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ENDIA Resource Availability and Supplementary Information

Accelerating Collaborative Research in Type 1 Diabetes Etiology and Pathogenesis using Biosamples from an Observational Clinical Study

1. The ENDIA cohort

The ENDIA study was approved by the Women's and Children's Hospital Network Human Research Ethics Committee (HREC/16/WCHN/066) under the Australian National Mutual Acceptance Scheme and the Women and Newborn Health Service Ethics Committee in Western Australia (RGS0000002639). ENDIA is registered in the Australia and New Zealand Clinical Trials Registry (ACTRN12613000794707). Recruitment commenced in 2013 and was completed in 2019, resulting in a study population of 1214 unique gestational mothers, 1217 unique biological fathers, and 1473 babies (Figure 1). The eldest ENDIA child was born in November 2012 and the youngest in July 2020 with a median birth date of 13/11/2017 (IQR: 16/7/2016-7/1/2019).

Eighty percent of the study participants were recruited during pregnancy with almost 60% recruited in the first or second trimester of pregnancy. The first-degree relative (FDR) T1D proband distribution is 62% maternal, 28% paternal, and 11% sibling. Two percent of children have more than one FDR with T1D. Families were recruited from every State and Territory in Australia. The recruitment strategy has been published[1].

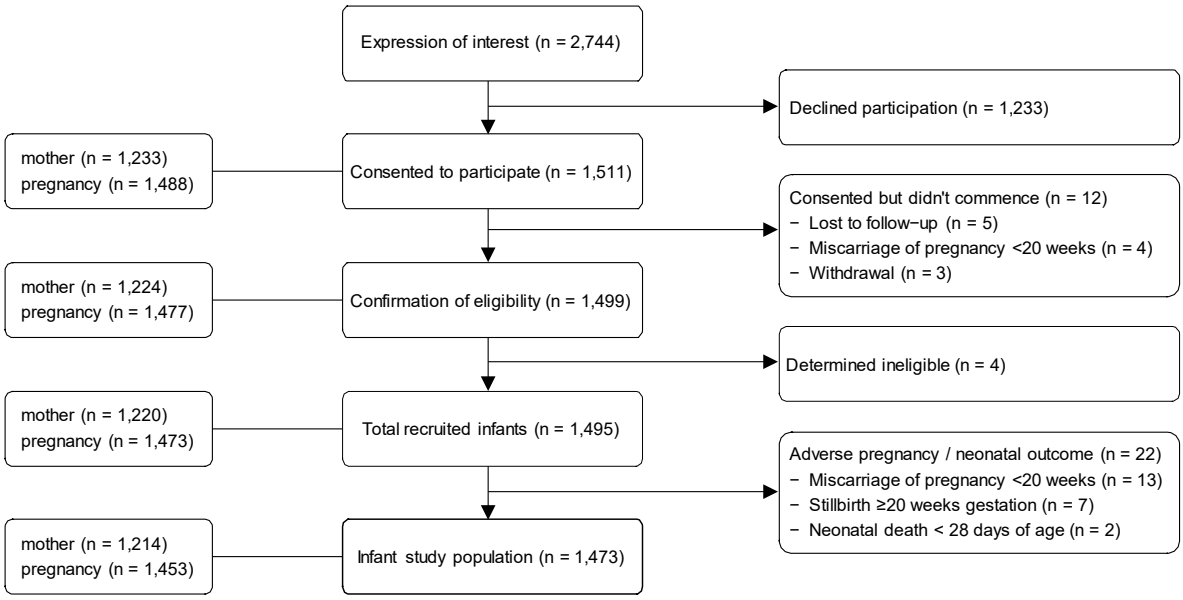

Figure 1. STOBE flow diagram of participant recruitment to the ENDIA study.

2. Summary of follow-up

Biospecimen and clinical data collection from the mothers during pregnancy and the infants in the first two years of life has occurred 3-monthly, then 6-monthly thereafter until 10 years of age. Samples and data were also collected from fathers and siblings (Figure 2). Ten years of age was determined to be an appropriate time to end the intensive follow-up as beyond this age the impact of insulin resistance in association with puberty may become increasingly relevant to disease progression. Further, while early life exposures are not irrelevant in this older group, the numbers of children developing islet autoimmunity during the pre-pubertal and pubertal periods in ENDIA is likely to be at a slower rate. The sample collection methods, including protocol changes made in response to the COVID-19 pandemic, and details of the questionnaires have been described previously[2-4].

