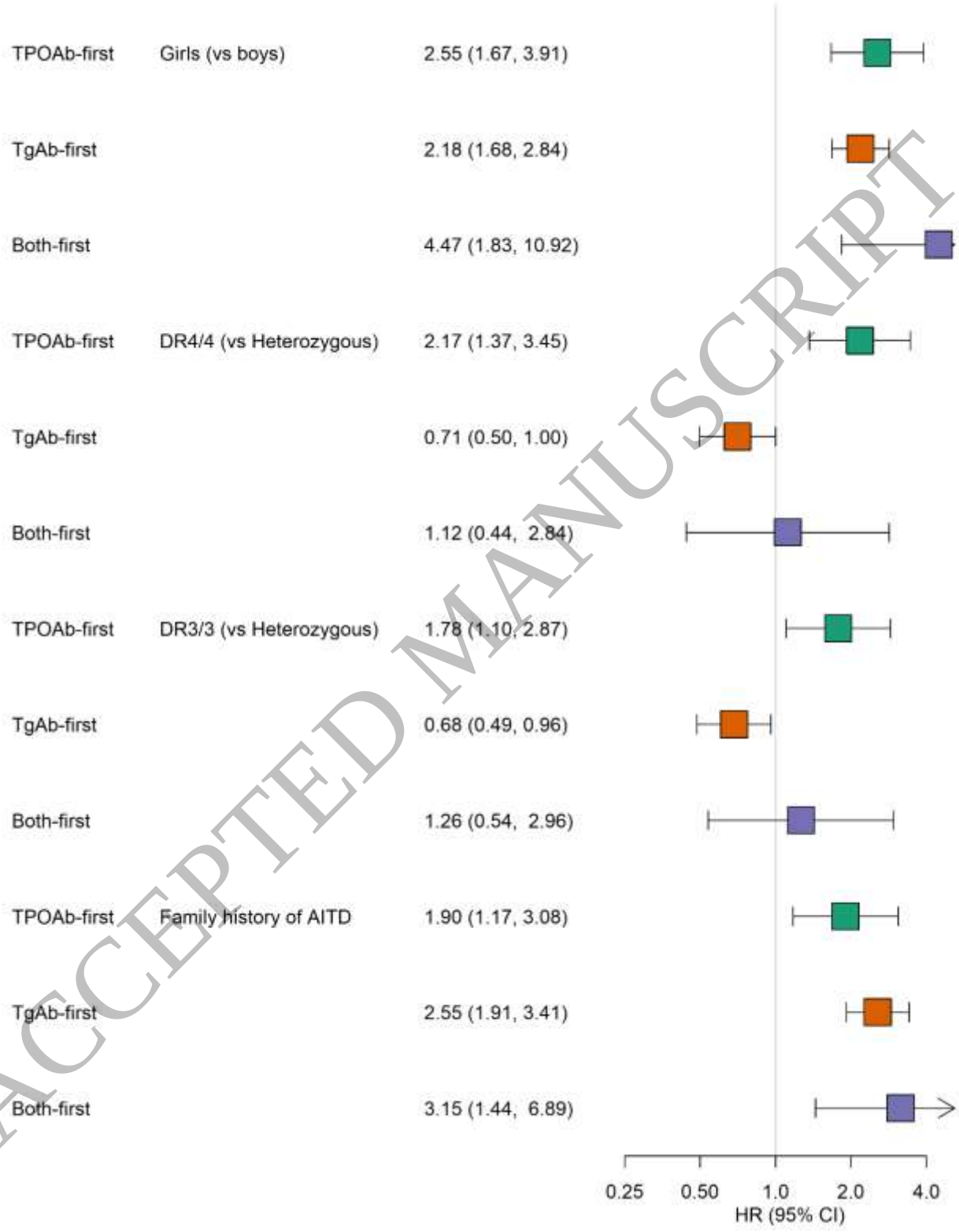

Figure 4
216x279 mm (x DPI)

