Untangling the Relationships Between Autism Spectrum Disorder and Non-Genetic Risk Factors

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ABSTRACT

Autism spectrum disorder (ASD) has been attributed to genetic and non-genetic risk factors. Of the non-genetic factors, prenatal and perinatal complications have been extensively investigated, though few associations have been replicated consistently. We selected 2,562 families with at least one individual with ASD and one unaffected sibling. We investigated the relationships between 29 prenatal and perinatal complications and ASD, while considering the influences of confounding factors, comorbid conditions, and different ASD definitions. Although many complications were associated with ASD in the pairwise comparisons, only haematological disorders of the newborn and lower Apgar scores remained significant after adjusting for the effects of the confounders. After removing individuals with congenital anomalies, only 5-minute Apgar scores were associated with ASD. In conclusion, after considering confounding effects and four ASD definitions, several perinatal complications were associated with ASD with moderate effect sizes. Furthermore, comorbid conditions with ASD appear to be intertwined in these relationships.

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DISCLAIMER

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LIST OF ABBREVIATIONS

5mC	5-methylcytosine
5hmC	5-hydroxymethylcytosine
5fC	5-formylcytosine
5caC	5-carboxylcytosine
ADDM	Autism and Developmental Disabilities Monitoring
ADI	Autism Diagnostic Interview
ADI-R	Autism Diagnostic Interview-Revised
ADOS	Autism Diagnostic Observation Schedule
ASD	Autism Spectrum Disorder
BER	Base Excision Repair
CI	Confidence Interval
CIHI	Canadian Institute for Health Information
CNV	Copy Number Variation
DNA	Deoxyribonucleic Acid
DNMTs	DNA Methyltransferases
DSM	Diagnostic and Statistical Manual of Mental Disorders
DZ	Dizygotic
EIBI	Early Intensive Behavioural Intervention
FXS	Fragile X Syndrome
ICD	International Classification of Diseases
ICD-9-CM	International Classification of Diseases, Clinical Modification
ICD-10-CA	International Classification of Diseases, Canadian Modification
IQ	Intelligence Quotient
LCL	Lower Confidence Limit
MCHP	Manitoba Centre for Health Policy
mRNA	Messenger Ribonucleic Acid
MZ	Monozygotic
OR	Odds Ratio
PBMC	Peripheral Blood Mononuclear Cells
PHIN	Personal Health Identification Number
PTM	Post-Translational Modification
RNA	Ribonucleic Acid
RTT	Rett's Syndrome
SD	Standard Deviation
SFARI	Simons Foundation Autism Research Initiative
TDG	Thymine DNA Glycosylase
TET	Ten-Eleven Translocation (Proteins)
UCL	Upper Confidence Limit

1. INTRODUCTION

1.1. AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that is characterized by impairments in social communication and interactions as well as the presence of restricted and repetitive activities, interests, and behaviours[1]. Impairments in social interactions and communication can include deficits in nonverbal behaviours (e.g. a lack of or abnormal eye contact, gestures, and facial expressions), lack of shared emotion, and difficulties in developing relationships. Restricted and repetitive activities, interests, and behaviours include stereotyped motor movements (e.g. hand flapping), repetitive speech, strict routines, resistance to change, and abnormal intensity and focus on specific interests (e.g. a strong attachment to an object). The initial symptoms of ASD typically appear between 12 and 24 months of age and may include abnormal joint attention and imitation, delayed babbling, and limited toy play[2]. Although ASD is characterized by two groups of symptoms, it is clinically heterogeneous; as its name implies, ASD collectively describes a broad spectrum of phenotypes with high variability in symptomology and severity[1], even among multiple individuals with ASD from the same family[3].

1.1.1. Diagnosis and Assessment

To date, many assessment tools have been developed to make or verify an ASD diagnosis, as described below. Notably, many of these assessments can be applied in both clinical and research settings.

1.1.1.1. Diagnostic and Statistical Manual of Mental Disorders

The Diagnostic and Statistical Manual of Mental Disorders (DSM) provides criteria for diagnosing ASD and other mental disorders[1]. In the previous edition of the DSM, the DSM-IV, autism spectrum disorder was called Pervasive Developmental Disorders[4]. This group of disorders included Autistic Disorder, Rett's Syndrome, Other Childhood Disintegrative Disorder, Asperger's Syndrome, and Pervasive Developmental Disorder Not Otherwise Specified, which differed in symptomology, severity, age of onset, and male to female ratio. In the DSM-IV, these disorders were defined by three groups of symptoms: social impairments, communication impairments, and the presence of restricted and repetitive activities, interests, and behaviours.

In the most recent edition of the DSM, the DSM-5, autism spectrum disorder is an umbrella disorder that encompasses individuals with varying degrees of severity[1]. Under the DSM-5, clinicians use two criteria to determine if an individual should receive a diagnosis of ASD: 1) impairments in social communication and social interaction are present, 2) restricted and repetitive activities, interests, or behaviours are present. Furthermore, these criteria must be qualified by the following three conditions: 1) symptoms are present during early development, 2) deficits in functioning (social, occupational, or otherwise) occur as a result of these symptoms, and 3) intellectual disability or developmental delay do not account for these symptoms. According to the severity of his/her impairments within the first two criteria, an individual is further categorized as level 1: requiring support; level 2: requiring substantial support; or level 3: requiring very substantial support.

1.1.1.2. Autism Diagnostic Observation Schedule

The Autism Diagnostic Observation Schedule (ADOS) is a gold standard diagnostic test for ASD that is designed to assess an individual's social communication, imaginative use of objects, and play[5]. The ADOS has four modules, each specific to a different level of language ability and developmental stage. A trained professional observes the individual during various activities (specific to each ADOS module) and scores his/her behaviours and symptoms on a scale from 0 to 2, where 0 indicates no evidence of abnormality and 2 indicates definite evidence of abnormality. Occasionally, an individual may receive a score of 3 for an activity, which indicates that the abnormality is so severe that it interfered with the observation. The scores are summed separately for the social behaviour and communication sections and then combined for the entire assessment; a score of 3 is converted to a 2 when summarizing the scores. Cut-off scores are provided for both autism and autism spectrum diagnoses. If an individual's scores exceed the cut-offs, this suggests the presence of classical autism or autism spectrum disorder, though clinical judgement is used to interpret the results.

1.1.1.3. Autism Diagnostic Interview-Revised

The Autism Diagnostic Interview-Revised (ADI-R) is an interview that is administered to primary caregivers of individuals who are suspected to have ASD[6]. The original ADI was designed to assess children five years or older with a mental age of at least two years. The ADI-R is a revised version that is designed to also assess younger children and to be more specific to impairments associated with ASD. The questions in the ADI-R are divided into five sections: 1) opening questions about early development and medical and family history, 2) questions regarding communication, 3) questions about social development and play, 4) questions

regarding restricted and repetitive behaviours, and 5) questions about behavioural problems. Based on the caregiver's description of the individual's symptoms, the interviewer assigns a score to each question. Similar to the ADOS, the scores usually range from 0 to 2, where a score of 0 indicates that the behaviour is not present, while a score of 2 indicates definite presence of the behaviour; a score of 3 may be assigned for extremely severe behaviours, but is converted to a 2 when totalling the scores. Cut-off scores are provided for ASD and were observed to have both high sensitivity and specificity (i.e. >0.90) when compared to the best estimate diagnosis for ASD. Similar to the ADOS, the ADI-R is a gold standard diagnostic test for ASD that is used in combination with informed clinical judgement.

1.1.1.4. Other Assessments

There are many other assessments that may be used to evaluate an individual's symptoms and/or to verify a diagnosis of ASD. Examples of other diagnostic tools include, but are not limited to: the Childhood Autism Spectrum Test[7], the Wechsler Abbreviated Scale of Intelligence[8], the Expressive and Receptive One-Word Picture Vocabulary Tests[9,10], and the Social Communication Questionnaire[11]. Some of these assessment tools (including the ADOS and ADI-R) were designed according to the diagnostic criteria outlined in the DSM-IV.

1.1.2. Prevalence

There have been a few recent estimates of the prevalence of ASD in Canada. Using data from 2002, Ouellette-Kuntz *et al.* estimated the prevalence of ASD in children 14 years of age or younger to be 28.4 per 10,000 (95% CI: 26.1-30.8) and 35.2 per 10,000 (95% CI: 28.2-43.4) in Manitoba and Prince Edward Island, respectively[12]. Using an administrative dataset, Brownell *et*

al. estimated the prevalence of ASD among children aged five to nine years in Manitoba to be 49 per 10,000 between 1996/1997 and 2000/2001[13]. Between 2001/2002 and 2005/2006, the prevalence was estimated to be 88 per 10,000, a two-fold increase above that during the previous time period. When the sample was expanded to include individuals 19 years of age or younger, the prevalence estimates were 30 per 10,000 from 1996/1997 to 2000/2001 and 60 per 10,000 from 2001/2002 to 2005/2006, again representing a doubling during this timespan. Using special education funding information from 2003, Fombonne *et al.* estimated the prevalence of ASD among school-aged children to be 64.9 per 10,000 (95% CI: 55.8-75.0) in Quebec[14].

An apparent increase in the prevalence of ASD has been reported consistently in the literature. The Autism and Developmental Disabilities Monitoring (ADDM) Network, which is funded by the Centers for Disease Control and Prevention, is a surveillance program that estimates the prevalence of developmental disabilities (including ASD) in the United States. The ADDM estimated the prevalence of ASD to be 66.7 per 10,000 8-year-old children in 2002[15], 90.9 per 10,000 in 2006[16], 113.6 per 10,000 in 2008[17], and 147.1 per 10,000 in 2010[18]. The prevalence estimates for ASD in Canada were similar to the estimates in the United States for similar aged children during similar time periods (i.e. 49 per 10,000 in 1996/1997 to 2000/2001 to 88 per 10,000 in 2001/2002 to 2005/2006 for children aged 5 to 9 years old in Manitoba[13] compared to 67 per 10,000 for 8-year-old children in 2002 in the United States[15]). Comparisons of the prevalence estimates from other countries are needed in order to assess whether the trend is worldwide.

Multiple explanations have been proposed to account for a higher incidence and prevalence of ASD. The ADDM Network postulated that an increase in the number of higher intellectual ASD cases may account for some of the apparent increase in ASD prevalence[18]. For example, in a study using the data from the National Survey of Children's Health, a significant proportion of children aged 10 to 17 were diagnosed with ASD at seven years of age or later and more than half of these children were reported to have "mild" ASD (based on parental report)[19]. Diagnostic substitution (e.g. intellectual disability) has also been hypothesized to account for the increase in ASD cases in recent years. However, only some studies have observed a decline in cases of intellectual disability concurrent with the increase in ASD cases[20], while others have observed a relatively stable prevalence of intellectual disability[21]. Other factors that have been theorized to explain the apparent increase in the prevalence of ASD include increased public awareness, changes in special education policies, and increased availability of services to individuals with ASD and their families[22,23]. Advanced maternal and paternal ages have also been associated with ASD and a trend of older parents has been observed in recent years[24-26].

Interestingly, the sex ratio of ASD is approximately 4:1 for males versus females[1]. Though the reasons behind the sex difference are largely unknown at this time, several potential mechanisms have been hypothesized to account for the sex difference among individuals with ASD. It has been proposed that a female protective effect may exist, resulting in an increased liability threshold in females compared to males[27]. Genetic and epigenetic effects from the sex chromosomes have also been conjectured to contribute to the sex difference in ASD; specifically, the hormonal effects associated with regions on the Y chromosome (e.g. SRY) may increase susceptibility in males, while epigenetic mechanisms in females (i.e. skewed X chromosome

inactivation and increased X chromosome dosage for genes escaping X inactivation) may contribute to their lower susceptibility[28]. However, most of the known ASD candidate genes are on the autosomes, thus genes on the sex chromosomes may only account for a small fraction of the risk for ASD. Hormone activity and maternal immune activation during the prenatal period have also been implicated in ASD and are thought to affect males and females differently[28]. Last, biases in the diagnostic criteria for ASD and differences in the core symptoms of ASD between males and females may lead to underdiagnosis of ASD in females[27,28]. For example, females with ASD tend to show increased social behaviours, more language development, and fewer or different repetitive behaviours than males with ASD. At this time, the reasons underlying the sex difference in ASD remain largely unknown, though it seems likely that multiple factors are involved.

1.2. GENETIC RISK FACTORS

1.2.1. Heritability

Heritability is the proportion of variance in a trait that is due to genetic variation[29]. Often, twin studies are used to estimate the heritability of a disease or trait. Identical or monozygotic (MZ) twins result from the splitting of one zygote to form two separate zygotes; as such, MZ twins theoretically share 100% of their genetic variants[30]. Alternatively, fraternal or dizygotic (DZ) twins are formed when two eggs are fertilized, leading to the formation and development of two separate zygotes. DZ twins share approximately half of their genetic variants, as is the case for siblings. If a disease is caused by genetic factors, its concordance rate (i.e. both twins are affected) will be greater in MZ twins than that in DZ twins because of the differences in shared genetic material between the twin types[29].

Twin studies have been used to estimate the heritability of both classical autism and ASD. Early studies reported very high concordance rates in MZ twins and low concordance rates in DZ twins, yielding heritability estimates of greater than 90%[31-33]. In more recent studies, the heritability estimates varied considerably, ranging from 21% to 95%[34-38]. Additionally, the sample sizes for some of these studies were small, leading to large confidence intervals for the heritability estimates[34,36]. In a recent meta-analysis, Tick *et al.* combined the results from seven twin studies to estimate the heritability of ASD[39]. Using six different models, which varied in the underlying prevalence assumption and the ASD phenotype (e.g. narrow or broad), the combined heritability estimates ranged from 64% to 91%. Recently, Sandin *et al.* conducted the largest heritability study for ASD (n=2,049,973) by including twin pairs, siblings, and first-cousins[40]. This study yielded heritability estimates of 50% (95% CI: 45%-56%) for ASD and 54% (95% CI: 44%-64%) for classical autism, which were lower than the estimates in most of the previous studies. Additionally, the 95% confidence intervals were much narrower than those in previous studies due to the large sample size. However, the major limitations of this study were that the ASD diagnoses were not validated and that it was a family study rather than a twin study, which is the gold standard for estimating heritability. Discrepancies among the heritability estimates from previous studies may be due to differences in: the case ascertainment methods (e.g. population-based or clinic-based), the diagnostic criteria used to define the ASD sample (e.g. narrow, broad, or subclinical), and the statistical methods employed[37,38]. Despite these disparities, the results from the previous heritability studies indicate that both genetic and non-genetic factors likely contribute to the risk for ASD. Currently, the non-genetic component is thought to include both epigenetic and environmental risk factors.

1.2.2. Candidate Genes and Loci for ASD

Approximately 10% of ASD cases are syndromic, meaning that the ASD phenotype is part of a known genetic syndrome[41]. Examples of these syndromes include tuberous sclerosis (caused by mutations in *TSC1* or *TSC2*), neurofibromatosis type 1 (due to mutations in *NF1*), and fragile X syndrome (associated with more than 200 triplet expansions in *FMR1*).

Different from syndromic ASD cases, a few idiopathic ASD cases have been associated with rare *de novo* and inherited genetic variants in several groups of genes, including synaptic genes (e.g. neuroligins and neurexins), genes regulating morphogenesis and growth (e.g. *HOX1A*), and genes regulating calcium homeostasis (e.g. *CACNA1C*)[41]. These rare variants are estimated to account for approximately 5% to 10% of ASD cases[42]. Besides rare variants, Klei *et al.* estimated that additive effects of common variants may explain approximately 40% to 60% of the variance in the liability for ASD[43], which would account for a significant proportion of the "missing" heritability in ASD. Numerous studies have aimed to identify both common and rare genetic variants that are associated with ASD, but these studies have yielded largely inconsistent results.

Besides single nucleotide variants, known copy number variations (CNVs) and other chromosomal rearrangements have been reported to be present in approximately 10% to 20% of ASD cases[42]. CNVs are DNA segments between 50 base pairs and several megabases in size that differ in the number of copies present between individuals[41]. Examples of genomic loci where CNVs have been associated with ASD include 16p11.2, 1q21.1, and 15q11.1-13.3[44]. Some CNVs that have been associated with ASD may be *de novo*, while others may be inherited from a parent; CNVs that are inherited from an unaffected parent suggest that some ASD-associated

CNVs may exhibit incomplete penetrance, further complicating efforts to identify these genetic risk factors[41].

Even though many genes have been reported to be associated with ASD in previous studies, the supporting evidence for each gene varies greatly. Several databases, such as the Simons Foundation Autism Research Initiative (SFARI) Gene database[45,46], AutismKB[47], and the Human Gene Mutation Database[48], are commonly used to identify the ASD candidate genes with high confidence. For example, SFARI Gene employed a gene scoring process to evaluate the evidence for over 500 genes implicated in ASD[49]. By combining information about sample size, statistical significance, replication across studies, functional consequences, and other characteristics of the previous studies, each gene was assigned to one of seven categories in the SFARI Gene database (Table 1.1). Fewer genes were assigned to the high and strong confidence candidate gene categories compared to the lower-ranked categories, indicating that there is limited evidence for most ASD candidate genes at this time[46]. SFARI has also created a database of more than 2,000 CNV loci that have been associated with ASD in previous studies[50], however a scoring process (similar to that for genetic variants) has not been developed.

Table 1.1. Genes classified using the SFARI Gene Scoring Module[49]

SFARI classification	# genes	Examples
S: Syndromic[a]	66	FMR1, MECP2, NF1, PRKD1
Category 1: High confidence	16	ANK2, GRIN2B, SCN2A, TBR1
Category 2: Strong candidate	38	FOXP1, NRXN1, RELN, SHANK2
Category 3: Suggestive evidence	110	AUTS2, FOXP2, OXTR, UBE3A
Category 4: Minimal evidence	210	ATRX, EN2, GABRA4, RAB39B
Category 5: Hypothesized but untested	116	ATG7, DLX1, MAP2, ROBO1
Category 6: Not supported	19	BCL2, GRM8, MED12, XIRP1

[a]If evidence suggested that a gene may also be associated with idiopathic autism, a numeric classification was also assigned

Many known ASD candidate genes (e.g. the genes in the SFARI Gene database) have exhibited modest effect sizes. Devlin *et al.* predicted that most common variants (which have been conjectured to account for approximately 40% to 60% of the liability in ASD[43]) will have odds ratios between 1.1 and 1.2 (or the inverse ratio), while a few may have odds ratios between 1.2 and 1.5[51]. Additionally, they predicted that no common variants would have an odds ratio greater than 1.5. Genetic studies require very large sample sizes in order to detect common variants associated with ASD with such modest effect sizes; accordingly, few associations with these variants have been replicated between independent studies.

1.2.3. Genetic Heterogeneity

ASD is a genetically heterogeneous disorder, as illustrated by the many genes that have been associated with ASD, in combination with the largely inconsistent results from previous genetic studies. Furthermore, few, if any, ASD genes are expected to account for more than 1% of ASD cases[42]. Due to the clinically and genetically heterogeneous nature of ASD, examining more homogeneous groups of cases (e.g. individuals with similar symptomology or individuals without exposure to environmental risk factors that have been associated with ASD) may help to untangle the genetic risk factors for ASD in future studies.