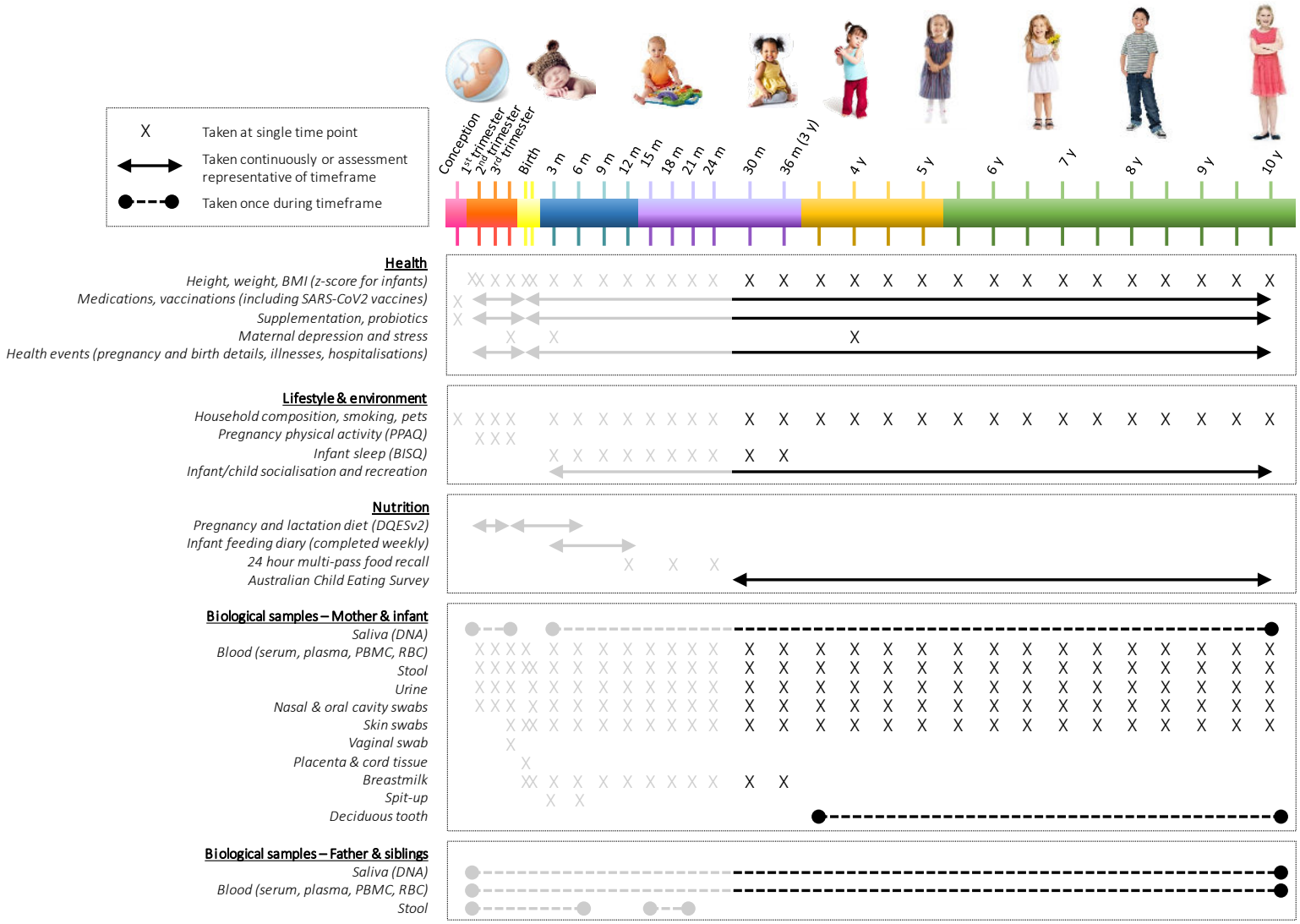

Figure 2. Graphical summary of follow-up in the ENDIA study. Time points shaded in light grey have been completed by all ENDIA children.