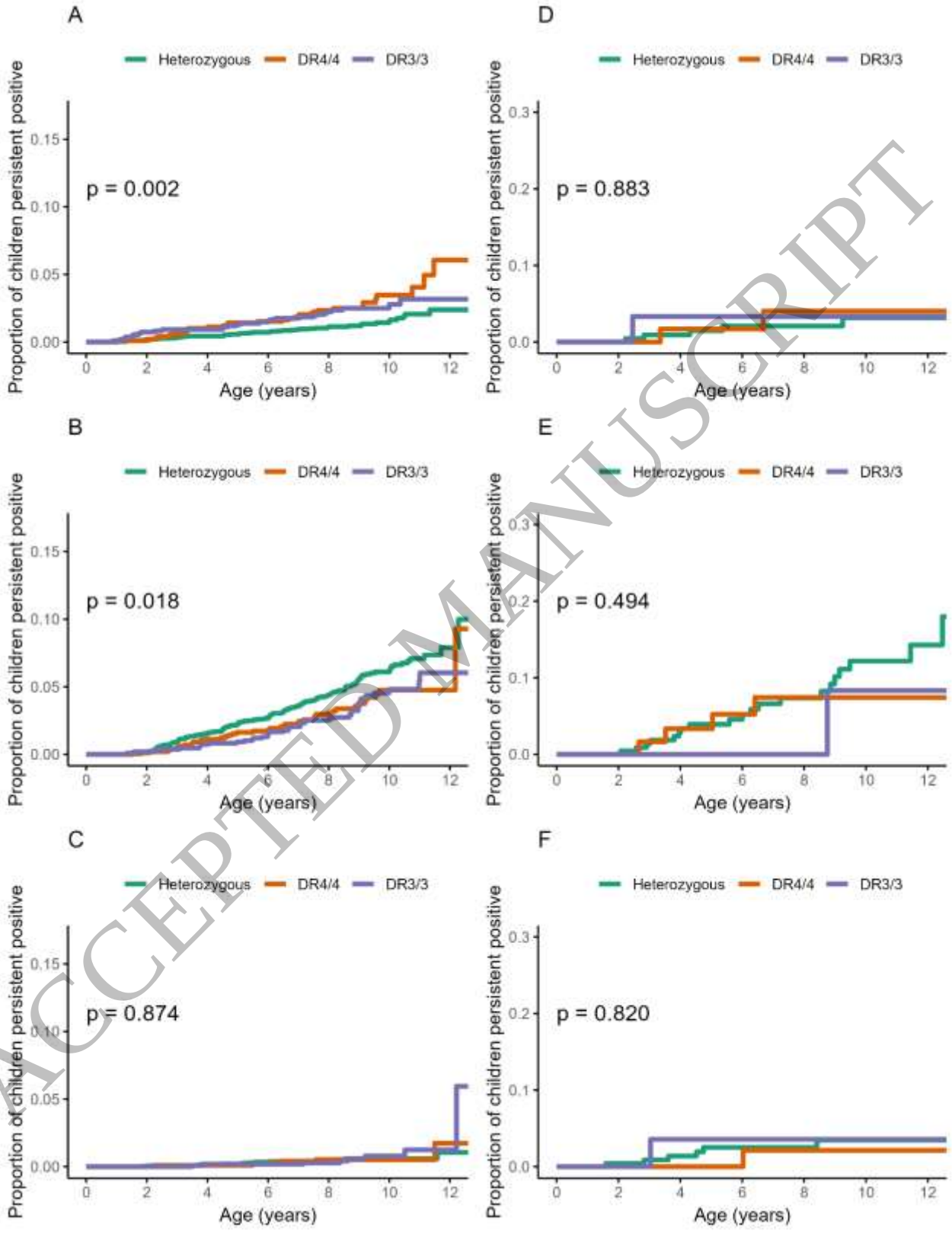

Figure 5
216x279 mm (x DPI)