1.3. EPIGENETIC RISK FACTORS

Epigenetics is the study of changes in gene expression that are not related to changes in the DNA sequence[52]. Epigenetic mechanisms include DNA methylation, histone modifications, and non-coding RNA. Epigenetic factors have been implicated in ASD because autistic symptoms are present in some Mendelian disorders (e.g. fragile X and Rett's syndromes) that have an

epigenetic aetiology[53]. Fragile X syndrome (FXS) is caused by large CGG triplet expansions in the 5' untranslated region of the fragile X mental retardation gene 1 or *FMR1* (located at Xq27.3)[54]. Normally, individuals have approximately 5 to 40 CGG repeats; 50 to 200 repeats are considered to be premutations, while more than 200 repeats are considered to be full mutations. The full mutation usually results in hypermethylation of the *FMR1* promoter, leading to inactivation of *FMR1* and the FXS phenotype. Rett's Syndrome (RTT) is characterized by autistic-like traits and was classified as a pervasive developmental disorder in the DSM-IV[4]. The majority of Rett's Syndrome cases are caused by loss-of-function mutations in the methyl-CpG binding protein 2 gene or *MECP2* (located at Xq28)[55,56]. MeCP2 contains a methyl binding domain, allowing it to bind to methylated cytosines, and a transcription repressor domain, which binds to AT-rich DNA[57]. As such, MeCP2 is thought to be involved in various biological processes, including transcription regulation, chromatin remodelling, RNA splicing, and microRNA regulation.

Epigenetic factors have also been linked to ASD because some MZ twins are discordant for ASD (i.e. only one twin is affected or each twin has different ASD-related traits[3]). Although MZ twins share the same genetic variants and similar prenatal and postnatal environments, previous studies have demonstrated that MZ twins can differ significantly in terms of epigenetics[58-63]; as such, some phenotypic differences between MZ twins may be due to epigenetic factors. Because MZ twins are matched for age, genetics, and early environment, discordant MZ twins are the ideal research subjects to investigate epigenetic contributors to disease. However, these studies are often underpowered due to the limited number of MZ twins who are discordant for some disorders, such as ASD.

1.3.1. DNA Methylation

DNA methylation is an epigenetic mechanism by which DNA methyltransferases add a methyl group to the fifth carbon position in cytosine, resulting in 5-methylcytosine (5mC) (Figure 1.1)[64]. In addition to 5mC, there are three other cytosine modifications, specifically 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), which can be generated from 5mC by TET (ten eleven translocation) proteins[65]; however, most research to date has focused on 5mC and 5hmC. DNA methylation typically occurs at CpG dinucleotides; approximately 60% to 90% of the CpGs in mammalian genomes are methylated[64]. Alternatively, CpG islands (genomic regions greater than 200 base pairs with a GC content greater than 50% and an observed to expected ratio of CpG greater than 0.6 that tend to occur at the promoters of housekeeping and developmental genes[66]) are usually not methylated. 5mC in the promoter region is typically associated with gene repression, while 5mC in the gene body may be associated with gene activation, depending on cell type and cell activity[67]. Opposed to 5mC, 5hmC is largely euchromatic and tends to be associated with increased gene expression[68]. It is also thought that 5hmC may block methyl binding proteins, relieving the effects of 5mC. While 5mC levels are similar among adult tissues, the highest levels of 5hmC are found in adult brain. The complex relationship between 5mC and 5hmC, as well as the cell-type specificity of 5hmC, indicate that it is important to investigate both 5mC and 5hmC levels in relation to ASD.

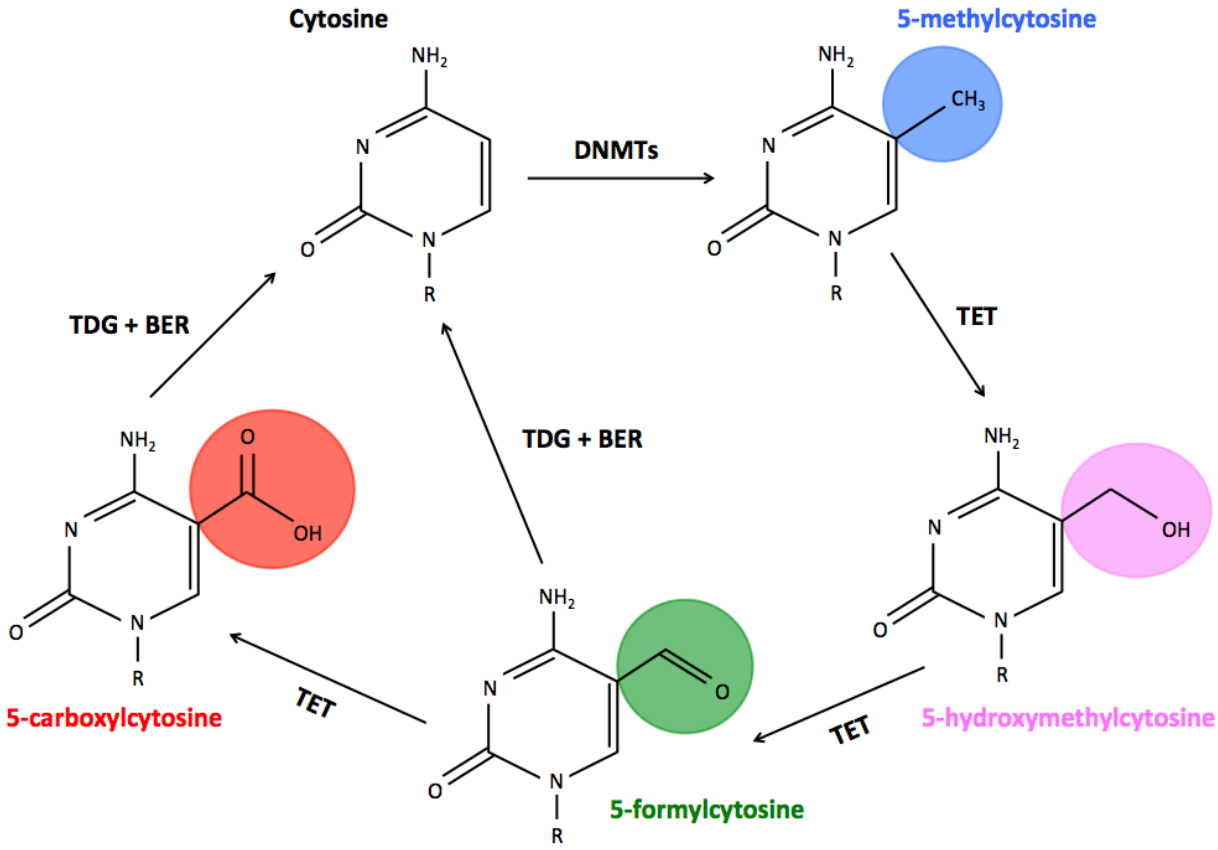


Figure 1.1. Pathways by which DNA methylation and other DNA modifications are made (based on Pastor, Aravind, and Rao[69]). DNMTs: DNA methyltransferases; TET: ten-eleven translocation proteins; TDG: thymine DNA glycosylase; BER: base excision repair.

To date, several studies have illustrated associations between DNA methylation levels and ASD disease status[70-74], including two twin studies[75,76]. To supplement their DNA methylation data, several of these groups also examined the correlations between DNA methylation and gene expression levels at differentially methylated sites. Three studies used brain samples, while four studies used blood or buccal cells. Collectively, these studies reported associations between ASD or ASD-related traits and DNA methylation levels at genes that have been previously associated with ASD (e.g. *NLGN2*[76] and *c11orf21/TSPAN32*[73]) or are involved in biological pathways that have been implicated in ASD (e.g. inflammation and synaptic regulation[75]), in addition to novel

associations (e.g. *RORA*[75]). In one of the twin studies, Wong *et al.* reported that most sites with large differences in DNA methylation levels between the twin with ASD and his/her unaffected co-twin were family-specific[76]. In combination with the fact that few associations have been replicated between studies, Wong *et al.*'s observation suggests that ASD may also exhibit epigenetic heterogeneity.

1.3.2. Histone Modifications

To package DNA compactly in a cell, DNA wraps around histone octamers to form nucleosomes, which are then further compacted into a 30 nm chromatin fiber[77]. The histone N- and C-terminal tails extend outside of the nucleosome, facilitating interactions with other histones, including those on other nucleosomes, which can aid in chromatin compaction. Post-translational modifications (PTMs), which are covalent modifications of the amino acids in histones, often occur on the histone tails; as such, PTMs can modulate interactions among nucleosomes, chromatin binding proteins, and co-factors to affect chromatin remodelling[78]. Examples of PTMs include the acetylation of lysine residues (facilitated by histone acetyltransferases and histone deacetylases), phosphorylation of serine, threonine, and tyrosine residues (facilitated by kinases and phosphatases), and methylation of lysine and arginine residues (facilitated by methyltransferases and demethylases), among others.

Several studies have examined the relationships between ASD and histone PTMs. For example, Shulha *et al.* compared H3K4me3 marks (trimethylated form of the lysine 9 residue on the histone H3, which is usually found at transcription start sites and is often associated with gene activation) in prefrontal cortex neurons between ASD cases and controls[79]. They observed that a

subset of the cases exhibited H3K4me3 marks extending bidirectionally from the transcription start sites (i.e. towards the gene bodies and the upstream promoter sequences). Altered levels of H3K3me3 were also associated with altered gene expression at some loci, potentially representing a mechanism by which the expression levels of some ASD candidate genes may be changed. James *et al.* examined DNA methylation, gene expression, and protein levels, and H3K4me3 and H3K27me3 marks (trimethylated form of the lysine 27 residue on the histone H3, which is usually associated with gene repression) for *EN2* (an ASD candidate gene) in cerebellum samples from autistic cases and controls[80]. Although the *EN2* promoter was hypermethylated in the autistic brains (which would typically be associated with decreased gene expression), *EN2* expression and protein levels were increased compared to the controls. However, they also observed that, compared to the controls, H3K27me3 was decreased at the *EN2* promoter in the autistic brains, while H3K4me3 was increased, which may have contributed to the observed changes in the gene expression and protein levels. Last, several ASD candidate genes from the SFARI database are known to be involved in the addition and maintenance of histone PTMs; among these are *HDAC4*, a histone deacetylase, and *EHMT1*, a histone methyltransferase[49], further suggesting that histone modifications may be important in establishing and/or maintaining epigenetic marks that are associated with ASD.

1.3.3. Non-Coding RNA

In recent years, researchers have examined the role of non-coding RNAs in ASD. Non-coding RNAs are not translated into proteins but instead function as RNA molecules[81]. Non-coding RNAs that are less than 200 nucleotides are considered to be short, while those that exceed 200 nucleotides are long. Short non-coding RNAs can be further divided into subgroups, such as

microRNAs, short interfering RNAs, and Piwi-interacting RNAs, among others. Short non-coding RNAs are typically involved in mRNA degradation, translation arrest, and chromatin-dependent gene silencing[82]. Subgroups of the long non-coding RNAs include long intergenic non-coding RNAs and natural antisense transcripts, as examples[81]. The long non-coding RNAs regulate gene activity by a number of different mechanisms, including through mRNA degradation, regulation of cellular transport, and chromatin remodelling[82]. Importantly, both short and long non-coding RNAs are thought to interact with other players involved in epigenetic programming and to mediate epigenetic mechanisms (i.e. DNA methylation and histone PTMs)[83].

As an example, many long non-coding RNAs were observed to be differentially expressed between ASD cases and controls in both brain (specifically prefrontal cortex and cerebellum)[84] and peripheral blood cells[85]. Some of these long non-coding RNAs were associated with pathways that have been previously implicated in ASD, including neurological regulation and inflammation[85]. While the results from these studies, as well as others, illustrate that non-coding RNAs may be involved in ASD, further research is required to elucidate the downstream effects of these non-coding RNAs and their interactions with other epigenetic mechanisms.

Taken together, researchers have made some progress in understanding the relationships between epigenetic risk factors and ASD. Importantly, future epigenetic research may help to identify the genes that are associated with ASD, as well as elucidate the mechanisms by which environmental risk factors contribute to the ASD phenotype. Future studies should consider and improve upon the limitations of the currently published research, including sample sizes, the

relevance of the biological samples used, and the technology employed (e.g. the number of CpG sites examined or the ability to differentiate between 5mC and 5hmC).

1.4. ENVIRONMENTAL RISK FACTORS

1.4.1. Prenatal and Perinatal Complications

Of the environmental risk factors for ASD, prenatal and perinatal complications have been extensively investigated. In these types of studies, the prenatal period is typically defined as the full intrauterine period (i.e. conception to birth)[86], while the perinatal period is defined as the period from birth to four weeks after birth[87]; the first 30 days after birth may also be referred to as the neonatal period[86] (Figure 1.2). Because the initial symptoms of ASD typically appear between 12 and 24 months of age[1], exposure to any causal environmental insults must occur prior to this time period. Thus, studies of exposure to prenatal and perinatal complications are especially relevant to ASD. Additionally, the prenatal and perinatal periods are a critical time for neurodevelopment, which is significantly altered in individuals with ASD[88].

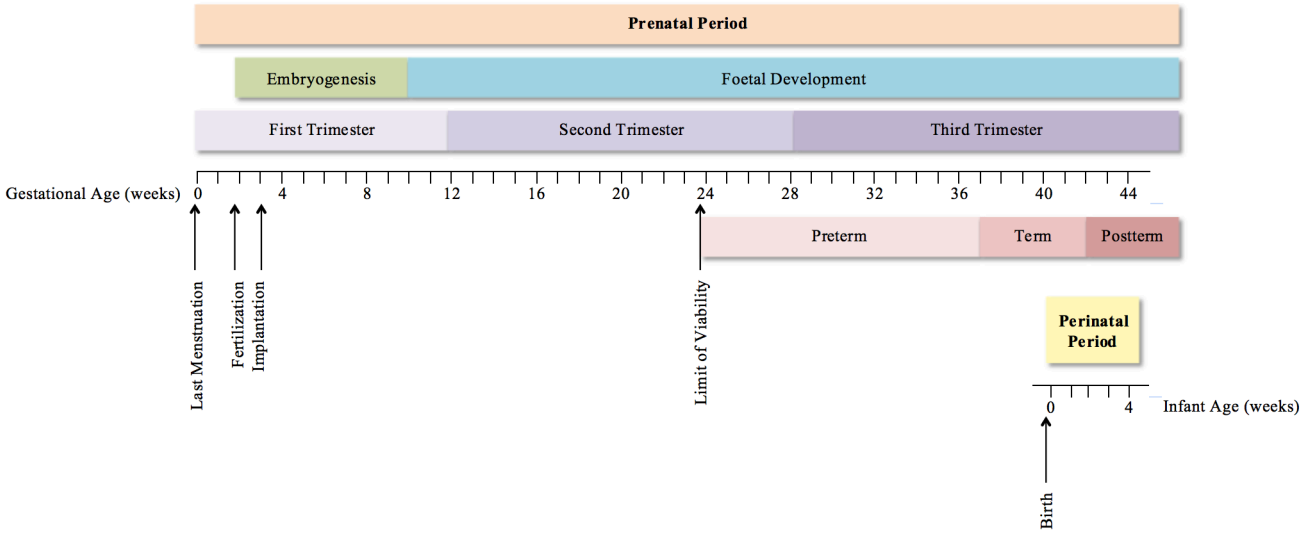


Figure 1.2. Timeline of the prenatal and perinatal periods (created based on information from Callahan and Caughey[89])

Due to the large number of studies that have investigated the relationships between ASD and prenatal and perinatal complications, some groups have combined the results from previous studies in meta-analyses. For example, Gardener *et al.* conducted two such meta-analysis studies, each with the results from 40 previous studies[90,91]. Among the prenatal complications and familial risk factors that were investigated, they found that advanced maternal and paternal age, being born first versus third or later, gestational diabetes, maternal medication use during pregnancy, and maternal bleeding were associated with an increased risk for ASD[90]. The estimated effect sizes, which were based on the relative risks from previous studies, ranged from 1.04 to 2.07. The largest estimated effects were observed for gestational diabetes (2.07; 95% CI: 1.24-3.47) and maternal age greater than or equal to 40 compared to maternal age less than 30 (2.06; 95% CI: 1.48-2.86). Among the perinatal and neonatal complications, abnormal or breech presentation, cord complications, foetal distress, birth injury or trauma, multiple births, maternal haemorrhage, summer births, congenital malformations, low 5-minute Apgar scores, feeding difficulties, aspirated meconium, neonatal anaemia, ABO or Rh incompatibility, and hyperbilirubinemia were associated with an increased risk for ASD[91]. The largest effect sizes were observed for neonatal anaemia (7.87; 95% CI: 1.43-43.36), aspirated meconium (7.34; 95% CI: 2.30-23.47), and birth injury or trauma (4.90; 95% CI: 1.41-16.94). More recent studies have also reported associations between ASD and a range of prenatal and perinatal complications, including threatened abortion, respiratory distress, markers of hypoxia, jaundice, intracranial haemorrhage, and short inter-pregnancy interval[92-95]. To a large extent, the results reported in previous studies have varied widely. However, there are consistent findings for some risk factors, such as preterm births, low birth weight, and advanced parental ages. Additionally, many

previous studies have observed that more perinatal complications were associated with ASD and had stronger effect sizes than prenatal complications.

Preterm births were associated with ASD in many previous studies[96-98], though gestational age was not associated with ASD in Gardener *et al.*'s meta-analysis[91]. Additionally, a higher prevalence of ASD was reported among children born extremely preterm (23 to 27 weeks) compared to the prevalence in the general population[99]. Many previous studies chose arbitrary cut-offs to categorize gestational age (e.g. <37 weeks as preterm births versus ≥37 weeks as term births); however, dichotomizations of gestational age may not be aetiologically relevant. To this end, Leavey *et al.* compared the risk for ASD at each gestational age between 23 to 42 weeks to the combined risk for all later gestational ages (e.g. 23 weeks versus >23 weeks) using over 200,000 singleton births in Alberta, Canada[100]. They observed that there was a gradual increase in the risk for ASD as gestational age decreased, especially for gestational ages between 29 and 37 weeks. It is well-known that more preterm and low birth weight children survive than in previous years due to advanced medical treatments. As such, Atladottir *et al.* examined the associations between gestational age and ASD over a 30-year time period (1980 to 2009) using approximately 1.7 million births in Denmark[101]. They observed that shorter gestational age was associated with a higher risk for ASD across each 10-year time period. However, despite the increase in premature births throughout the study period, the strongest association between preterm births and ASD was in 1980 to 1989, while more ASD cases were born at term in the more recent time periods. Last, some groups have examined the effect of gestational age on specific ASD symptoms and symptom severity. For example, using an online research database in the United States, Movsas and Paneth observed that individuals who were born pre- or post-

term had higher scores on diagnostic tests for ASD (indicating more social deficits and other characteristic symptoms of ASD) than children who were born at term[102]. Additionally, preterm individuals tended to be older when they took their first steps or spoke their first words, and were more likely to have lower IQ and exhibit self-injurious behaviours than children born at term.

Low birth weight has also been consistently associated with ASD[103-105], including in Gardener *et al.*'s meta-analysis[91]. Similar to the results reported by Joseph *et al.* for gestational age[99], Pinto-Martin *et al.* reported a higher prevalence of ASD among children born at low birth weights compared to children born at normal birth weights[106]. Additionally, in a twin population, Losh *et al.* reported that in 24 of 36 same-sex twin pairs discordant for ASD, the twin born at a lower birth weight had higher/more severe scores on diagnostic tests for ASD and higher odds of exhibiting traits that are characteristic of ASD than his/her co-twin[107]. In addition to ASD, other developmental disabilities, such as cerebral palsy, learning disabilities, and stuttering, have been associated with lower birth weights[108]. Last, associations between birth weight for gestational age and ASD have been reported in some previous studies, whereby being small for gestational age is associated with a higher risk for ASD[91,105,109]. These demonstrated the independent effects of low birth weight on ASD while controlling for the effects from gestational age.

1.4.1.1. Limitations of Previous Studies

Despite the large number of studies that have investigated prenatal and perinatal risk factors for ASD, most have limitations that may explain some of the heterogeneity among the results from previous studies. First, most studies do not adjust for the effects of multiple known confounders[110-112]. Confounders are variables that are related to both the predictor (i.e. prenatal

and perinatal complications) and the outcome (i.e. ASD). If known confounders are not included in the statistical model, false associations may be observed between the predictor and the outcome. For example, because advanced maternal age has been repeatedly associated with ASD[90] and older mothers are also at a higher risk for some prenatal and perinatal complications than younger mothers[113,114], maternal age is a confounder in the relationships between ASD and prenatal and perinatal complications and should be adjusted for in these statistical analyses.