3. Data availability

A summary of the validated data collection tools utilized in ENDIA is provided in **Table 1**. Islet autoantibodies (IAA, GADA, ZnT8A, IA2A) are measured in mothers during pregnancy and the first visit postpartum, in cord blood, and in infants at all study visits. Other available longitudinal clinical data can be divided into eight main categories with 47 subcategories (Table 2).

Table 1. Validated data collection tools used in the ENDIA cohort

Data type	**Who**	**When**
Edinburgh postnatal depression scale (EPDS)[5, 6]	Mother	Pregnancy third trimester, 3m postpartum
Perceived stress scale (PSS)[7]	Mother	Pregnancy third trimester, 3m postpartum, 4 y postpartum
Pregnancy physical activity questionnaire (PPAQ)[8]	Mother	First, second, and third trimester
Basic infant sleep questionnaire (BISQ)[9]	Infant	3m, 6m, 9m, 12m, 15m, 18m, 21m, 24m, 30m, 36m
DQES v2 food frequency questionnaire[10]	Mother	Pregnancy third trimester, 3m, and 6m postpartum during lactation
24-hour multi-pass food recall[11, 12]	Infant	12m, 18m, 24m
Australian child eating survey (ACES)[13]	Infant	2-10 years (6 monthly)

In addition to those categories outlined in Table 2, other variables undergoing cleaning and codifying include:

- Infant health events in accordance with ICD11 system
- COVID infections and vaccinations in children and households
- Medications in accordance with Australian Monthly Index of Medical Specialties (MIMS) classifications
- Detail infant feeding practices from birth to 12m
- Complete Blood Count data for most blood draws

Table 2. Summary of available clinical data. The 47 subcategories represent 391 variables (most longitudinal)

1. Demographics	2. Conception	3. Pregnancy	4. Birth	5. Medications	6. Infant	7. Lifestyle	8. Clinical
Demographics: Family history of T1D	Conception: Assisted	Pregnancy: Diet & Exercise	Birth: Age at birth	Medications: Antenatal magnesium sulphate	Infant: Anthropometry^	Lifestyle: Household members	Clinical: HbA1c
Demographics: Maternal	Conception: Dates	Pregnancy: GDM Management	Birth: Complications maternal postnatal	Medications: Antenatal steroids	Infant: Congenital defects	Lifestyle: Infant socialisation	Clinical: Iron
Demographics: Maternal anthropometry^		Pregnancy: Hospital admissions	Birth: Complications of infant	Medications: Antibiotic Use	Infant: Feeding	Lifestyle: Pets	Clinical: Vitamin D
Demographics: Paternal		Pregnancy: Hypertensive Conditions in Pregnancy	Birth: Complications of labour	Medications: Vaccination at birth	Infant: Sleep	Lifestyle: Smoking	Clinical: HLA Status
Demographics: Paternal anthropometry		Pregnancy: Infections	Birth: Infant anthropometry				
Demographics: Probands		Pregnancy: Obstetric history	Birth: Infant sex				
		Pregnancy: Plurality	Birth: Labour duration				
		Pregnancy: Pre-existing Health Conditions	Birth: Labour hospital admission				
		Pregnancy: T1D Management	Birth: Labour indication for Caesarean				
			Birth: Labour indication for induction				
			Birth: Labour mode of birth				
			Birth: Labour onset				
			Birth: Labour rupture of membranes				
			Birth: Rupture of membranes				

^ includes weight, height/length, BMI/BMI Z-score

4. Biospecimen availability

The number of unique samples in the ENDIA biobank for the cohort of 1,511 consented mother-infant dyads and other family members as of December 2022 is provided in Table 3.

The composition of the NCC biobank, which comprising 190 unique mother-infant dyads, is provided in Table 4. In the ENDIA NCC study, cases were defined as children who had either developed persistent IA (single or multiple) or had progressed to clinical T1D with onset by 31st December 2019 (inclusive). The 54 children who met this case definition were matched 1:3 based on age and sex with children who were confirmed antibody negative at the time of seroconversion in the case. Matching of eligible controls to cases was performed using incidence density sampling[14] as has been describe for the TEDDY Study[15] (Figure 3). A manuscript regarding the NCC protocol and analysis plan has been submitted for publication.