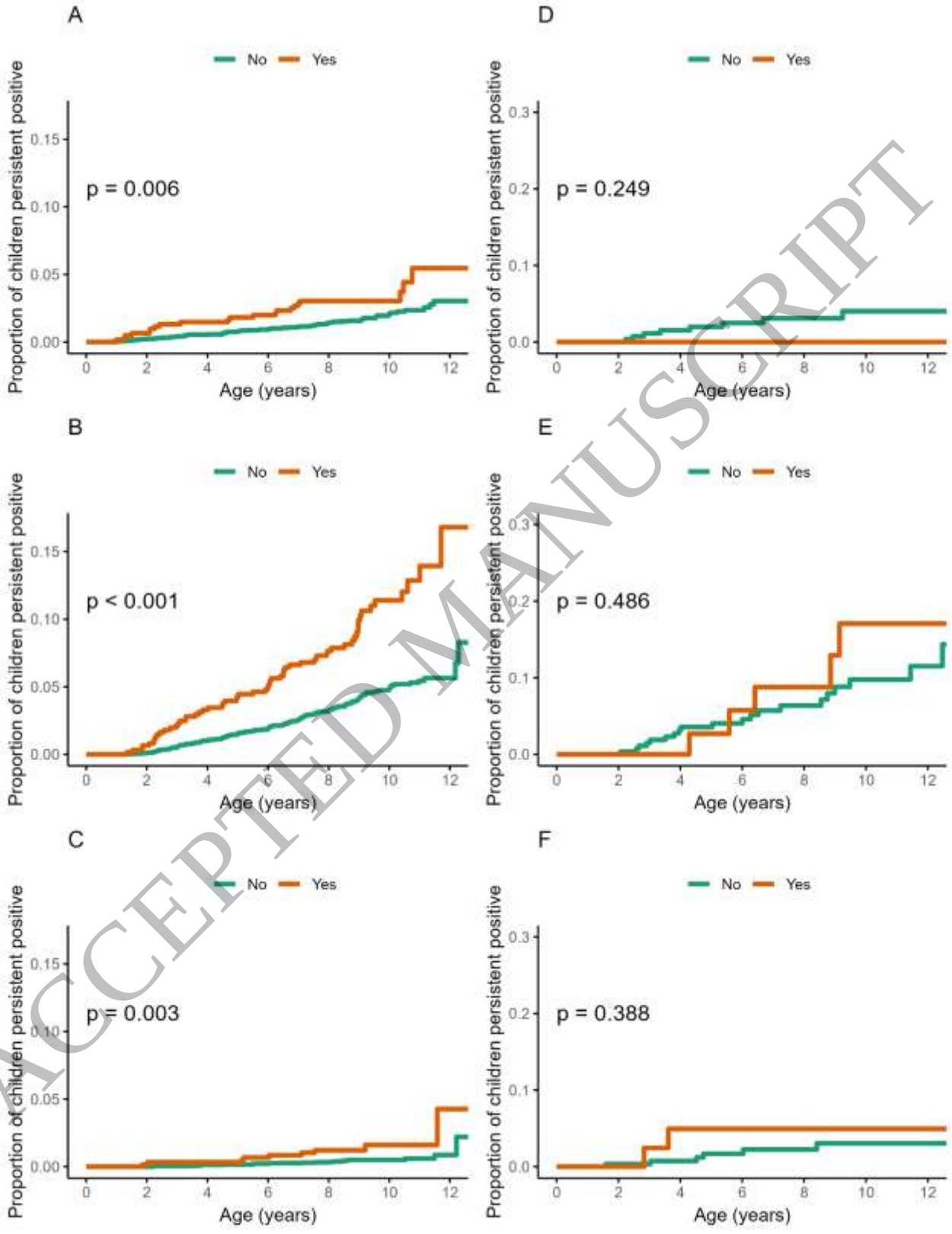

Figure 6
216x279 mm (x DPI)