Second, the majority of previous studies on the relationships between prenatal and perinatal complications and ASD compared unrelated cases and controls, while affected and unaffected siblings are clearly better matched than unrelated cases and controls due to their shared genetics and home environment. For this reason, comparing siblings allows researchers to control for both known and unknown confounding factors. A few studies have used siblings to examine such relationships, however the sample sizes were usually small[115-117].

1.4.1.2. Potential Confounders for the Relationships Between Prenatal and Perinatal Complications and ASD

In addition to the widely reported effects of birth year and sex (section 1.1.2.), as well as birth weight and gestational age (section 1.4.1.) on the risk for ASD, multiple births, advanced parental ages, birth order, and family size may also be confounders for the relationships between prenatal and perinatal complications and ASD.

Multiple Births

It is widely accepted that twins experience more prenatal and perinatal complications than singletons[118-121]. If such complications are related to ASD, then it follows that twins and higher-order multiples should be at an increased risk for ASD. To date, studies investigating the association between multiple births and ASD have reported variable results. Two groups observed a higher than expected rate of twins among ASD sib pairs: Greenberg *et al.* observed that 30 of 166 (18%) sibling pairs were twins[122] and Betancur *et al.* observed that 11 of 79 (14%) sibling pairs were twins[123]. These proportions were much higher than the expected rate of twins (2.4%), suggesting that twinning may increase the risk for ASD. In their meta-analysis study, Gardener *et al.* combined the results for the association between ASD and multiple births from 10 previous studies[91]. Of these, three studies reported an increased risk for ASD, while seven studies did not. Their meta-analysis revealed a statistically significant estimated effect of 1.77 (95% CI: 1.23-2.55). However, their analysis also revealed significant heterogeneity in relative risks among the previous studies. Conversely, other studies have not found a statistically significant relationship between twin or multiple births and ASD[104,111,124,125]. Taken together, these results suggest that better-designed studies with large sample sizes may give us more information about the true relationship between multiple births and ASD.

It is important to investigate whether twins and other multiples are at a higher risk for ASD because there has been a dramatic increase in the incidence of multiple births in recent decades (from 1.9% in 1980 to 3.3% in 2009), which is likely explained by older maternal age at birth, as well as an increase in the use of infertility treatments[126]. If multiples are at an increased risk for ASD, this may explain a portion of the apparent increase in ASD prevalence. Additionally, if the

prevalence of ASD is higher in multiples than in singletons, this growing population should be followed to facilitate early assessment and intervention.

Advanced Parental Ages

As mentioned above, advanced parental age has been consistently associated with an increased risk for ASD. To examine the relationship between ASD and parental ages, some studies used categorical age groups rather than treating age as a continuous variable. When age groups were used, the results were not directly comparable between studies as different research groups defined advanced parental age differently. For example, one study defined advanced maternal age as 35 to 44 years and advanced paternal age as 30 to 40 years[103], while a different study defined advanced maternal or paternal ages as greater than 40 years[127]. In the meta-analysis study by Gardener *et al.*, nine out of 12 previous studies reported a significant association between advanced maternal age and ASD. Similarly, four out of seven previous studies reported a significant relationship between advanced paternal age and ASD[90]. In addition to the studies included in the meta-analysis paper, many other studies have reported relationships between ASD and advanced parental ages[103,128-131]. Regardless of the categorical definitions used for parental age groups, the majority of recent studies support a positive association between both advanced maternal and paternal ages and the risk for ASD.

Recently, several groups have investigated the dependent effects of maternal and paternal ages on the risk for ASD. The studies tended to agree that, independently, increasing maternal or paternal age was associated with a higher risk for ASD[131-133], although Sandin *et al.* observed a U-shaped association between maternal age and the risk for ASD with the lowest risk for

mothers at approximately 30 years of age[134]. When looking at the dependent effects, both Shelton *et al.* and Sandin *et al.* observed some of the highest risks in association with large parental age differences (e.g. fathers less than 25 and mothers 35 to 39 or fathers 25 to 29 and mothers 40 or older[132]; fathers less than 29 and mothers 40 or older[134]). Most of the previous studies reported that increasing maternal age was always associated with an increasing risk for ASD, regardless of paternal age. Alternatively, increasing paternal age tended only to be associated with an increased risk for ASD when the mother was younger (e.g. less than 30[132,134] or less than 35[131,133]), with a non-apparent or non-significant trend in couples with older mothers. Examining the dependent effects of maternal and paternal age is important as it may help us to identify the mechanisms by which parental ages affect the risk for ASD and whether the age of one parent is more important than the age of the other.

Although the biological relationship between advanced parental ages and ASD is currently unknown, several explanations have been proposed. Increased maternal age may be associated with ASD due to expansion mutations of unstable trinucleotide repeats[135], which are thought to result from loop formation during base and nucleotide excision repair in oocytes[136]. Additionally, as previously mentioned, older mothers are at an increased risk for prenatal and obstetrical complications[113,114], which may also be associated with ASD. Increased paternal age may be related to a higher risk for ASD because spermatogonia accumulate *de novo* mutations over time, as a result of continuous DNA replication[135]. Last, environmental exposures in both the mother and father may induce *de novo* mutations and/or affect the epigenetic profile of their gametes[130].

Birth Order

Many studies have reported significant associations between ASD and birth order; however, the direction of this relationship has varied between studies. In their meta-analysis, Gardener *et al.* combined the results for the relationships between ASD and birth order from 20 previous studies, of which 11 observed no significant association, one observed a higher risk for ASD associated with higher birth order, two observed a lower risk for ASD associated with higher birth order, and six observed a mixed trend. The meta-analysis results showed that first-born children were at an increased risk for ASD compared to third- or later-born children; they also examined seven other birth order comparisons (e.g. first-born versus second-born children), none of which were significant[90]. Several other studies also reported that first-born children were at the greatest risk for ASD[128,130,137], while some groups did not find any association between ASD and birth order[111,125,138].

The effects of family/sibship size on the relationships between ASD and birth order have also been investigated. Cheslack-Postava *et al.* observed a significantly lower risk for ASD associated with increasing parity for ASD, Asperger's Syndrome, and PDD-NOS and a significantly higher risk for classical autism in second-born children compared to first-born children[139]. However, they did not observe a significant association between ASD and family size or differences in associations between ASD and parity by family size. On the other hand, Carballo *et al.* reported an increased risk for ASD associated with increasing family size (when the family included three or more children), but no significant associations between ASD and birth order[140]. In several earlier studies, it was reported that the first-born sibling was more likely to be affected with ASD in families with two children, while the later-born siblings were more likely to have ASD in

families with four or more children[141-143]. This observation has been attributed to reproductive stoppage, where parents stop having children after one child receives a diagnosis of ASD[144]. In two more recent studies, Hoffman *et al.* and Grønborg *et al.* observed significant stoppage effects whereby parents were less likely to have additional children after having a child with ASD[145,146]. As such, family size and reproductive stoppage effects may confound the relationships between birth order and ASD and should be considered in statistical analyses, when possible.

1.4.2. Other Environmental Exposures

Exposure to chemicals and air pollutants have also been hypothesized to increase the risk for ASD since foetuses and neonates are more susceptible to toxins than adults during early development, especially early brain development[147]. Specifically, Roberts *et al.* investigated the relationship between maternal exposure to agricultural pesticides and ASD in California[148]. Exposure to organochlorines immediately before and during the prenatal period of central nervous system development (days -7 [7 days prior to fertilization] to 49) was associated with an increased risk for ASD. Dicofel and endosulfan, two pesticides that are commonly applied to fruits, vegetables, beans, nuts, and cotton, accounted for the majority of the organochlorines that were applied.

Groups have also investigated the associations between hazardous air pollutants and ASD, though these studies have yielded varying results. For example, Windham *et al.* observed significantly increased odds of ASD associated with exposure to solvents (i.e. methylene chloride, trichloroethylene, and vinyl chloride), metals (i.e. cadmium, mercury, and nickel), and

diesel particulate matter in the San Francisco Bay area[149]. Alternatively, Kalkbrenner *et al.* observed elevated odds ratios for several pollutants in North Carolina and West Virginia, though none reached statistical significance[150].

Last, McCanlies *et al.* investigated the relationship between maternal or paternal occupational exposure to various chemical agents[147]. They observed an increased risk for ASD associated with exposure to lacquer, varnish, xylene, solvents, and asphalt, although none of the associations remained statistically significant after correcting for multiple testing.

To address the discrepancies in previous results and further our understanding about the effects of the above-mentioned environmental exposures, future studies need to consider important confounding factors, such as urbanicity, correlation among pollutants, and levels and duration of chemical exposure.

Taken together, the studies described above illustrate the potential risks that environmental factors may pose during early brain development. Identifying environmental risk factors for ASD will: 1) facilitate earlier assessment and intervention (if needed) for individuals at a higher risk of developing ASD, and 2) inform subgrouping of ASD cases in future genetic studies (by exposure or lack of exposure to environmental risk factors) to better identify genetic variants that are associated with ASD.

1.5. COMORBID DISORDERS

1.5.1. Congenital Anomalies

Previous studies have investigated the prevalence of congenital anomalies among individuals with ASD because identifying comorbid conditions may help to pinpoint the aetiology, risk factors, and important developmental stages associated with ASD[151-153]. In these studies, the prevalence of congenital anomalies has been consistently reported to be approximately 5% to 10% among individuals with ASD, compared to approximately 1.5% to 6% in population-based controls[97,151-155]. Additionally, one study reported the prevalence of birth defects to be approximately 8% in the unaffected siblings of individuals with ASD[153]. In studies that reported the frequencies of individual congenital anomalies, anomalies of the nervous system, heart, genital organs, urogenital system, or musculoskeletal system, and chromosomal anomalies occurred the most frequently, with reported prevalences ranging from approximately 1.5% to 6.5%[97,152,153].

1.5.2. Other Mental and Behavioural Disorders

Many other mental and behavioural or psychiatric disorders tend to be comorbid with ASD. Approximately 70% of individuals with ASD were previously reported to have at least one other mental or behavioural disorder[1,156,157]. These studies also reported that approximately 40% to 60% of individuals with ASD had two or more comorbid psychiatric disorders[1,156,157] and approximately 20% to 35% had three or more comorbid disorders[156, 157]. Of the mental and behavioural disorders, social anxiety, specific phobias, attention deficit hyperactivity disorder, obsessive compulsive disorder, and oppositional defiant disorder occurred the most frequently in individuals with ASD, with prevalences ranging from approximately 30% to 45%. Identifying

the psychiatric disorders that tend to be comorbid with ASD may help to elucidate the brain mechanisms and functions that are altered in ASD, as well as help to identify genetic variants associated with both ASD and the other disorders[156]. However, IQ and communication abilities vary widely among individuals with ASD, making it difficult for clinicians to diagnose comorbid psychiatric disorders in some of these individuals[158]. Consequently, the reported prevalence for these comorbid disorders may be underestimated.

1.6. ASD DEFINITIONS USING ADMINISTRATIVE DATA

Many research groups have taken advantage of the rich information available for large populations in administrative databases. Those that use administrative datasets to identify individuals with ASD often use multiple algorithms because the ASD diagnoses have not been validated. For example, Burstyn *et al.* identified ASD cases in Alberta, Canada by ICD-9 codes for ASD using four separate algorithms: 1) one claim made by any physician, 2) one claim made by a pediatrician or a psychiatrist, 3) two claims made by any physician, and 4) two claims made by a pediatrician or a psychiatrist[137]. Pinborough-Zimmerman *et al.* identified ASD cases from administrative education and health data sources in Utah, United States using three algorithms: 1) one special education code, 2) one ICD-9 health diagnosis, or 3) one special education code and one health diagnosis[159]. Recently, Coo *et al.* identified ASD cases by ICD-9 codes in the hospital and medical records, applications for special needs education funding, and records from Children's Special Services, a program funded by the provincial government to support children with disabilities, using the administrative data from the Manitoba Centre for Health Policy[95]. Three algorithms were used: 1) individuals with at least one ASD claim, 2) individuals with at least two ASD claims from the hospital, medical, or education datasets, and 3) individuals in the

Children's Special Services records. These research groups observed that the results varied between the least stringent case definition and the more stringent definitions.

Two research groups have examined the validity of ASD diagnoses from administrative health data in North America, which corroborates the use of different ASD case definitions. Dodds *et al.* identified ASD cases in Nova Scotia, Canada from hospital discharge abstracts, physician billings, and a mental health outpatient information system[160]. They validated the ASD claims using a clinical database of diagnoses made by the Autism Team from the IWK Health Centre. They compared seven algorithms using combinations of diagnoses from the three data sources. The algorithm based on one claim in any of the three databases was selected as the best algorithm because it yielded the highest sensitivity (69.3%); however, this algorithm had the lowest specificity (77.3%) of the seven algorithms, indicating that 22.7% of the unaffected individuals were identified as ASD cases. Other algorithms yielded higher specificity rates, though at the cost of lower sensitivity (e.g. the algorithm that required at least two claims from the physician billings or at least one claim from the hospital records had specificity=93.2% and sensitivity=36.9%). Burke *et al.* examined the validity of autism diagnoses based on a large private health insurance plan in the United States[161]. To confirm the validity of the administrative claims, they reviewed each subject's medical records for documentation of an ASD diagnosis and compared algorithms using at least one ASD claim or more than one ASD claim. The positive predictive value for the algorithm using individuals with more than one ASD claim was 87.4% and was higher than that for the algorithm using at least one ASD claim (74.2%). The results from these two studies are inconsistent about which algorithm is better; as such, more research is needed on this topic.

1.7 HYPOTHESES AND OBJECTIVES

We hypothesize that individuals with ASD experienced more prenatal and perinatal complications than their unaffected siblings. Furthermore, because twins typically experience more prenatal and perinatal complications than their singleton siblings, we hypothesize that twins are at an increased risk for ASD.

The objectives of this study are to:

1) examine the associations between prenatal and perinatal complications and ASD, while adjusting for the effects of confounding factors, and

2) investigate the relationships between twin status and ASD.

2. METHODS

2.1. DATA SOURCES AND COLLECTED INFORMATION

2.1.1. Manitoba Population Health Research Data Repository

The information used for this study was from the Population Health Research Data Repository at the Manitoba Centre for Health Policy (MCHP). The repository consists of registry, health, education, social, and justice administrative databases about residents in the province of Manitoba, Canada[162].

All Manitoba residents are enrolled in and receive health services through the Manitoba Health Insurance Registry, a health insurance plan that is funded by the provincial and federal governments[163]. Each resident is assigned a unique personal health identification number (PHIN), either at birth or upon entry into the province. For the data repository at MCHP, each individual is assigned an encrypted PHIN, allowing access to individual-level data and linkage across datasets while maintaining confidentiality[164].

The information used for this study was from four data sources within the provincial data repository: 1) the Manitoba Health Insurance Registry, 2) hospital discharge abstracts, 3) medical services claims, and 4) education enrolments, marks, and assessments (Table 2.1).

Table 2.1. Information collected from each data source

Data source (years)	Information collected for children	Information collected for parents
Manitoba Health Insurance Registry (1979-2013)	• Scrambled ID • Birthdate • Sex • Parents' scrambled ID's • Health care coverage start and end dates • Reason for loss of coverage (if applicable)	• Scrambled ID • Birthdate • Sex
Hospital Discharge Abstracts (1979-2013)	• Diagnosis (full ICD-9/ICD-10 code) • Admission date • Discharge date • Birth weight • Gestational age • 1- and 5-minute Apgar scores	• Diagnosis (full ICD-9/ ICD-10 code) • Admission date • Discharge date
Medical Services Claims (1979-2013)	• Diagnosis (3-digit ICD-9 code) • Service date • Specialist type	• Diagnosis (3-digit ICD-9 code) • Service date
Education Enrolment, Marks, and Assessments (1994-2013)	• Level of special needs funding • Disability classification • Academic year of funding	———

2.1.2. Manitoba Health Insurance Registry Data

Manitoba Health provided the Manitoba Health Insurance Registry data. The dataset included demographic data, as well as information about family structure and registration in the healthcare system for all residents in Manitoba since 1970[164,165].

Some demographic information (i.e. sex and birth year) and health care coverage start and end dates were selected from the registry dataset. Other information (i.e. birth order and parental ages at birth) was calculated for each family using the information about family structure. The registry

information was also used to identify twins (defined as children born to one mother on the same day or consecutive days).

2.1.3. Hospital Discharge Abstracts and Medical Services Claims Data

Manitoba Health provided the hospital discharge abstracts and medical services claims data. The hospital dataset included demographic and clinical information that was extracted from hospital discharge abstracts at both acute and chronic care facilities[166]. The data encompassed three groups of individuals: 1) Manitoba residents treated in Manitoba, 2) Manitoba residents treated out of province, and 3) out of province residents treated at Manitoba facilities. The medical dataset contained claims submitted by healthcare providers to Manitoba Health for reimbursement of physician visits and medical tests[167].

Diagnostic information was available in the form of the International Classification of Diseases (ICD) codes. Because this data was collected over time, the ICD codes came from both the ninth and tenth editions. The diagnostic codes from ICD-10 were converted to ICD-9 using the 2007/2008 Canadian Institute for Health Information (CIHI) conversion tables (Canadian Institute for Health Information, Canada), which convert the ICD-10-CA (Canadian modification) codes to the ICD-9-CM (clinical modification) codes. Several prenatal and perinatal diagnostic codes that were not converted using the CIHI conversion tables were converted using an online resource[168]. Complete diagnostic codes (comprised of the first three digits and the fourth- and fifth-digit subclassifications) were available from the hospital dataset, while the medical dataset only included the three-digit ICD code. The admission and discharge

dates were selected for each claim in the hospital dataset. The service dates and specialist types were collected for each claim in the medical dataset.

The individuals with ASD were identified using the ICD-9 code 299 in the hospital and medical records (Table 2.2). Claims for congenital conditions (ICD-9 codes 740 to 759 [Appendix 1]) and other mental and behavioural disorders (ICD-9 codes 290 to 319, excluding 299 [Appendix 2]) were also selected from the hospital and medical records.

Table 2.2. ICD-9-CM codes for pervasive developmental disorders[169]

ICD-9-CM code	Pervasive developmental disorder
299.0	Autistic disorder
299.1	Childhood disintegrative disorder
299.8	Other specified pervasive developmental disorder
299.9	Unspecified pervasive developmental disorder

Birth weight, gestational age, and 1- and 5-minute Apgar scores were selected from the hospital records if they were recorded within 30 days of birth. Apgar scores are reported for newborns at one and five minutes after birth and indicate the newborn's condition, specifically heart rate, respiration, reflex irritability, muscle tone, and colour[170]. The scores range from 0 to 10; a score between 7 to 10 is considered normal, while scores between 4 to 6 and 0 to 3 are considered moderately abnormal and very abnormal, respectively[171].

For each individual, his/her mother's prenatal complications (ICD-9 codes 640 to 679, with some exclusions [Appendix 3]) were selected from the hospital and medical records if the admission or service dates fell within his/her gestation period or within 24 weeks before his/her birth when gestational age was unavailable. Each child's perinatal complications (ICD-9 codes 760 to 779,

with some exclusions [Appendix 3]) were selected from the hospital and medical records if the admission or service dates were within 30 days of birth.

2.1.4. Education Enrolments, Marks, and Assessments Data

Manitoba Education provided the education data. The dataset included information about approved applications for special needs funding from the Manitoba government. Two levels of funding are available: level 2 funding is provided to students who require specialized instruction for most of the day, while level 3 funding is provided to those who require more support than is available with level 2, including specialized instruction for the entire day, as well as additional supports and programming[172].