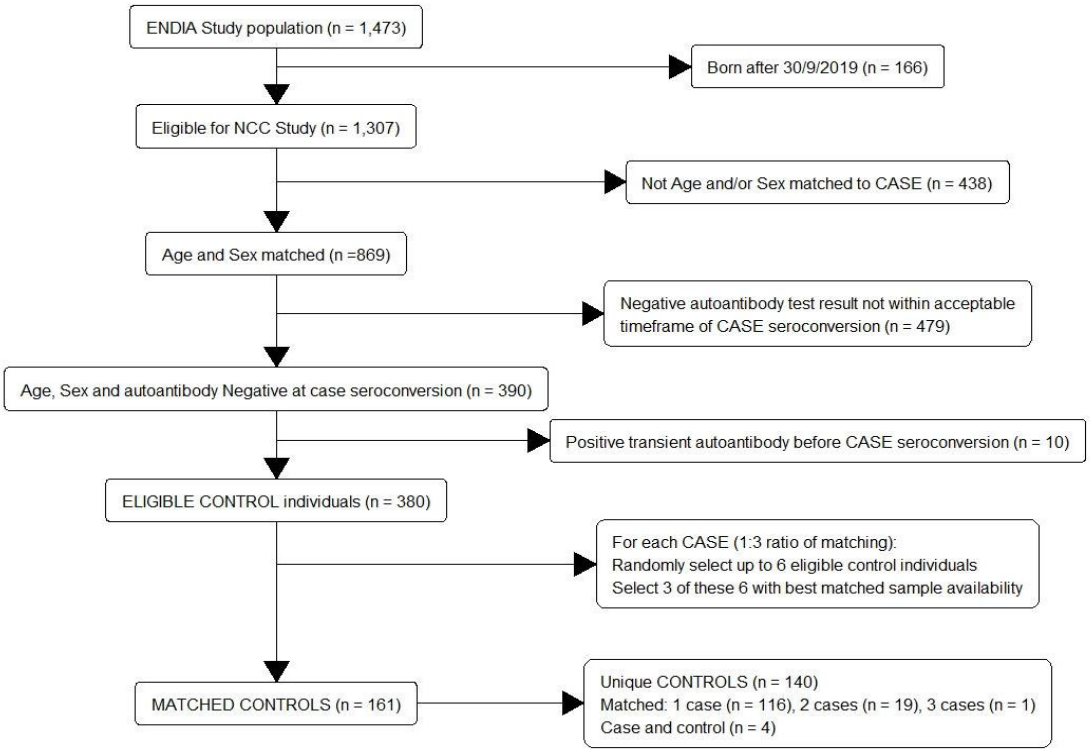

Figure 3. Flow diagram for inclusion of children in the NCC. 53 cases had 3 matched controls, 1 case had 2 matched controls.

Table 3. Composition of the ENDIA biobank at December 2022 (n=1,1511) with expected numbers of samples remaining for collection.

	Stool	Nasal	Tongue	Buccal	Throat	Mat skin	Inf skin	Vagina	Placenta	NBSC	Serum	Buffy	RBC	Plasma	PBMC	Urine	Breast milk	Baby teeth	TOTAL
Pregnancy	1671	2337	2341	2332	2258	1046	0	961	0	0	2180	476	2158	2172	1628	2261	0	0	
Birth	1479	913	910	911	846	1766	1723	0	305	509	829	154	731	751	532	471	1102	0	
3 m-2 years	6393	7893	7894	7853	7343	2111	7930	0	0	0	4272	679	3492	3686	2852	5300	2654	0	
2.5-5 years	2485	4022	4063	4037	3643	0	4092	0	0	0	2657	83	2299	2389	2219	3286	46	0	
5.5-10 years	590	1076	1082	1084	994	0	1088	0	0	0	789	6	731	746	726	1001	0	106	
Other family members	1601	0	0	0	0	0	0	0	0	0	0	236	852	1284	1628	0	0	0	
Current total	14219	16241	16290	16217	15084	4923	14833	961	305	509	10727	1634	10263	11028	9585	12319	3802	106	159046
Estimate remaining	*6848*	*9396*	*9431*	*9384*	*8665*	*1527*	*9482*	*0*	*0*	*0*	*5582*	*555*	*4717*	*4933*	*4193*	*6934*	*1953*	*914*	84515
Total expected	21067	25637	25721	25601	23749	6450	24315	961	305	509	16309	2189	14980	15961	13778	19253	5755	1020	243561
Completion	67%	63%	63%	63%	64%	76%	61%	100%	100%	100%	66%	75%	69%	69%	70%	64%	66%	10%	65%