Information about the level of special needs funding, the disability classification for the funding, and the academic year of the funding was collected from the education records. The individuals with ASD were identified by approved applications for level 2 or level 3 funding, where the disability was classified as ASD.

2.1.5. Ethical and Privacy Considerations

This study was approved by the University of Manitoba Research Ethics Board, the Health Information Privacy Committee from Manitoba Health, Manitoba Education, the Manitoba Centre for Health Policy, and the Health Sciences Centre Department of Research.

Because the data repository contains individual-level information, measures are in place to protect the confidentiality of the data. All relevant regulatory bodies and the data providers must

approve each study before the researchers can access the repository. The data is maintained in a secure environment and is de-identified, meaning that identifying information (e.g. name or PHIN) is removed prior to data acquisition. To further prevent the identification of any individuals, data representing less than six individuals cannot be presented. Prior to the release of any analysis results, MCHP reviews all output for consistency with the approved project and agreement with their privacy policy. Last, the data providers review all presentations and publications prior to public dissemination.

2.2. STUDY SAMPLE

All individuals with ASD and twins who were born in Manitoba between 1979 and 2013 were selected from the provincial data repository. Information about the mothers and fathers was selected when available. If multiple females were associated with one child's registration in the health care system, the most likely mother was identified by prenatal or delivery claims (ICD-9 codes 640 to 679) within 30 days before a child's birth. If multiple males were associated with one child's registration, the father's information was set to missing. Siblings (defined as individuals sharing the same mother) of the individuals with ASD and the twins were also selected.

Two sets of families were identified: 1) the ASD families, and 2) the twin families. The ASD families included at least one individual with ASD and one unaffected sibling, who were covered by Manitoba Health from birth to a minimum of five years of age. A minimum coverage of the first five years was used to select the ASD families to ensure that individuals could be identified

as ASD-affected in the hospital, medical, and education records. The twin families included at least one twin and one singleton sibling, who were covered by Manitoba Health at birth.

2.2.1. Quality Control

2.2.1.1. Study Subjects

Quality control was performed for the subjects from the selected ASD and twin families (Table 3.1). First, higher-order multiples (i.e. triplets or higher) were identified by the ICD-9 codes 651 and V27 in the mother's hospital and medical records within 24 weeks before a child's birth and the ICD-9 codes V30 to V39 in the child's hospital and medical records within 30 days after birth (Appendix 4). Higher-order multiples were removed because these individuals were expected to experience more prenatal and perinatal complications than twins and singletons. Twin status was set to missing if an individual was not identified as a twin but the ICD codes indicated twins (Appendix 4) in the mother's hospital or medical records (ICD-9 codes 651 and V27) within 24 weeks before the child's birth or in the child's records (ICD-9 codes V30 to V39) within 30 days after birth. Second, ASD disease status was set to missing if an individual did not have any ASD claims after two years of age, as diagnoses before this age are considered to be unreliable[173,174]. This removed some ASD families that did not contain any other individuals with ASD. Third, if information about registry coverage at birth was discrepant between a twin and his/her co-twin, the coverage start date was set to missing for both twins. This removed some twin families because it was not clear whether the twins were covered at birth. Fourth, to remove possible half-siblings or non-siblings, individuals were removed if maternal or paternal age at his/her birth was less than 13 years old. Siblings with less than three weeks between their mother's pregnancies (i.e. the start date for the pregnancy of a younger sibling – the end date for

the pregnancy of the older sibling = (birth date – gestational age for the younger sibling) – (birth date of the older siblings) < 3 weeks) or less than 27 weeks between their birth dates (when gestational age was unavailable) were also removed; the minimum gestational age selected in the study sample was 24 weeks. Last, children with a chromosomal anomaly (ICD-9 code 758) were removed because a chromosomal condition may be the cause of the ASD phenotype.

2.2.1.2. Collected Information

Quality control was also performed for the collected information. Prenatal and perinatal complications were excluded if they affected the mother only and likely had no effect on the foetus/newborn (e.g. infections of the breast and nipple associated with childbirth [ICD-9 code 675]), occurred during the postpartum period (e.g. postpartum haemorrhage [666]), encompassed a mixture of conditions (e.g. other complications of pregnancy, not elsewhere classified [646]), were specific to twins (e.g. multiple gestation [651]), overlapped with other conditions that were already included in the analyses (e.g. disorders relating to short gestation and low birth weight [765]), or occurred at a frequency of less than 1% in the selected ASD families (e.g. perinatal disorders of the digestive system [777]) (Appendix 3). A birth weight less than 500 grams or greater than 6500 grams or a gestational age shorter than 24 weeks were set to missing because these are considered to be the limits of foetal viability[89]. These values were also set to missing if multiple and discrepant values for birth weight and/or gestational age were listed for one individual. Last, gestational age was set to missing if there was a discrepancy between a twin and his/her co-twin. For discrepancies in the start and end dates of an individual's registry coverage, the admission dates from the hospital discharge abstracts and the service dates from the medical services claims were used to determine the most likely coverage start and/or end dates. For

parents with discrepant birthdates in the registry dataset, if one birth date was the first day of the month and/or year of the other birth date, the more likely birth date (later in the month and/or year) was selected. If the discrepant birth dates were less than 31 days apart, one birth date was randomly selected. When discrepancies in a parent's birth date could not be resolved, parental age at each of his/her children's births was set to missing.

2.2.2. ASD Case Definitions

The sources of the ASD claims, the number of ASD claims, and the clinical specialists who submitted the ASD claims were examined for the affected individuals in the ASD families that were selected after the quality control procedures. All ASD claims submitted before a child reached two years of age were excluded. The number of ASD claims was restricted to one claim per day and one claim per hospital stay. Four definitions were then used to select the ASD cases: 1) at least one claim from any source, 2) at least two claims from any source or combination of sources, 3) at least one claim made by a selected specialist (i.e. geneticist, neurologist, paediatrician, or psychiatrist), and 4) at least two specialist claims; this yielded four sets of ASD families for analysis. Numbers 2 to 4 were more stringent than number 1 and were expected to reduce diagnostic misclassification among the study subjects.

2.3. STATISTICAL METHODS

All data management and statistical analyses were conducted using SAS, version 9.4 (SAS Institute Inc., Cary, NC).

2.3.1. Descriptive Statistics

The frequencies of categorical variables (e.g. prenatal and perinatal complications) were tabulated for both the ASD and twin families. For continuous variables, the mean, standard deviation, and range were calculated for each set of families.

2.3.2. Statistical Tests

For all association tests, mixed models with both fixed and random effects were used to account for the relatedness among family members. In these models, family membership (defined as individuals born to the same mother) was treated as a random variable, such that covariance between outcomes was permitted among the individuals from the same family. Generalized linear mixed models with Gauss-Hermite quadrature estimation were used to examine the relationship between a categorical outcome (e.g. ASD status) and a predictor. Linear mixed models with residual maximum likelihood estimation were used to investigate the association between a continuous outcome (e.g. birth weight) and a predictor.

2.3.2.1. Characteristics of the Selected Families

The demographic and birth information was compared between the ASD and twin families. Additionally, the prevalences of congenital conditions and mental and behavioural disorders were compared between the individuals with ASD and their unaffected siblings in the ASD families.

As illustrated in Figure 2.1, the following relationships were tested:

2.3.2.2. Prenatal and Perinatal Complications and Covariates (#1 to #3 in Figure 2.1)

Using the ASD families, the pairwise relationships 1) among the prenatal and perinatal complications and Apgar scores, 2) between the prenatal and perinatal complications, as well as the Apgar scores, and the covariates (i.e. sex, twin status, parental age, birth year, birth order, gestational age, and birth weight), and 3) among the covariates, were examined. The relationships among the prenatal complications and between the prenatal complications and some covariates (i.e. parental ages, birth year, birth order, and gestational age) were examined per pregnancy, while all of the other relationships were examined per individual.

Since the number of twins in the ASD families was small, the pairwise relationships between twin status and the prenatal and perinatal complications and Apgar scores, as well as between twin status and the other covariates, were also examined using the twin families. The relationships between twin status and the other covariates that were estimated using the twin families were expected to be more reliable due to the larger sample size.

2.3.2.3. ASD and Covariates (#4 and #5 in Figure 2.1)

The pairwise relationships between ASD and each covariate were examined in the ASD families. Additionally, the relationships between ASD and each covariate, adjusted for the effects of the other covariates (including either maternal or paternal age), were also examined.

2.3.2.4. ASD and Prenatal and Perinatal Complications (#6 and #7 in Figure 2.1)

The pairwise relationships between ASD and the prenatal and perinatal complications, as well as the 1- and 5-minute Apgar scores, were investigated in the ASD families using each of the four ASD case definitions. Subsequently, these relationships were adjusted for the effects of the covariates (i.e. twin status, sex, maternal or paternal age, birth year, birth order, gestational age, and birth weight percentile). The relationships between ASD and subtypes of the statistically significant complications (from the adjusted analyses) (e.g. anaemia of prematurity from haematological disorders of the newborn) were examined, when possible.

Last, the relationships between ASD and the significant complications were re-examined after removing individuals with a claim for any congenital anomaly that occurred significantly more among the ASD cases than among their unaffected siblings (i.e. $p<0.05$). The relationships between these complications and congenital anomalies were also investigated.

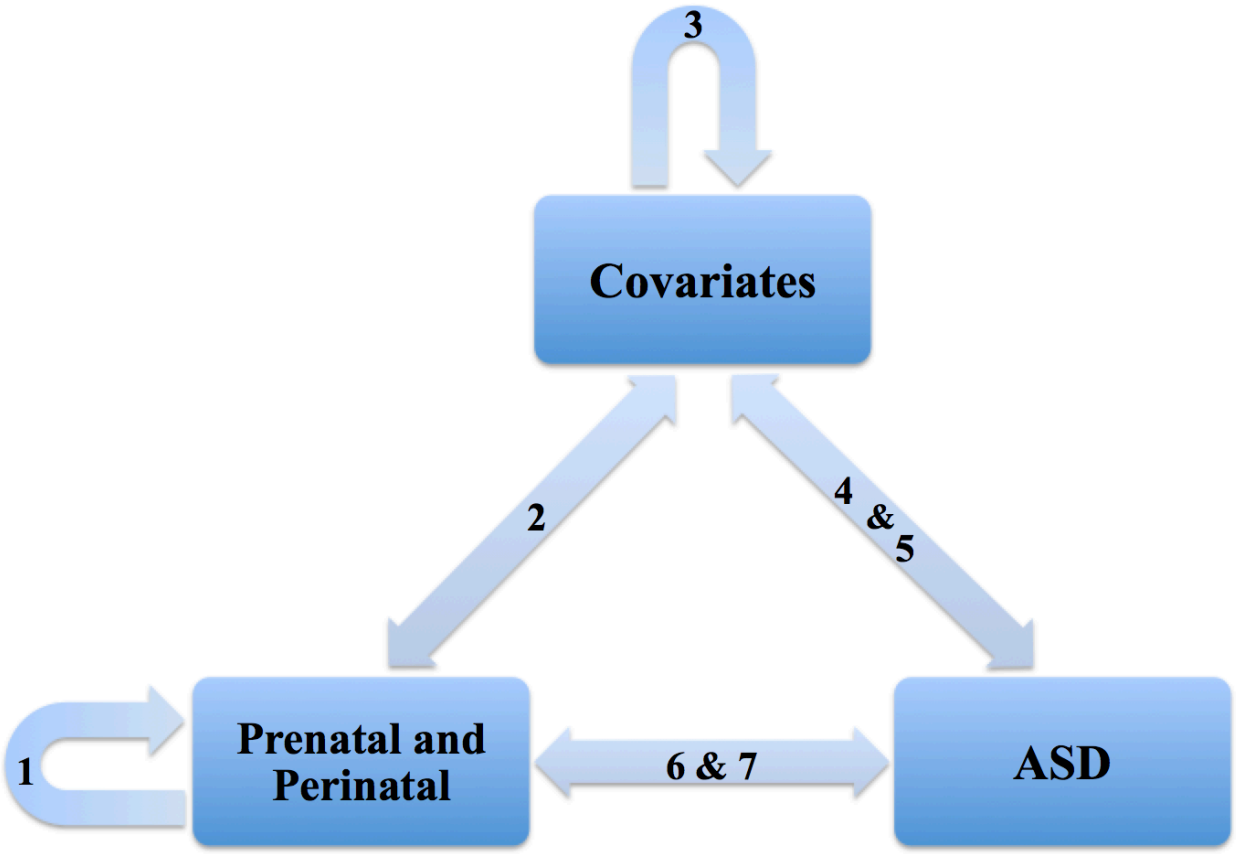


Figure 2.1. Relationships examined in this study: 1) pairwise relationships among the prenatal and perinatal complications, 2) pairwise relationships between the prenatal and perinatal complications and the covariates, 3) pairwise relationships among the covariates, 4) pairwise relationships between ASD and the covariates, 5) adjusted relationships between ASD and all of the covariates, 6) pairwise relationships between ASD and the prenatal and perinatal complications, and 7) relationships between ASD and the prenatal and perinatal complications, adjusted for all of the covariates.

2.3.3. Solutions for Collinearity Among the Covariates

Birth year and parental age exhibit collinearity because they both increase on the same linear scale in siblings. To avoid collinearity between these covariates, parental ages and birth year from the first child were used for all the children in a family. Birth order was then used to account for the differences in these two variables between siblings. If the first child was removed during quality control or birth order was missing, parental age and birth year were set to missing

for all children in the family. Birth order was set to missing for all children in a family if a child was removed for a reason other than a chromosomal anomaly. This is because the other exclusion criteria removed possible non-siblings, as well as potentially duplicated records for the same individual. Winsorization was performed for birth order due to the rare events of large families. The 98 percentile of birth order in the ASD families (birth order of six) was used as a cut-off.

As described in the Introduction (section 1.4.1.2.), maternal and paternal ages are highly correlated with one another, though both may contribute to the risk for ASD. Due to the small sample sizes for the categories with older maternal age and younger paternal age and vice versa (Appendix 5), similar tests to those used in previous studies could not be performed; as such, the effects of maternal and paternal ages were examined separately.

Birth weight and gestational age are also known to be highly correlated with one another. To adjust for birth weight and gestational age in the same statistical models, birth weight percentiles were determined by sex and twin status for each gestational age. Birth weight percentile for the twins was calculated using all of the twins born in Manitoba between 1979 and 2013. Birth weight percentile for the singletons was calculated using all of the singletons from the ASD and twin families. Individuals were excluded from the calculation of birth weight percentile if they were from a higher-order multiple pregnancy (i.e. triplet or higher), had inconsistent twin status (as described in the Quality Control section), or had missing or discrepant birth weight or gestational age information. Additionally, twin pairs were excluded if there were discrepancies in gestational age between the co-twins.

Because the majority of the ASD families contained three or fewer children, it was important to re-examine the relationship between ASD and birth order while considering the effect of family size. To separate the effects of birth order and family size, a birth order index was constructed based on the method described in Booth and Kee[175]. This birth order index was calculated as $B = \phi/A$, where ϕ is the birth order of the individual (ranging from 1 to 6 in the ASD families) and A is the average birth order in the family, calculated as $(N + 1)/2$. Theoretically, $B = 1$ is the mean in each family, regardless of family size; therefore, birth order index is independent of family size.

The pairwise relationships among ASD, birth order index, and family size were examined. Adjusted models were then constructed for the relationships between ASD and all of the covariates, including birth order index and family size. Last, the relationships between ASD and the prenatal and perinatal complications were re-examined with adjustments for the effects of all of the covariates, including birth order index and family size.

2.3.4. Combined Effects of the Significant Predictors for ASD

To ascertain the individual importance of each of the covariates and predictors, as well as their collective effects, the Cragg-Uhler/Nagelkerke pseudo R-square[176,177] was calculated using a logistic regression model.

3. RESULTS

3.1. STUDY SUBJECTS

Originally, 5,369 individuals with at least one ASD claim and 15,040 twins (7,520 twin pairs) were identified in the MCHP Data Repository; of the twins, 136 individuals had at least one ASD claim. From the original sample, 2,794 ASD families (including 7,609 siblings) and 4,092 twin families (including 16,206 siblings) were identified (Table 3.1).

Table 3.1. Quality control for the study subjects

Exclusion criteria	ASD families		Twin families	
	Families	Individuals[a]	Families	Individuals[a]
Initial sample size	*2,794*	*7,609*	*4,092*	*16,206*
Higher-order multiples (i.e. triplet or higher) or inconsistent twin status[b]	7	24	39	155
No ASD claims after two years of age	46	122	0	0
Discrepant coverage start date between twins	0	0	78	223
Maternal or paternal age <13 years at a child's birth; <3 weeks between a mother's pregnancies (calculated using gestational age) or <27 weeks between siblings' birth dates[c]	5	14	10	51
Chromosomal anomalies[d]	174	506	22	198
Selected sample	**2,562**	**6,943**	**3,943**	**15,579**

[a]The number of individuals removed at each step was the total of the number of individuals who failed the quality control measure and the number of siblings who were removed because the family no longer met the criteria as a twin or ASD family.
[b]Identified by the ICD-9 codes in Appendix 4
[c]27 weeks between birthdates was used when gestational age was unavailable
[d]Identified by the ICD-9 code 758, which indicates chromosomal anomalies

3.1.1. Quality Control

During quality control, 232 ASD families (8.3%) and 149 twin families (3.6%) were removed (Table 3.1). A larger proportion of the ASD families were removed than the twin families due to a higher prevalence of chromosomal anomalies in the ASD families. In the ASD families, 6% of the individuals with ASD had a chromosomal anomaly compared to 0.98% of their unaffected siblings ($p<0.0001$). In the twin families, 1% of twins had a chromosomal anomaly compared to 0.76% of their singleton siblings ($p=0.03$). After quality control, 2,562 ASD families (2,673 affected individuals and their 4,270 unaffected siblings) and 3,943 twin families (7,948 twins and their 7,631 singleton siblings) remained.

3.1.2. Characteristics of the ASD Claims and ASD Case Definitions

Of the 2,673 individuals with ASD from the selected ASD families, the majority (2,284 individuals, 85.4%) had at least one ASD claim from the medical services dataset (Figure 3.1). Fewer individuals had an ASD claim from the hospital records (420 individuals, 15.7%). Interestingly, 282 individuals (10.6%) were only identified in the education records.

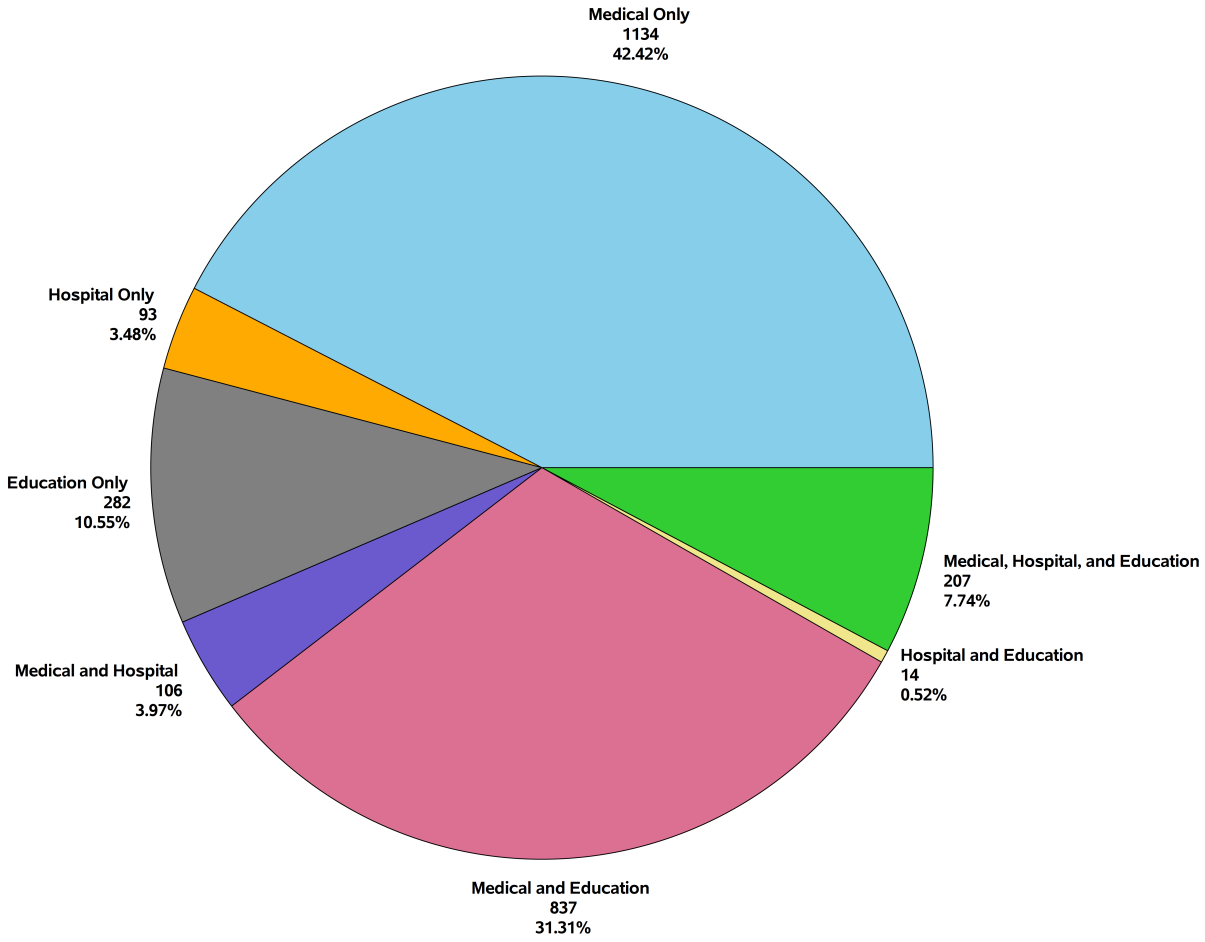


Figure 3.1. Frequency (%) of the individuals with ASD from the selected ASD families (n=2,673) with ASD claims from each data source (i.e. medical service claims, hospital discharge abstracts, or special needs funding applications) or combination of sources

Among the individuals with ASD, 2,391 (89.5%) had at least one claim in the hospital discharge abstracts or medical services claims, which unlike the education records, can contain more than one claim per individual. Of these, 1,416 individuals (59.2%) had more than one ASD claim (Figure 3.2).

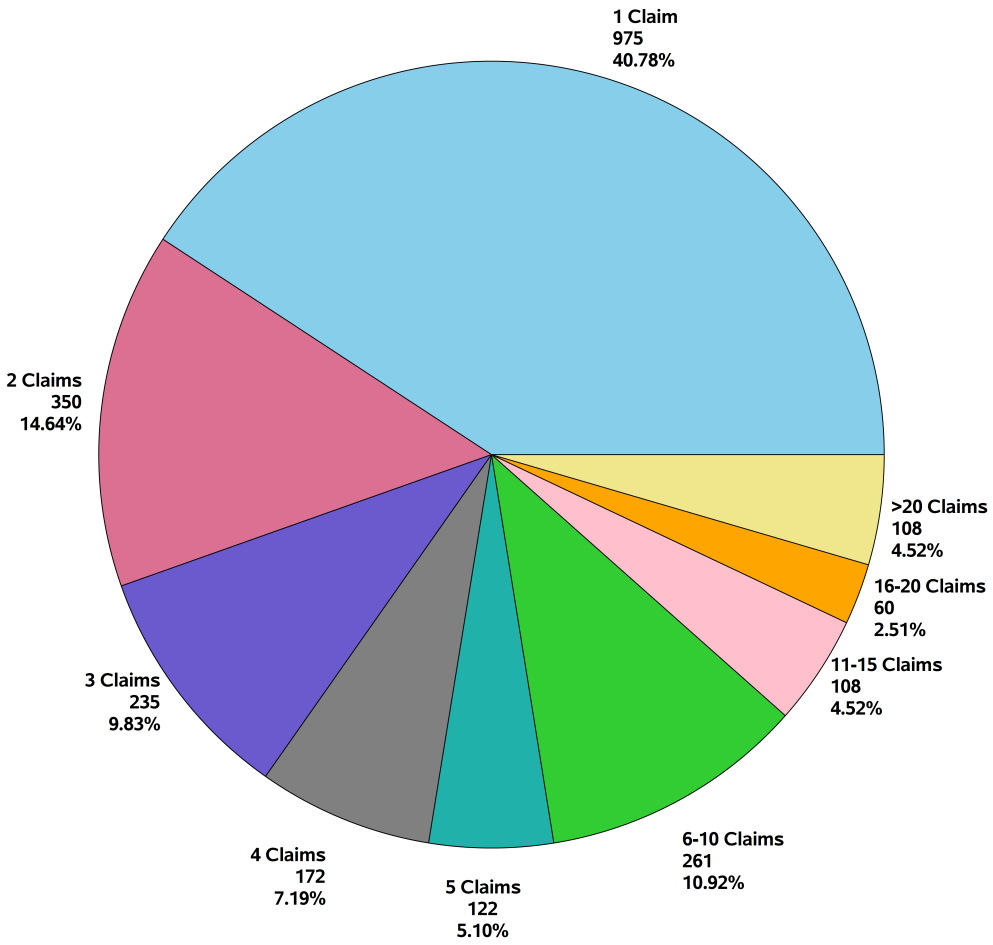


Figure 3.2. Frequency (%) of the individuals with ASD from the selected ASD families (n=2,391) by the number of ASD claims from the hospital and/or medical records

Of the 2,284 individuals with ASD with an ASD claim from the medical services dataset, 1,968 (86.2%) had at least one claim from a selected specialist (i.e. geneticist, neurologist, paediatrician, or psychiatrist); the majority of these individuals (1,901, 96.6%) had a claim from a paediatrician and/or psychiatrist (Figure 3.3). Specialist information was not available from the hospital discharge abstracts or the education records.

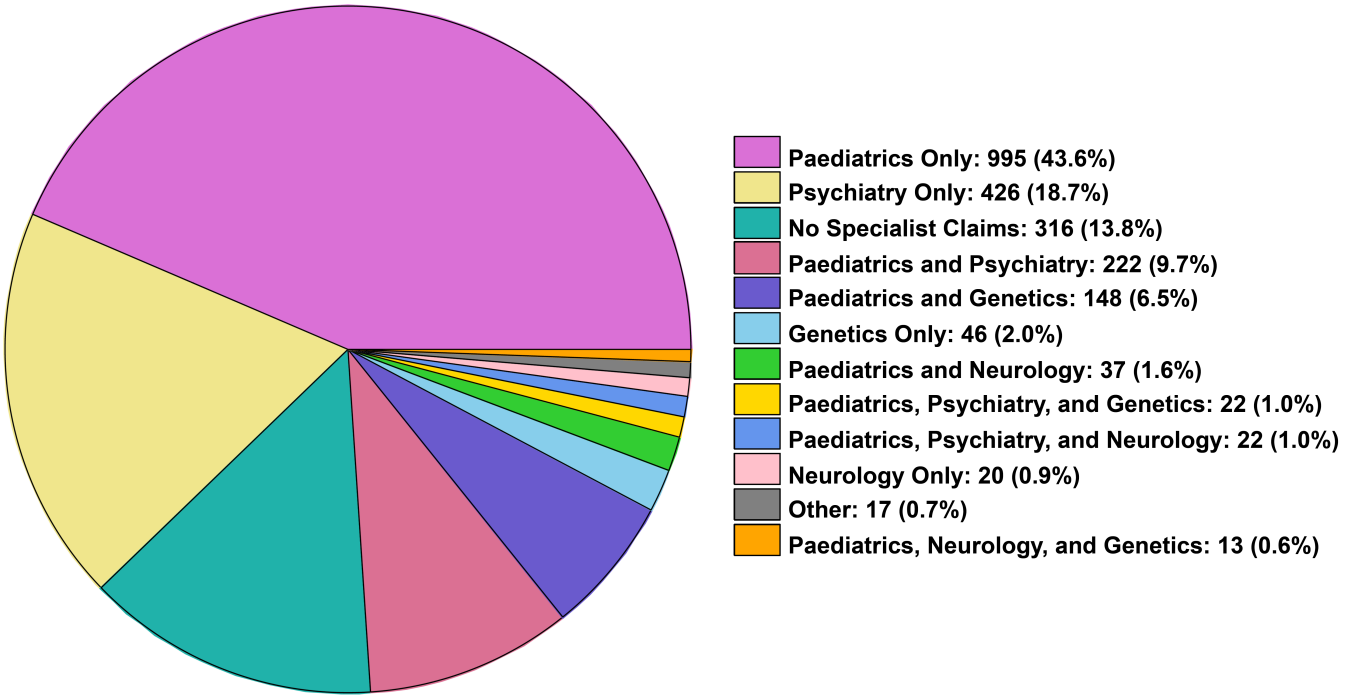


Figure 3.3. Frequency (%) of the individuals with ASD from the selected ASD families with ASD claims from the medical records (n=2,284) submitted by selected specialists (i.e. geneticist, neurologist, paediatrician, or psychiatrist)

Based on the four ASD case definitions, the following number of ASD families were selected for the statistical analyses: 1) any ASD claim (2,562 families, 6,943 individuals), 2) at least two ASD claims (1,563 families, 4,215 individuals), 3) any claim from a selected specialist (1,881 families, 5,073 individuals), and 4) at least two specialist claims (1,112 families, 2,941 individuals). The distributions for the number and sources of ASD claims were similar between the families with any ASD claim and any specialist claim, as well as between those with at least two ASD claims and at least two specialist claims (Appendices 6 and 7).

3.2. CHARACTERISTICS OF THE SELECTED FAMILIES

3.2.1. Family Structures

Most of the selected ASD families (2,458 families, 95.9%) contained only one individual with ASD after quality control (Table 3.2A). Similar proportions were observed in the families selected using the more stringent case definitions (Tables 3.2B to 3.2D). The majority of the selected twin families (3,834 families, 97.2%) contained one set of twins after quality control (Table 3.3). However, in some families, one twin was removed due to a chromosomal anomaly, while his/her co-twin was not removed. As a result, 52 of the selected twin families (1.3%) contained one twin after quality control. In addition, 57 of the twin families (1.5%) contained more than two twins after quality control.

Table 3.2. Structures of the selected ASD families by case definition: A) any ASD claim, B) at least two ASD claims, C) any specialist claim, and D) at least two specialist claims

A)

Frequency (%)		ASD-affected		**Total**
		1	2 or more	
Unaffected	1	1,440 (56.2)	63 (2.5)	**1,503 (58.7)**
	2 or more	1,018 (39.7)	41 (1.6)	**1,059 (41.3)**
Total		**2,458 (95.9)**	**104 (4.1)**	**2,562**

B)

Frequency (%)		ASD-affected		**Total**
		1	2 or more	
Unaffected	1	882 (56.4)	36 (2.3)	**918 (58.7)**
	2 or more	623 (39.9)	22 (1.4)	**645 (41.3)**
Total		**1,505 (96.3)**	**58 (3.7)**	**1,563**

C)

Frequency (%)		ASD-affected		**Total**
		1	2 or more	
Unaffected	1	1,058 (56.2)	52 (2.8)	**1,110 (59.0)**
	2 or more	741 (39.4)	30 (1.6)	**771 (41.0)**
Total		**1,799 (95.6)**	**82 (4.4)**	**1,881**

D)

Frequency (%)		ASD-affected		**Total**
		1	2 or more	
Unaffected	1	648 (58.3)	23 (2.0)	**671 (60.3)**
	2 or more	430 (38.7)	11 (1.0)	**441 (39.7)**
Total		**1,078 (97.0)**	**34 (3.0)**	**1,112**

Table 3.3. Structures of the selected twin families

Frequency (%)		Twins			**Total**
		1	2	3 or more	
Singletons	1	24 (0.6)	1,987 (50.4)	26 (0.7)	**2,037 (51.7)**
	2	16 (0.4)	1,033 (26.2)	12 (0.3)	**1,061 (26.9)**
	3 or more	12 (0.3)	814 (20.6)	19 (0.5)	**845 (21.4)**
Total		**52 (1.3)**	**3,834 (97.2)**	**57 (1.5)**	**3,943**

3.2.2. Demographic Information

As expected, the sex ratio for the individuals with ASD was approximately four males to one female in the ASD families (Table 3.4), while the sex ratio among the unaffected siblings was approximately even (47.9% male and 52.1% female). The ASD families were mostly comprised of singletons, although some twins were included (236 individuals, 3.4%). The majority of the children in the ASD families were third- or earlier-born, though 11.6% of children were fourth- or later-born. The mean paternal age (31.3 years, SD=6.0) was slightly higher than the mean maternal age (28.1 years, SD=5.6), though its standard deviation was larger, probably due to a smaller sample size for which paternal information was available compared to maternal information. The average age for all of the individuals at the end of the study period was 17.9 years in the ASD families, with ages ranging from 5.0 to 34.9 years old. The mean age at the first ASD claim was 8.4 years (SD=5.5), with 1,199 children (44.9%) having a claim for ASD before 6 years of age (Figure 3.4), though the ages ranged from 2.0 to 33.3 years. As birth year increased, the age at the first ASD claim steadily decreased (Figure 3.5). For example, the mean age at the first ASD claim was approximately 16.4 years for individuals born in 1980, 10.4 years for children born in 1990, and 6.1 years for children born in 2000. Most of the demographic information was similar in the families selected using the more stringent ASD case definitions (Table 3.5). However, the mean age at the first ASD claim was lower when using the more stringent ASD case definitions, especially two or more specialist claims (6.6 years, SD=4.9).

Table 3.4. Description of the demographic variables in the ASD families

Variable[a]	Category	**ASD-affected individuals** Frequency (%) / Mean (SD, Range)	**Unaffected siblings** Frequency (%) / Mean (SD, Range)	**Total** Frequency (%) / Mean (SD, Range)
Sex	Male Female	2,117 (79.2) 556 (20.8)	2,044 (47.9) 2,226 (52.1)	4,161 (59.9) 2,782 (40.1)
ASD status	Affected Unaffected	2,673 (100.0) 0 (0.0)	0 (0.0) 4,270 (100.0)	2,673 (38.5) 4,270 (61.5)
Twin status	Twin Singleton	82 (3.1) 2,585 (96.7)	154 (3.6) 4,113 (96.3)	236 (3.4) 6,698 (96.5)
Birth order	First Second Third Fourth Fifth Sixth or later	1,009 (38.3) 1,006 (38.1) 407 (15.4) 135 (5.1) 39 (1.5) 34 (1.3)	1,357 (32.4) 1,454 (34.7) 772 (18.4) 326 (7.8) 135 (3.2) 126 (3.0)	2,366 (34.7) 2,460 (36.0) 1,179 (17.3) 461 (6.8) 174 (2.5) 160 (2.3)
Maternal age at birth (years)	—	28.3 (5.5, 14.4-45.4)	27.9 (5.6, 14.7-45.7)	28.1 (5.6, 14.4-45.7)
Paternal age at birth (years)	—	31.6 (6.0, 13.8-57.6)	31.2 (6.0, 13.5-57.7)	31.3 (6.0, 13.5-57.7)
Age[b]	—	17.5 (6.7, 5.0-34.6)	18.1 (7.3, 5.0-34.9)	17.9 (7.1, 5.0-34.9)
Age at first ASD claim	—	8.4 (5.5, 2.0-33.3)	—	8.4 (5.5, 2.0-33.3)

[a]Sex, ASD status, and twin status are presented per individual; birth order, maternal age, paternal age, and age are presented per pregnancy
[b]Age on November 30, 2013 (end of study period)

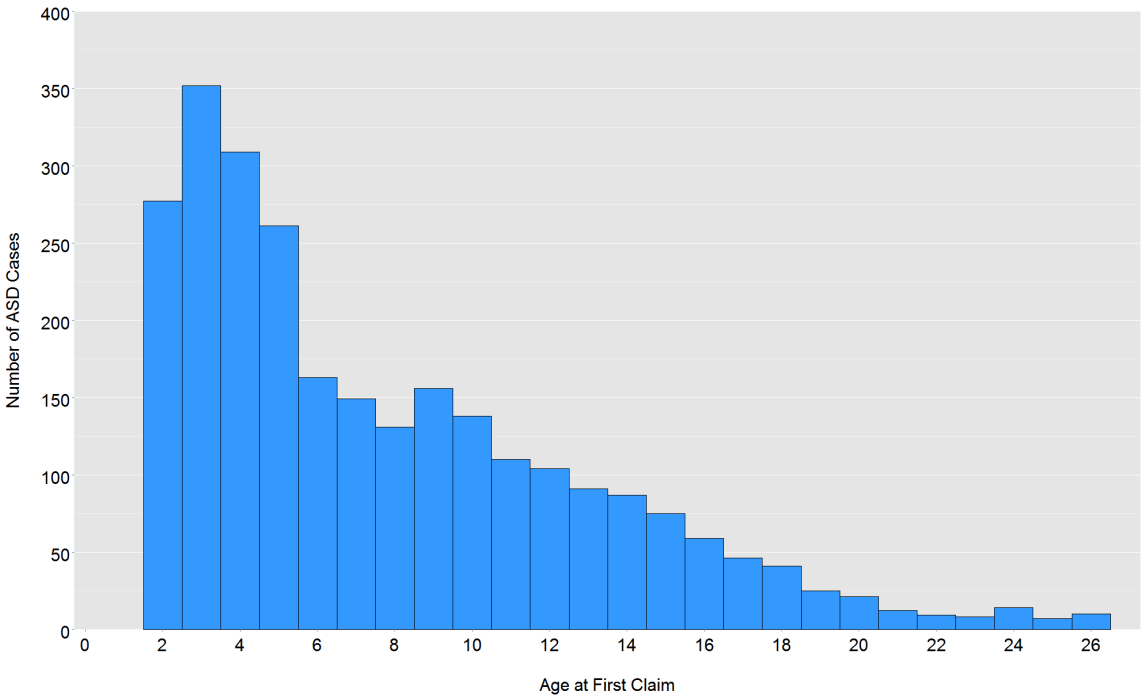


Figure 3.4. Distribution of age at the first ASD claim in the ASD families. Age at the first ASD claim was only displayed up to 26 years because the frequencies at older ages were less than six and could not be presented due to privacy restrictions.