Table 4. Composition of the ENDIA NCC biobank

	Stool	Nasal	Tongue	Buccal	Throat	Mat skin	Inf skin	Vagina	Placenta	NBSC	Serum	Buffy	RBC	Plasma	PBMC	Urine	Breast milk	Baby teeth	TOTAL
Pregnancy	245	302	301	302	297	146	0	135	0	0	288	120	285	287	149	294	0	0	
Birth	231	141	140	140	133	264	260	0	18	83	114	29	87	88	48	74	186	0	
3 m-2 years	775	904	904	897	890	317	904	0	0	0	683	200	612	626	393	662	383	0	
2.5-5 years	89	141	141	141	139	0	140	0	0	0	128	10	116	124	110	112	0	0	
Other family members	237	0	0	0	0	0	0	0	0	0	0	59	99	194	270	0	0	0	
Total	1577	1488	1486	1480	1459	727	1304	135	18	83	1213	418	1199	1319	970	1142	569	0	16587

5. Funded ENDIA projects

The past and current efforts within ENDIA can be divided into five key activity areas (Table 5).

Table 5. Key Activity Areas of ENDIA

Key Activity Area	Description of effort
1. Cohort follow-up	Recruitment and follow-up of the ENDIA cohort encompasses the research coordinators, research officers, project and data management team, all consumables required for sample/data collection at visits, clinical tests including for islet autoantibodies, and other direct research costs. The original ENDIA protocol[2] was modified to incorporate a Regional Participation Program[16] and framework for the conduct of visits during COVID-19[17]. We have also published on the ENDIA recruitment strategy[18] and method development[19-22]. *Funded projects (unpublished)* • ENDIA cohort profile • Experiences of caregivers and children in ENDIA • HLA typing of the ENDIA cohort
2. Impact of T1D in pregnancy on risk and protection	As 62% of ENDIA recruits are women with T1D, we have compared mothers with and without T1D in pregnancy, and the impact on their offspring, including investigation of the virome[23, 24], microbiome[25] and mycobiome[26]. Understanding distinct omics changes associated with T1D during pregnancy is relevant to the concept of "maternal protection" and has generated new hypotheses in this area. *Funded projects (unpublished)* • Maternal conditioning of the infant immune system • Maternal–infant transmission of the microbiome • Vaginal microbiome and mycobiome in pregnancy • Oral microbiome in pregnancy • Full length enterovirus sequencing in ENDIA children
3. Early life determinants of IA	The ENDIA NCC study has been established to investigate preliminary associations between omics signatures and biomarkers (including pancreatic exocrine function[27, 28]) during pregnancy and early-life, and IA risk. Publication of the statistical analysis plan of the NCC is in preparation. Future studies will involve two designs: (i) a case-control analysis comparing cases with all non-cases in the total cohort (n=1473), and (ii) a case-cohort study incorporating all cases and a control group selected as a subset of all participants at baseline (n≈450). *Funded projects (unpublished)* • Gut microbiome • Gut metaproteome and metabolome • Gut and respiratory virome • Viral serology • Plasma proteome, lipidome, glycome, cytokines, miRNAs • Immunogenomics of peripheral and cord blood T cells • Genome array profiling of the NCC by Immunochip • Impact of weight gain in early life
4. Improving care and outcomes for people with T1D	Data on pregnancy outcomes for women with and without T1D recruited Australia-wide have provided opportunities to examine mental health[29], nutrition and exercise, and mechanisms associated with pregnancy complications including hypertensive disorders of pregnancy. The ongoing CGM project in IA cases and matched controls provides the first international data in young children to understand the value of CGM in the care of children progressing to T1D and is extended beyond insulin initiation. *Funded projects (unpublished)* • Maternal and fetal outcomes in relation to maternal nutrition in T1D • Associations between the gut microbiome and hypertensive disorders of pregnancy • CGM in ENDIA • Antibody screening in the home using dried blood spots
5. Contributions beyond T1D	ENDIA children have coeliac autoantibodies measured serially from 3 months of age. Children referred for duodenal biopsy provide tissue for future research.

6. References

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