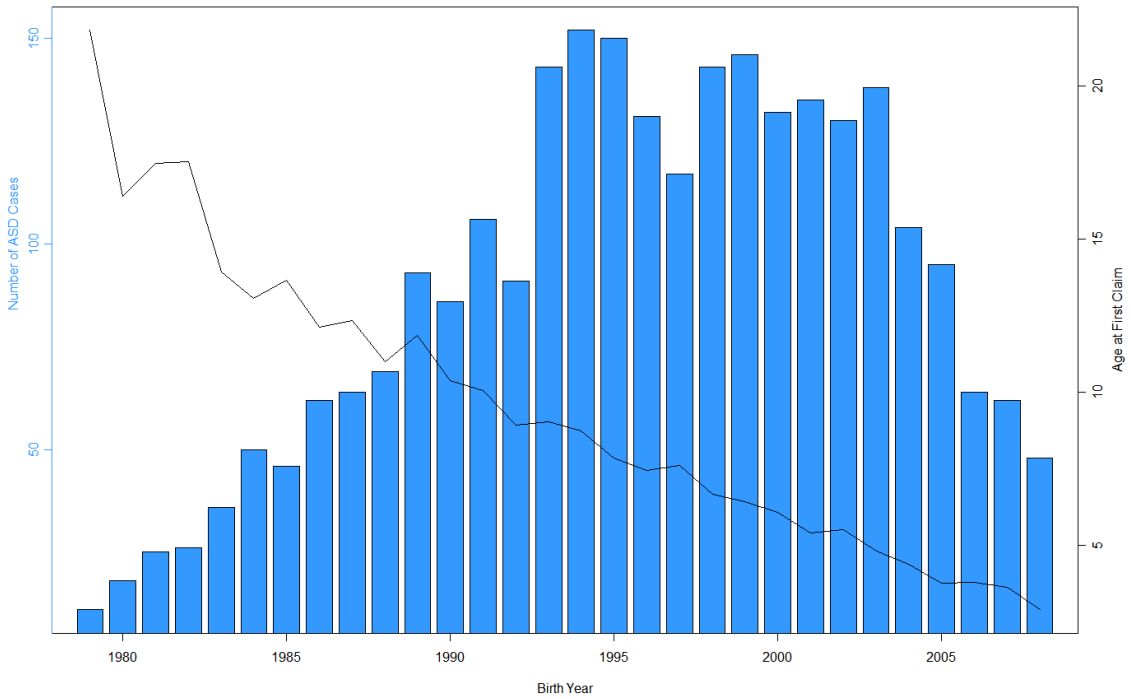


Figure 3.5. Mean age at the first ASD claim (black line) by birth year in the ASD families

Table 3.5. Description of the demographic variables in the ASD families by ASD case definition

Variable[a]	Category	Any ASD claim	≥2 ASD claims	Any specialist claim	≥2 specialist claims
Sex	Male	4,161 (59.9)	2,533 (60.1)	3,036 (59.8)	1,777 (60.4)
	Female	2,782 (40.1)	1,682 (39.9)	2,037 (40.2)	1,164 (39.6)
ASD status	Affected	2,673 (38.5)	1,623 (38.5)	1,968 (38.8)	1,147 (39.0)
	Unaffected	4,270 (61.5)	2,592 (61.5)	3,105 (61.2)	1,794 (61.0)
Twin status	Twin	236 (3.4)	154 (3.7)	181 (3.6)	107 (3.6)
	Singleton	6,698 (96.5)	4,054 (96.2)	4,886 (96.3)	2,830 (96.2)
Birth order	First	2,366 (34.7)	1,438 (34.8)	1,726 (34.8)	1,012 (35.1)
	Second	2,460 (36.0)	1,488 (36.0)	1,801 (36.3)	1,047 (36.3)
	Third	1,179 (17.3)	736 (17.8)	875 (17.6)	512 (17.7)
	Fourth	461 (6.8)	271 (6.5)	330 (6.6)	181 (6.3)
	Fifth	174 (2.5)	97 (2.3)	124 (2.5)	69 (2.4)
	Sixth or later	160 (2.3)	89 (2.2)	107 (2.2)	49 (1.7)
Maternal age at birth (years)	—	28.1 (5.6, 14.4-45.7)	28.3 (5.5, 14.4-45.7)	28.4 (5.5, 14.4-45.7)	28.7 (5.4, 14.7-45.7)
Paternal age at birth (years)	—	31.3 (6.0, 13.5-57.7)	31.4 (6.0, 15.1-56.0)	31.5 (6.0, 13.5-56.0)	31.8 (6.0, 15.1-55.9)
Age[b]	—	17.9 (7.1, 5.0-34.9)	18.1 (7.1, 5.0-34.9)	17.6 (7.1, 5.0-34.9)	18.3 (7.1, 5.0-34.9)
Age at first ASD claim	—	8.4 (5.5, 2.0-33.3)	7.2 (5.0, 2.0-32.4)	7.3 (5.1, 2.0-31.8)	6.6 (4.9, 2.0-31.7)

[a]Sex, ASD status, and twin status are presented per individual; birth order, maternal age, paternal age, and age are presented per pregnancy
[b]Age on November 30, 2013 (end of study period)

The twin families contained approximately even proportions of males (51.0%) and females (49.0%), as well as even proportions of twins (51.0%) and singletons (49.0%) (Table 3.6). Among the individuals in the twin families, 138 individuals (0.9%) had ASD. Similar to the ASD families, the majority of the children were third- or earlier-born, with 18.7% of children born fourth or later. Both the mean maternal and paternal ages were higher for the twins (maternal age: 28.8, SD=5.3; paternal age: 32.0, SD=5.5) than for their singleton siblings (maternal age: 26.6, SD=5.6; paternal age: 30.6, SD=5.8). The average age at the end of the study period was 17.0 years (SD=9.1), with ages ranging from 0.6 to 34.9 years old; the mean age of the twins

(16.1 years, SD=9.3) was slightly lower than the mean age of their singleton siblings (17.5 years, SD=9.0). Interestingly, the mean age at the first ASD claim was lower in the twins (6.7 years, SD=5.1) than in the singletons (8.9 years, SD=5.9), though only 68 twins (0.9%) and 70 singletons (0.9%) had a claim for ASD.

Table 3.6. Description of the demographic variables in the twin families

Variable[a]	Category	**Twins** Frequency (%) / Mean (SD, Range)	**Singleton siblings** Frequency (%) / Mean (SD, Range)	**Total** Frequency (%) / Mean (SD, Range)
Sex	Male Female	4,001 (50.3) 3,947 (49.7)	3,943 (51.7) 3,688 (48.3)	7,944 (51.0) 7,635 (49.0)
ASD status	Affected Unaffected	68 (0.9) 7,879 (99.1)	70 (0.9) 7,559 (99.1)	138 (0.9) 15,438 (99.1)
Twin status	Twin Singleton	7,948 (100.0) 0 (0.0)	0 (0.0) 7,631 (100.0)	7,948 (51.0) 7,631 (49.0)
Birth order	First Second Third Fourth Fifth Sixth or later	753 (18.8) 1,730 (43.2) 820 (20.5) 362 (9.0) 154 (3.8) 156 (3.9)	2,790 (36.6) 2,028 (26.6) 1,253 (16.4) 640 (8.4) 389 (5.1) 473 (6.2)	3,543 (30.5) 3,758 (32.3) 2,073 (17.8) 1,002 (8.6) 543 (4.7) 629 (5.4)
Maternal age at birth (years)	—	28.8 (5.3, 14.4-47.8)	26.6 (5.6, 13.1-55.3)	27.4 (5.6, 13.1-55.3)
Paternal age at birth (years)	—	32.0 (5.5, 17.9-57.8)	30.6 (5.8, 15.2-62.4)	31.1 (5.8, 15.2-62.4)
Age[b]	—	16.1 (9.3, 0.7-34.6)	17.5 (9.0, 0.6-34.9)	17.0 (9.1, 0.6-34.9)
Age at first ASD claim	—	6.7 (5.1, 2.0-21.8)	8.9 (5.9, 2.0-29.6)	7.8 (5.6, 2.0-29.6)

[a]Sex, ASD status, and twin status are presented per individual; birth order, maternal age, paternal age, and age are presented per pregnancy
[b]Age on November 30, 2013 (end of study period)

3.2.3. Congenital Anomalies

After removing 171 (6.0%) individuals with ASD and 45 (1.0%) of their unaffected siblings due to chromosomal anomalies during the final quality control step, 919 (34.4%) and 1,061 (24.8%) of the ASD-affected and unaffected siblings, respectively, had at least one claim for any other congenital anomaly. Other anomalies of the heart, genital organs, musculoskeletal system, and limbs were the most commonly observed among the individuals with ASD with frequencies of 6.5%, 5.3%, 8.6%, and 6.1%, respectively (Figure 3.6). The individuals with ASD were more likely to have most of the congenital anomalies compared to their unaffected siblings. However, the prevalences of ear, face, and neck anomalies, cleft palate, other digestive anomalies, and integument anomalies were not significantly different between the individuals with ASD and their unaffected siblings. Because it was not clear whether these congenital anomalies could be responsible for the ASD phenotype, individuals with congenital anomalies (other than chromosomal anomalies) were not removed for the majority of the statistical analyses. However, the relationships between the prenatal and perinatal complications and ASD were re-examined after removing individuals with claims for congenital anomalies (as described in the Methods section 2.3.2.4).

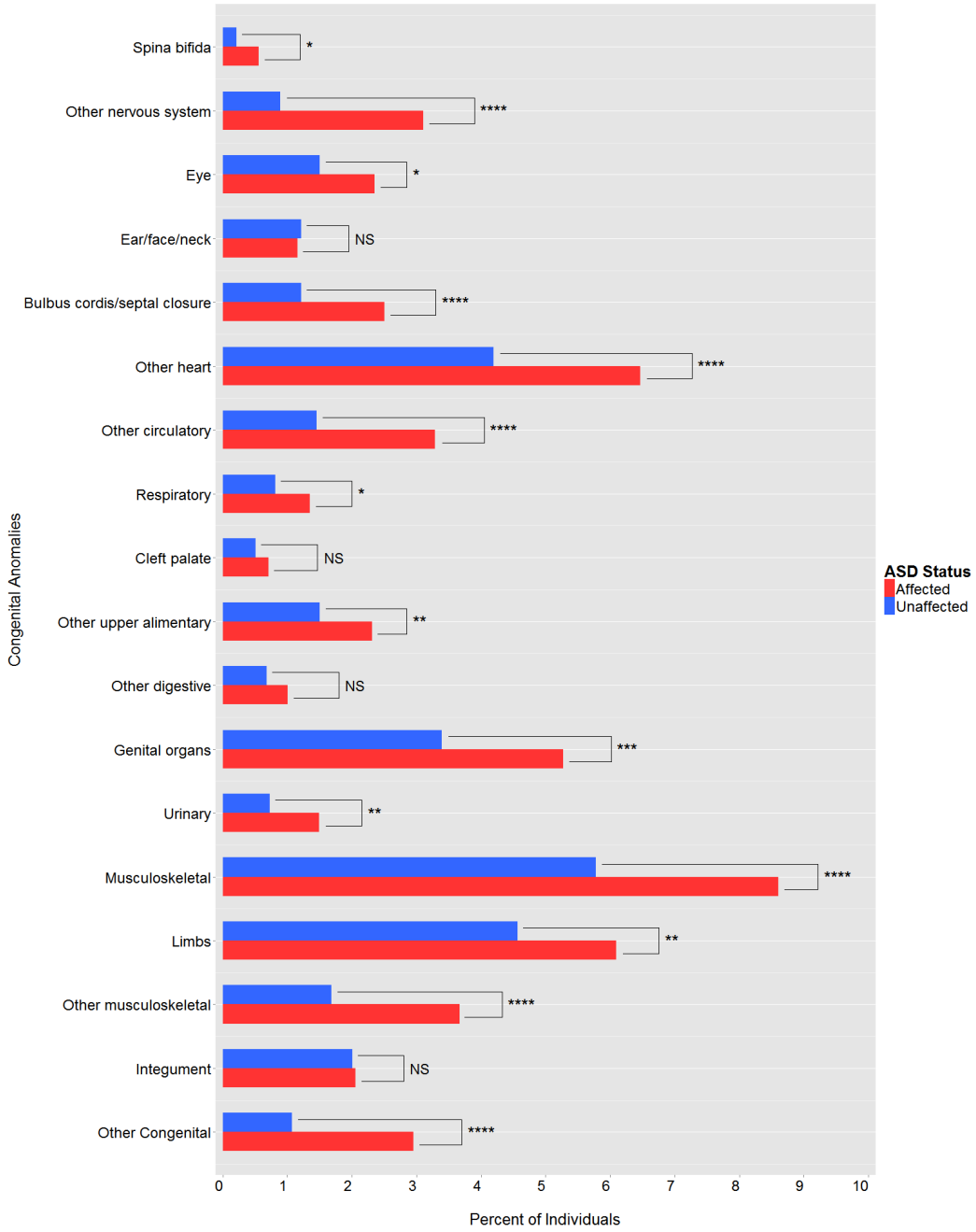


Figure 3.6. Prevalences of congenital anomalies in the ASD families. * $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $0.0001 < p \leq 0.001$; **** $p \leq 0.0001$; NS, not significant.

3.2.4. Other Mental and Behavioural Disorders

The other mental and behavioural disorders that were observed the most frequently among the individuals with ASD were neurotic disorders (36.0%), conduct disorder (37.4%), hyperkinetic

syndrome of childhood (48.2%), and developmental delay (43.5%) (Figure 3.7). The individuals with ASD experienced more of most of the mental and behavioural disorders than their unaffected siblings. Most of these disorders are expected to be comorbid with ASD (e.g. developmental delay, mild intellectual disability, and unspecified intellectual disability)[1]. The prevalences of alcoholic psychoses, persistent mental disorders, alcohol dependence, drug dependence, and disorders due to brain damage were not significantly different between the individuals with ASD and their unaffected siblings. The only disorder that occurred significantly more in the unaffected individuals than in their siblings with ASD was non-dependent drug abuse ($p=0.02$).

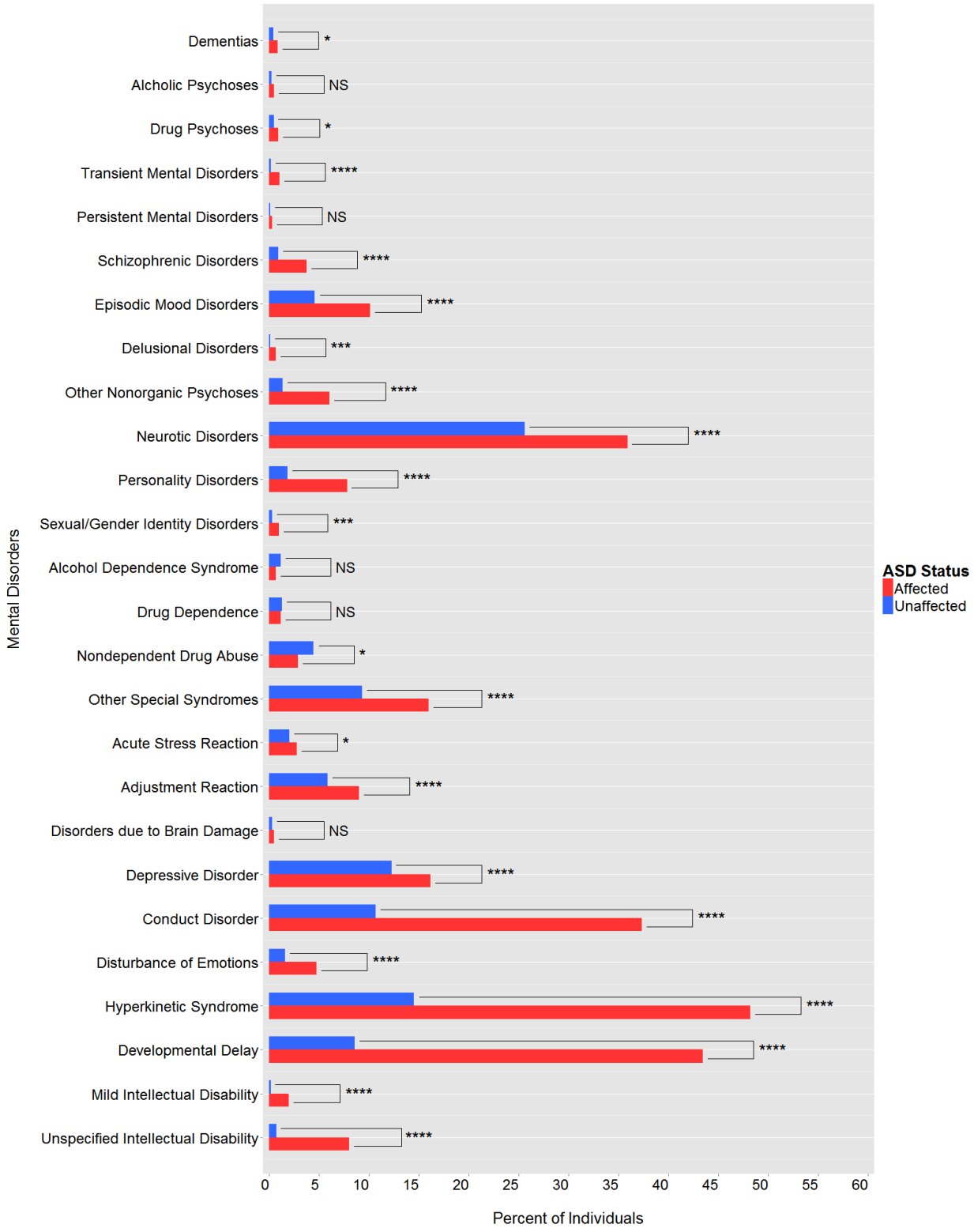


Figure 3.7. Prevalences of other mental and behavioural disorders in the ASD families. * $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $0.0001 < p \leq 0.001$; **** $p \leq 0.0001$; NS, not significant.

Of the individuals with ASD, 2,349 (87.9%) had a claim for at least one other mental or behavioural disorder (Figure 3.8). Specifically, 52.8% had a claim for at least one other mental disorder after his/her most recent ASD claim, and 71.9% had at least one other claim before his/her most recent ASD claim. The mean age among the individuals without a claim for another mental or behavioural disorder (13.2, SD=5.8) was significantly younger than the mean ages among individuals with a claim for at least one other disorder (all $p<0.0001$). Intriguingly, when using two of the more stringent case definitions (i.e. more than one ASD claim and more than one specialist claim), a smaller proportion of individuals had a claim for another mental or behavioural disorder after the most recent ASD claim than the samples selected based on any ASD claim or any specialist claim (Table 3.7).

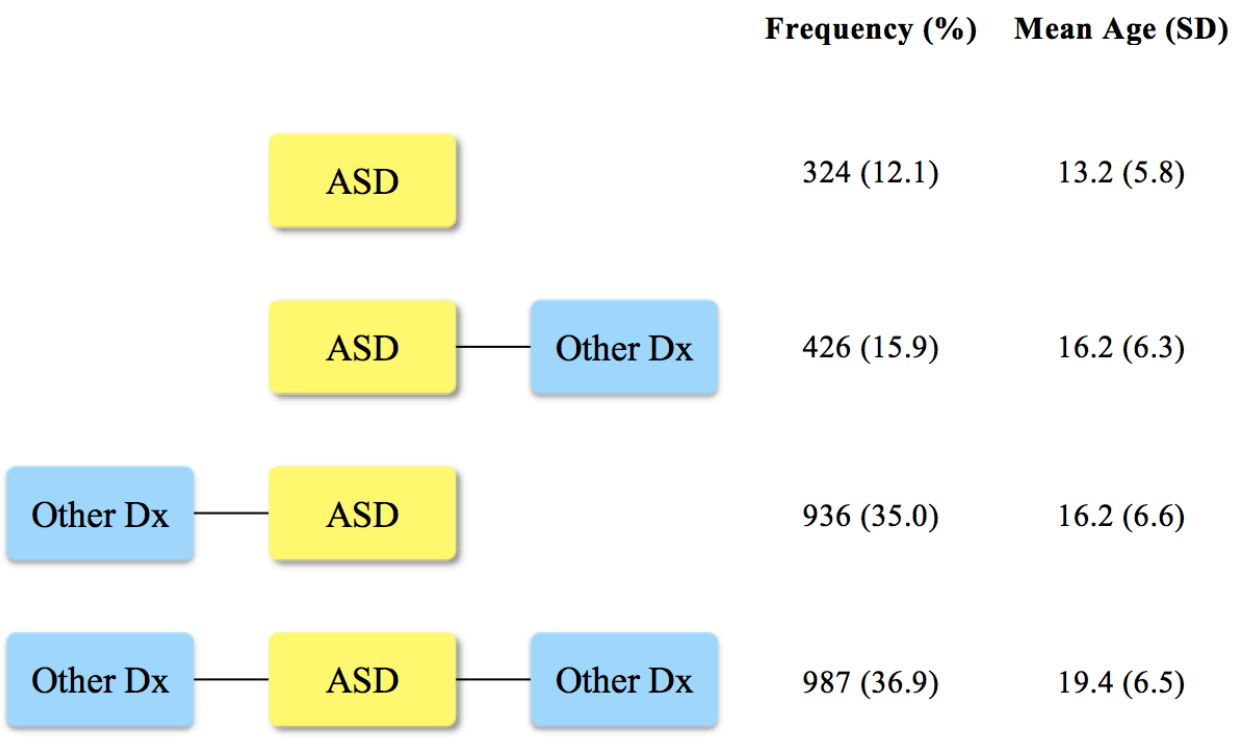


Figure 3.8. Descriptive statistics of the different temporal orders of the last claim for other mental and behavioural disorders in relation to the last claim for ASD

Table 3.7. Frequencies (%) of different temporal orders of the last claim for other mental and behavioural disorders in relation to the last claim for ASD by ASD case definition

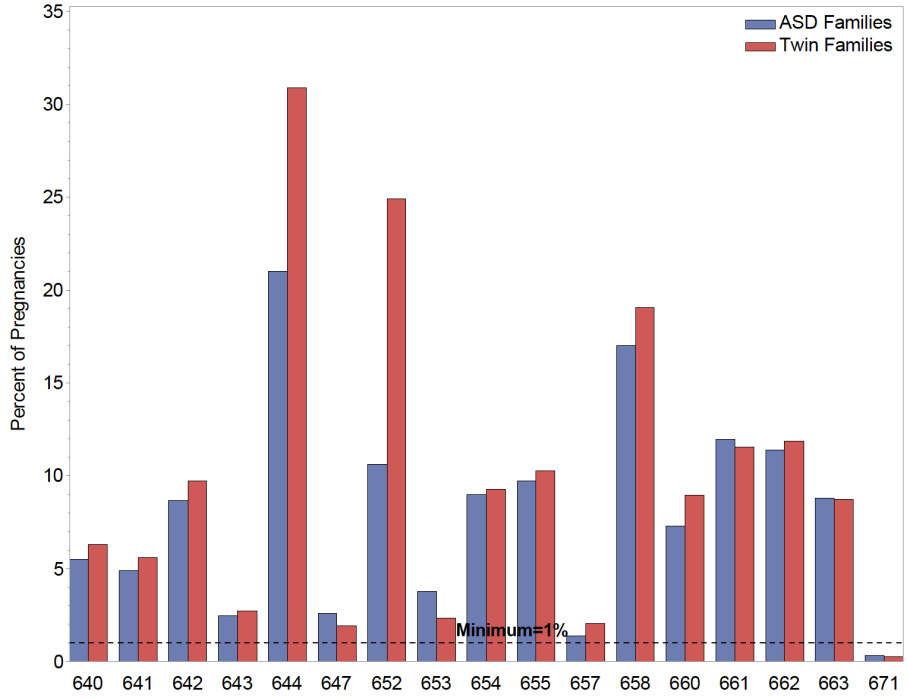
Temporal order[a]	Any ASD claim	>1 ASD claim	Any specialist claim	>1 specialist claim
None	324 (12.1)	190 (11.7)	249 (12.7)	136 (11.9)
After	426 (15.9)	161 (9.9)	278 (14.1)	102 (8.9)
Before/same	936 (35.0)	698 (43.0)	757 (38.5)	504 (43.9)
Before/same and after	987 (36.9)	574 (35.4)	684 (34.8)	405 (35.3)

[a]Timing of the last claim for any other mental or behavioural disorder in relation to the time of the last ASD claim

3.2.5. Prenatal and Perinatal Complications

Early or threatened labour (ICD-9 code 644) and complications of the amniotic cavity and membranes (658) were the prenatal complications with the highest frequencies in the ASD families (21.0% and 17.1%, respectively) (Figure 3.9A and Appendix 3). In the twin families, in addition to early or threatened labour (30.9%) and amniotic cavity and membrane complications (19.1%), malpresentation and malposition of the foetus (652) also occurred at a high prevalence (24.9%). The most frequently observed perinatal complications were other respiratory conditions of the foetus and newborn (770) and other perinatal jaundice (774) in both the ASD and twin families (Figure 3.9B and Appendix 3). The frequencies of other respiratory conditions were 10.5% in the ASD families and 16.6% in the twin families. The frequencies of other perinatal jaundice were 14.4% and 18.0% in the ASD and twin families, respectively.

A)



B)

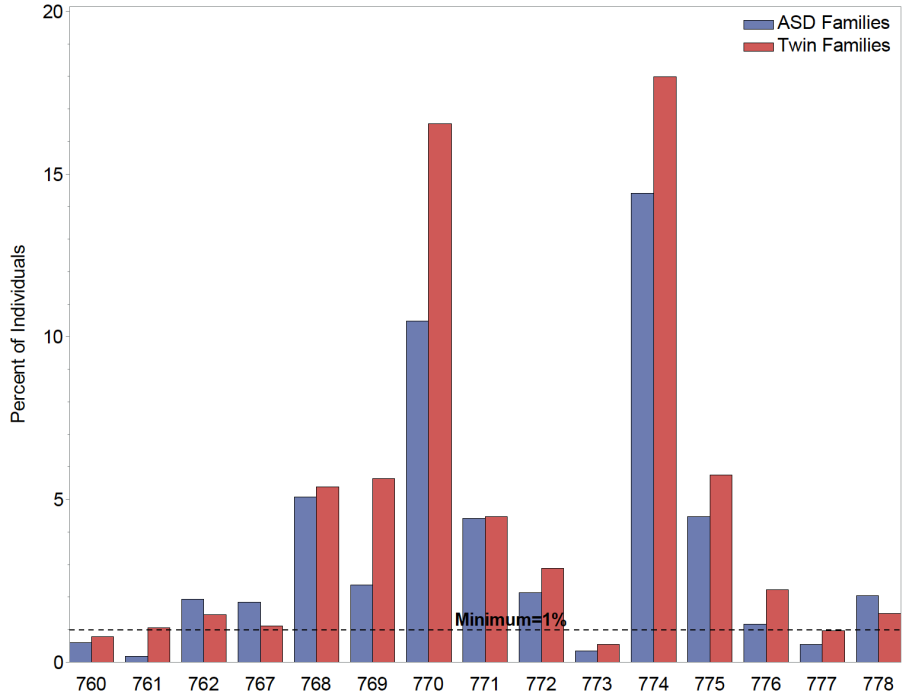


Figure 3.9. Prevalences of A) prenatal complications and B) perinatal complications in the ASD and twin families. The ICD-9 codes for each prenatal or perinatal complication is indicated on the X-axis. The prevalences in the ASD families are indicated in blue and the prevalences in the twin families are indicated in red. The prevalence of each prenatal complication was calculated per pregnancy, while the prevalence of each perinatal complication was calculated per individual. The dotted line indicates the minimum 1% prevalence that was required in the ASD families for a complication to be included in the study.

Venous complications in pregnancy and the puerperium (671) and obstetrical pulmonary embolism (673) were the only prenatal complications that were excluded due to a frequency of less than 1% among the pregnancies in the ASD families. The prevalence of obstetrical pulmonary embolism was not presented in Figure 3.9A because the frequency was less than six pregnancies and could not be presented due to privacy restrictions. Of the perinatal complications, maternal conditions that may be unrelated to pregnancy affecting the foetus or newborn (760), maternal complications of pregnancy affecting the foetus or newborn (761), haemolytic disease due to isoimmunisation (773), and perinatal disorders of the digestive system (777) were excluded due to a frequency of less than 1% in the ASD families.

3.2.6. Other Birth Information

In the ASD families, the mean birth weight was 3.4 kilograms (SD=0.6 kg) and the mean gestational age was 39.1 weeks (SD=2.0 weeks) (Table 3.8). The mean 1- and 5-minute Apgar scores were 7.9 (SD=1.5) and 8.9 (SD=0.7), respectively, which are both within the "normal" range (greater than or equal to 7). Similar means were observed in the ASD families selected using the more stringent case definitions (Table 3.9).

As expected, the mean birth weight (3.0 kg, SD=0.8 kg) and gestational age (38.1 weeks, SD=2.6 weeks) were lower in the twin families than in the ASD families (Table 3.10). Similarly, the average 1- and 5-minute Apgar scores in the twin families were lower than those in the ASD families, though both still fell within the "normal" range (1-minute Apgar score=7.7, SD=1.7; 5-minute Apgar score=8.7, SD=1.0).

Table 3.8. Description of the other birth information in the ASD families

Variable[a]	**ASD-affected individuals** Mean (SD, Range)	**Unaffected siblings** Mean (SD, Range)	**Total** Mean (SD, Range)
Birth weight (grams)	3,439 (635, 541-5,480)	3,441 (594, 700-5,772)	3,440 (610, 541-5,772)
Gestational age (weeks)	39.0 (2.2, 24-44)	39.1 (1.9, 25-43)	39.1 (2.0, 24-44)
1-minute Apgar score	7.8 (1.5, 1-10)	8.0 (1.4, 1-10)	7.9 (1.5, 1-10)
5-minute Apgar score	8.8 (0.8, 1-10)	8.9 (0.7, 1-10)	8.9 (0.7, 1-10)

[a]Birth weight and Apgar scores are presented per individual; gestational age is presented per pregnancy

Table 3.9. Description of the other birth information in the ASD families by ASD case definition

Variable[a]	Any ASD claim	≥2 ASD claims	Any specialist claim	≥2 specialist claims
Birth weight (grams)	3,440 (610, 541-5,772)	3,447 (602, 600-5,772)	3,443 (610, 541-5,772)	3,454 (607, 600-5,772)
Gestational age (weeks)	39.1 (2.0, 24-44)	39.1 (2.0, 24-43)	39.0 (2.1, 24-44)	39.1 (2.0, 24-43)
1-minute Apgar score	7.9 (1.5, 1-10)	7.9 (1.4, 1-10)	7.9 (1.4, 1-10)	7.9 (1.4, 1-10)
5-minute Apgar score	8.9 (0.7, 1-10)	8.9 (0.7, 1-10)	8.9 (0.7, 1-10)	8.8 (0.7, 1-10)

[a]Birth weight and Apgar scores are presented per individual; gestational age is presented per pregnancy

Table 3.10. Description of the other birth information in the twin families

Variable[a]	**Twins** Mean (SD, Range)	**Singleton siblings** Mean (SD, Range)	**Total** Mean (SD, Range)
Birth weight (grams)	2,540 (596, 500-4,918)	3,491 (581, 550-6,120)	3,012 (757, 500-6,120)
Gestational age (weeks)	36.1 (2.7, 24-42)	39.2 (1.9, 24-44)	38.1 (2.6, 24-44)
1-minute Apgar score	7.5 (1.8, 1-10)	7.9 (1.6, 1-10)	7.7 (1.7, 1-10)
5-minute Apgar score	8.6 (1.1, 1-10)	8.9 (0.8, 1-10)	8.7 (1.0, 1-10)

[a]Birth weight and Apgar scores are presented per individual; gestational age is presented per pregnancy

Birth weight percentile was calculated based on gestational age, sex, and twin status (Appendix 8). The number of individuals with a gestational age less than 35 weeks or larger than 42 weeks among the singletons and less than 28 weeks or greater than 40 weeks among the twins constituted less than 1% of the respective samples; however, these were not set to missing as the majority of these groups contained more than 10 individuals and were used to calculate birth weight percentiles.

3.3. RELATIONSHIPS AMONG PRENATAL AND PERINATAL COMPLICATIONS AND COVARIATES

3.3.1. Relationships Among Prenatal and Perinatal Complications

As expected, some of the prenatal and perinatal complications were associated with one another. Among the prenatal complications, early or threatened labour (644), complications of the amniotic cavity and membranes (658), obstructed labour (660), abnormal forces of labour (661), and long labour (662) were the most commonly associated with other prenatal complications (i.e. 4 or more positive associations with $p \leq 0.0001$) (Appendix 9). Alternatively, abnormalities of the organs and soft tissues of the pelvis (654) were negatively associated with several complications, such as complications of the amniotic cavity and membranes (658) and abnormal forces of labour (661).

The majority of the perinatal complications were significantly associated with one another and had p-values less than 0.0001 (Appendix 10). The odds ratios were exceedingly large for some of the complications, especially among respiratory distress syndrome (769), neonatal haemorrhage (772), and haematological disorders (776) (OR ranged from 69.9-424; all $p \leq 0.0001$) (Table

3.11). Haematological disorders were also strongly associated with complications of the placenta, cord, and membranes (762), other respiratory conditions (770), perinatal infections (771), perinatal jaundice (774), and endocrine and metabolic disturbances (775) (all OR>10; all $p \leq 0.0001$). Higher 1- and 5-minute Apgar scores were associated with a decreased risk for many perinatal complications; the strongest associations were observed with intrauterine hypoxia and birth asphyxia (768), respiratory distress syndrome (769), other respiratory conditions (770), and haematological disorders (776) (all $p \leq 0.0001$).

Table 3.11. Contingency tables for: A) respiratory distress syndrome and neonatal haemorrhage, B) respiratory distress syndrome and haematological disorders, and C) neonatal haemorrhage and haematological disorders in the ASD families

A)

Frequency (%)		Neonatal haemorrhage	
		Present	Absent
Respiratory distress syndrome	Present	53 (0.8)	112 (1.6)
	Absent	95 (1.4)	6,683 (96.3)

B)

Frequency (%)		Haematological disorders	
		Present	Absent
Respiratory distress syndrome	Present	51 (0.7)	114 (1.6)
	Absent	31 (0.4)	6,747 (97.2)

C)

Frequency (%)		Haematological disorders	
		Present	Absent
Neonatal haemorrhage	Present	31 (0.4)	117 (1.7)
	Absent	51 (0.7)	6,744 (97.1)

Some of the prenatal and perinatal complications were significantly associated with one another (Appendix 11); some of these associations were likely due to overlaps between the prenatal and perinatal ICD codes. For example, the prenatal ICD-9 code 663 indicates umbilical cord complications, while the perinatal ICD-9 code 762 indicates complications of the placenta, cord,

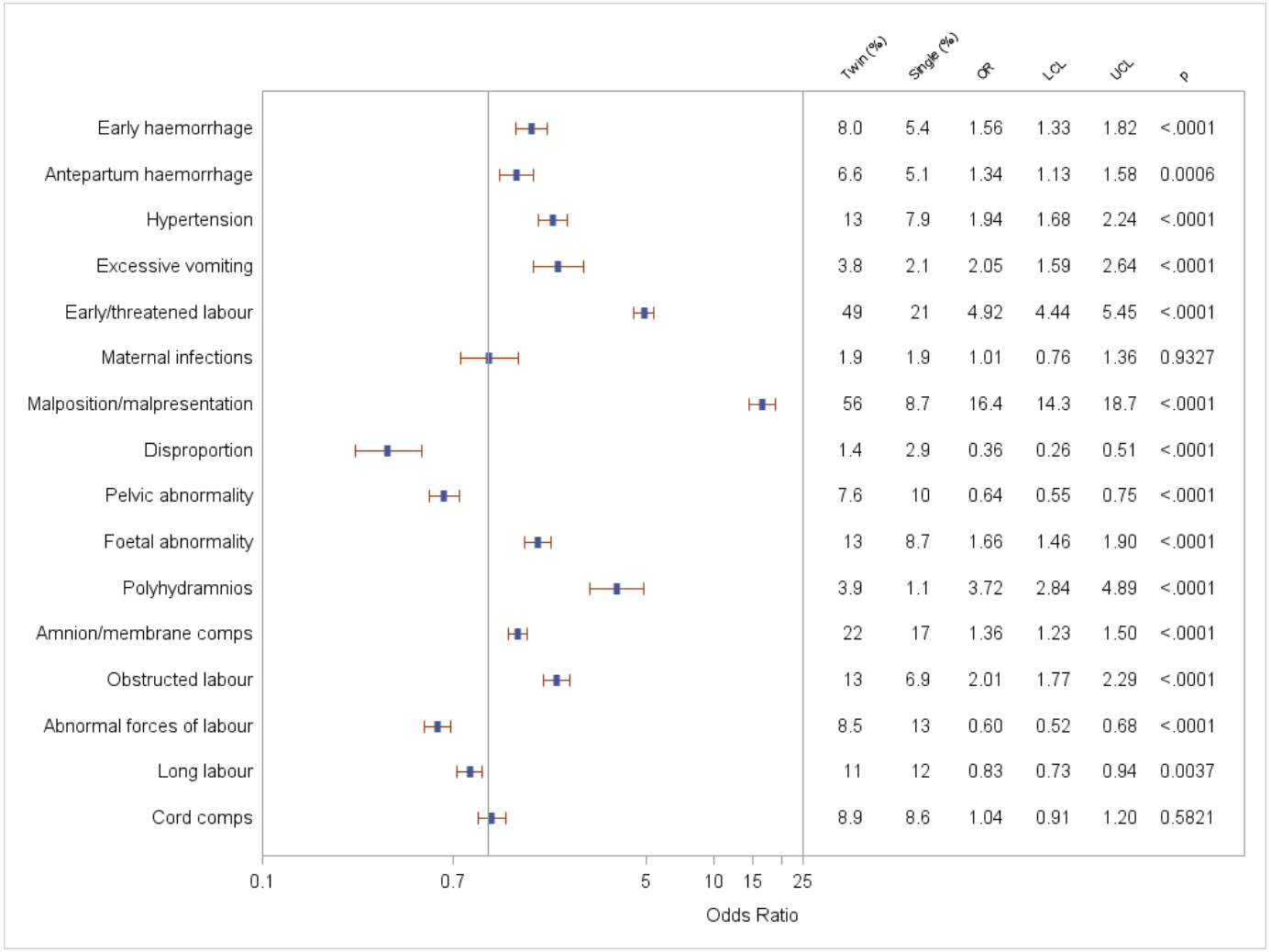
and membranes (OR=57.5; 95% CI: 34.5-96.4; p<0.0001). Of the prenatal complications, early or threatened labour (644) and malposition and malpresentation of the foetus (652) were the most commonly associated with perinatal complications. However, some prenatal complications, such as excessive vomiting during pregnancy (643) and abnormalities of the organs and soft tissues of the pelvis (654) were associated with few perinatal complications. Among the perinatal complications, respiratory distress syndrome (769) and other respiratory conditions (770) were the most commonly associated with prenatal complications. Some of the largest odds ratios were observed between haematological disorders and prenatal complications, such as placental complications (641; OR=4.44), early or threatened labour (644; OR=7.16), malposition and malpresentation of the foetus (652; OR=3.96), and polyhydramnios (657; OR=5.45). Similar to the perinatal complications, higher 1- and 5-minute Apgar scores were associated with a decreased risk for many prenatal complications, including placental complications (641), early or threatened labour (644), obstructed labour (660), and cord complications (663) (all p≤0.0001).

3.3.2. Relationships Between Prenatal and Perinatal Complications and Twin Status

In the twin families, mothers were more likely to experience most of the prenatal complications during their pregnancies with twins compared to their singleton pregnancies, as expected (Figure 3.10A). The twins were also more likely to have most of the perinatal complications (Figure 3.10B) and had lower birth weights (p<0.0001), shorter gestational ages (p<0.0001), and lower 1- and 5-minute Apgar scores (both p<0.0001) than their singleton siblings. In particular, twin status was strongly associated with an increased risk for malposition and malpresentation of the foetus (652) (OR=16.4; 95% CI: 14.3-18.7; p<0.0001), respiratory distress syndrome (769) (OR=17.6; 95% CI: 13.4-23.0; p<0.0001), and haematological disorders (776) (OR=14.1; 95%

CI: 9.47-21.1; $p<0.0001$). However, during twin pregnancies and deliveries, several complications (e.g. abnormality of the forces of labour [661] and birth trauma [767]) were encountered significantly less frequently than during singleton pregnancies and deliveries. This may be because twin pregnancies are at an increased risk for caesarean section[119,121], and these complications are less likely to be encountered during a caesarean section delivery.

A)


Twin (%)
Single (%)
OR
LCL
UCL
p
Early haemorrhage 8.0 5.4 1.56 1.33 1.82 <.0001
Antepartum haemorrhage 6.6 5.1 1.34 1.13 1.58 0.0006
Hypertension 13 7.9 1.94 1.68 2.24 <.0001
Excessive vomiting 3.8 2.1 2.05 1.59 2.64 <.0001
Early/threatened labour 49 21 4.92 4.44 5.45 <.0001
Maternal infections 1.9 1.9 1.01 0.76 1.36 0.9327
Malposition/malpresentation 56 8.7 16.4 14.3 18.7 <.0001
Disproportion 1.4 2.9 0.36 0.26 0.51 <.0001
Pelvic abnormality 7.6 10 0.64 0.55 0.75 <.0001
Foetal abnormality 13 8.7 1.66 1.46 1.90 <.0001
Polyhydramnios 3.9 1.1 3.72 2.84 4.89 <.0001
Amnion/membrane comps 22 17 1.36 1.23 1.50 <.0001
Obstructed labour 13 6.9 2.01 1.77 2.29 <.0001
Abnormal forces of labour 8.5 13 0.60 0.52 0.68 <.0001
Long labour 11 12 0.83 0.73 0.94 0.0037
Cord comps 8.9 8.6 1.04 0.91 1.20 0.5821
0.1
0.7
5
10
15
25
Odds Ratio

B)

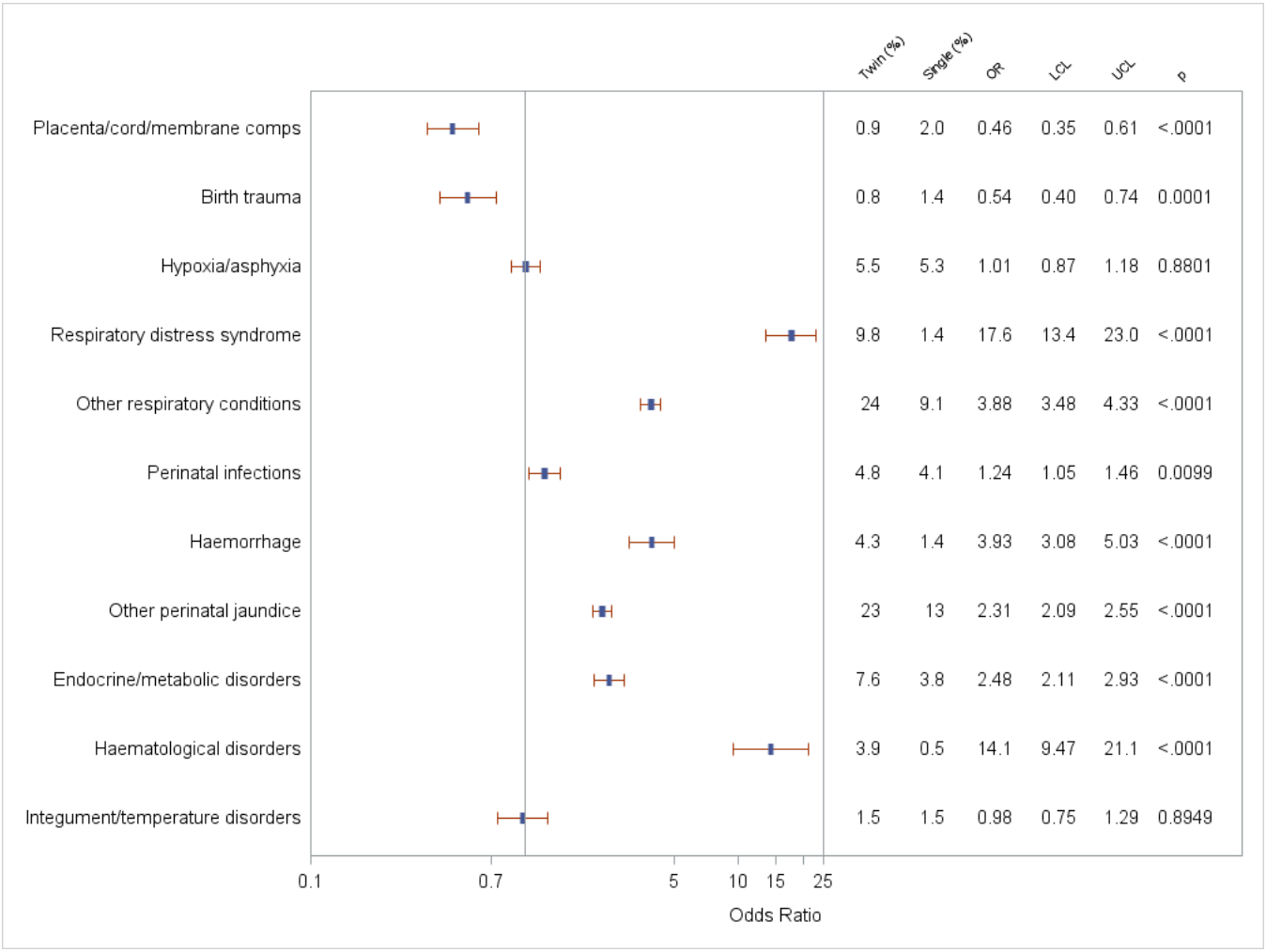


Figure 3.10. Pairwise relationships between twin status and A) prenatal complications and B) perinatal complications in the twin families. Twin (%) and single (%) indicate the frequency of each complication in the twins and singletons, respectively. The frequencies were calculated per pregnancy for the prenatal complications and per individual for the perinatal complications. An odds ratio >1 indicates that twins are at an increased risk for the complication, while an odds ratio <1 indicates that twins are at a decreased risk. OR=odds ratio; LCL=lower 95% confidence limit; UCL=upper 95% confidence limit; p=p-value.

In the ASD families, fewer significant relationships were observed between twin status and prenatal and perinatal complications (Appendices 12 to 14), likely due to the small number of twins (n=236). However, as in the twin families, similar significant relationships were observed between some of the complications (e.g. umbilical cord complications [663] and endocrine and metabolic disorders [775]) and twin status.

3.3.3. Relationships Between Prenatal and Perinatal Complications and the Other Covariates

The associations between the prenatal and perinatal complications and the other covariates (i.e. sex, maternal age at birth, paternal age at birth, birth year, birth order, gestational age, and birth weight) are presented in Appendices 12 to 14.

Some prenatal and perinatal complications were associated with **sex**, whereby both prenatal and perinatal complications were less likely to occur during a pregnancy with a female child than with a male child. Examples include infectious and parasitic conditions in the mother (647), other respiratory conditions (770), other perinatal jaundice (774), and conditions involving the integument and temperature regulation (778).

Many prenatal complications (e.g. excessive vomiting in pregnancy [643], early or threatened labour [644], umbilical cord complications [663]) and perinatal complications (e.g. intrauterine hypoxia and birth asphyxia [768] and other perinatal jaundice [774]) were more prevalent among younger mothers and their children. However, increased **maternal age** was also associated with an increased risk for several prenatal and perinatal complications (e.g. placental complications [641], malposition and malpresentation of the foetus [652], endocrine or metabolic disturbances

[775], and lower 5-minute Apgar scores). Although maternal age was more likely to be related to prenatal complications than paternal age, it is expected that **paternal age** would demonstrate similar associations due to the high correlation between maternal and paternal ages. However, some complications were associated with maternal age but not with paternal age, probably due to the reduced sample size for which paternal age was available.

Many of the prenatal complications (e.g. haemorrhage in early pregnancy [640], abnormality of forces of labour [661], and long labour [662]) were also associated with **birth year**, including the complications that were associated with younger maternal age (e.g. excessive vomiting in pregnancy [643], early or threatened labour [644], and amniotic cavity and membrane complications [658]); these complications were more frequent among the mothers giving birth in recent years. Intrauterine hypoxia and birth asphyxia (768) was the only perinatal complication that was significantly associated with birth year ($p<0.0001$), whereby the risk for this complication was decreased in more recent birth years.

Some of the prenatal complications were consistently associated with **birth order**. For example, during their pregnancies with the second- or later-born siblings, mothers were at an increased risk for abnormalities of the organs and soft tissues of the pelvis (654) and foetal abnormalities affecting management of the mother (655), compared to their pregnancies with the first-borns. During their later pregnancies, mothers were at a lower risk for hypertension (642), complications of the amniotic cavity and membranes (658), obstructed labour (660), and long labour (662). Alternatively, few perinatal complications (e.g. birth trauma [767] and perinatal jaundice [774]) were consistently associated with birth order. For example, there was a lower

risk of haematological disorders (776) in the second-born children and a higher risk in the fifth-born children, compared to the first-borns; similarly, 5-minute Apgar scores were higher in second- and fourth-born children only, compared to the first-borns. This is likely explained by a lower prevalence of perinatal complications compared to prenatal complications and the small number of ASD families that contained more than three children.

Birth weight and gestational age were significantly associated with the majority of the prenatal and perinatal complications, as expected. Among the prenatal complications, those that occur during pregnancy (e.g. early or threatened labour [644] and complications related to the amniotic cavity and membranes [658]) were typically associated with lower birth weight and shorter gestational age, while the complications encountered during labour and delivery (e.g. long labour [662]) were significantly associated with higher birth weight and longer gestational age. Almost all of the perinatal complications (e.g. haematological disorders [776] and low Apgar scores) were significantly associated with lower birth weight and shorter gestational age. Birth trauma (767) and conditions involving the integument and temperature regulation (778) were the only perinatal complications that were associated with a higher birth weight, though they were not associated with gestational age.

3.3.4. Relationships Among Covariates

Similar to the prenatal and perinatal complications, the covariates were also associated with one another (Table 3.12).

As expected, **birth weight and gestational age** were highly associated; for this reason, birth weight percentile by gestational age, sex, and twin status was used in all of the analyses that included both birth weight and gestational age in the same statistical model. Twins had significantly lower birth weights and shorter gestational ages than singletons. Females had significantly lower birth weights than males, although gestational age was not significantly different between males and females. Higher birth weights and shorter gestational ages were significantly associated with increased maternal or paternal age and later birth years. Similarly, later-born children had significantly higher birth weights and shorter gestational ages than their first-born siblings.

Older mothers were at an increased risk to have **twins**, however paternal age was not associated with twin status. There was no significant difference in sex, birth year, or birth order between twins and their singleton siblings. A trend of increasing numbers of twins over the study period was expected but was not observed, probably due to the small number of twins (n=236) in the ASD families. For this reason, the relationships between twin status and the other covariates were also examined in the twin families (Table 3.13). Similar to the ASD families, twins had significantly lower birth weights and shorter gestational ages than their singleton siblings. In the twin families, older mothers and fathers were more likely to have twins than younger parents. The prevalence of twins was also higher in more recent birth years. Last, twins were more likely to be born second or later; however, this relationship may be observed due to confounding among parental ages, birth year, and birth order, as described below.

As expected, **sex** was not significantly associated with birth year or birth order. Interestingly, older mothers were significantly more likely to have male children than female children; this relationship was likely observed in the ASD families because: 1) individuals with ASD are more likely to be males than females and 2) increased maternal age is a risk factor for ASD. A similar trend was also observed for older fathers, though it did not reach statistical significance ($p=0.08$). In the twin families, both maternal and paternal age were not associated with sex, further corroborating the potential confounding effect of ASD on the relationship between sex and parental age in the ASD families.

Maternal age, paternal age, birth year, and birth order were highly associated with one another. This is because as birth order increases, parental age and birth year also increase. For this reason, parental ages and birth year at the first child's birth were used for all children in the family in all further statistical analyses, while birth order was used to account for the differences in these variables between siblings.

Table 3.12. Pairwise relationships between the covariates in the ASD families

Test statistic (df) p-value	Outcome[a]							
Predictor[a]	Birth weight	Gest age	Twin status	Sex	Maternal age	Paternal age	Birth year	Birth order
Birth weight								
Gestational age	62.6 (4,053) <0.0001							
Twin status	-24.9 (91) <0.0001	-21.0 (91) <0.0001						
Sex	-9.72 (1,564) <0.0001	0.84 (1,572) 0.40	0.33 (1,649) 0.74					
Maternal age	2.70 (4,065) 0.007	-7.53 (3,989) <0.0001	2.65 (4,225) 0.008	-3.18 (4,360) 0.002				
Paternal age	2.74 (2,627) 0.006	-5.31 (2,560) <0.0001	0.52 (2,746) 0.61	-1.76 (2,839) 0.08	203 (2,741) <0.0001			
Birth year	4.63 (4,081) <0.0001	-6.89 (4,005) <0.0001	0.93 (4,245) 0.35	-1.30 (4,380) 0.19	892 (4,243) <0.0001	190 (2,759) <0.0001		
Birth order								
Second	7.55 (3,889) <0.0001	-1.87 (3,918) 0.06	0.36 (4,150) 0.72	-0.48 (4,168) 0.63	61.0 (4,148) <0.0001	45.9 (2,704) <0.0001	60.1 (4,168) <0.0001	
Third	4.75 (3,889) <0.0001	-4.05 (3,918) <0.0001	-0.45 (4,150) 0.65	1.10 (4,168) 0.27	86.3 (4,148) <0.0001	64.7 (2,704) <0.0001	85.6 (4,168) <0.0001	
Fourth	3.96 (3,889) <0.0001	-3.98 (3,918) <0.0001	0.65 (4,150) 0.52	0.59 (4,168) 0.56	80.7 (4,148) <0.0001	58.9 (2,704) <0.0001	80.6 (4,168) <0.0001	
Fifth	1.28 (3,889) 0.20	-5.03 (3,918) <0.0001	0.02 (4,150) 0.99	1.13 (4,168) 0.26	66.5 (4,148) <0.0001	47.6 (2,704) <0.0001	66.1 (4,168) <0.0001	
Sixth	1.80 (3,889) 0.07	-4.41 (3,918) <0.0001	-1.02 (4,150) 0.31	0.94 (4,168) 0.35	74.7 (4,148) <0.0001	54.4 (2,704) <0.0001	74.4 (4,168) <0.0001	

[a]When both the predictor and the outcome variables were specific to each pregnancy (i.e. gestational age, twin status, maternal age, paternal age, birth year, or birth order), the values for each pregnancy were used, rather than for each individual.

Table 3.13. Pairwise relationships between twin status and the other covariates in the twin families

Test statistic (df) p-value	Outcome[a]		
Predictor[a]	Twin status	Birth weight	Gest age
Twin status		-120 (3,492) <0.0001	-75.2 (3,360) <0.0001
Sex	1.66 (11,635) 0.10		
Maternal age	19.2 (7,666) <0.0001		
Paternal age	8.72 (3,735) <0.0001		
Birth year	7.15 (7,688) <0.0001		
Birth order			
Second	21.9 (7,625) <0.0001		
Third	14.6 (7,625) <0.0001		
Fourth	9.54 (7,625) <0.0001		
Fifth	3.69 (7,625) 0.0002		
Sixth	1.98 (7,625) 0.047		

[a]When both the predictor and the outcome variables were specific to each pregnancy (i.e. gestational age, twin status, maternal age, paternal age, birth year, or birth order), the values for each pregnancy were used, rather than for each individual.

3.4. RELATIONSHIPS BETWEEN ASD AND COVARIATES

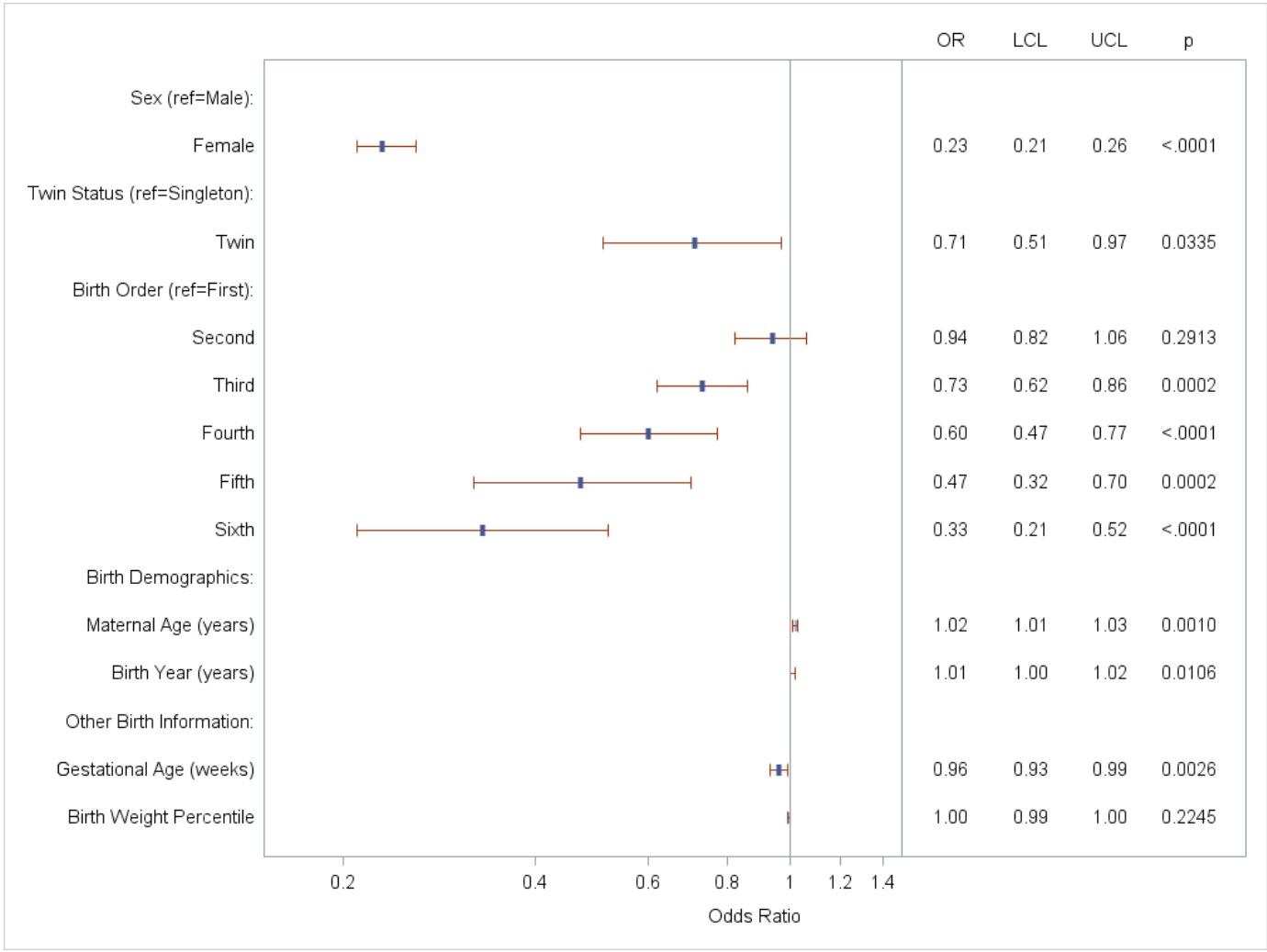
3.4.1. Pairwise Relationships

All of the covariates except birth weight and twin status were significantly associated with ASD at the 5% significance level in the pairwise association analyses (Appendix 15). As expected, the sex ratio for the ASD cases was approximately four males to one female; correspondingly, the odds of a male having ASD were approximately four times the odds for a female. There was no significant difference in the risk for ASD between the first- and second-born siblings; however, children born third or later were at a decreased risk for ASD compared to their first-born siblings. Children born to older mothers and fathers, as well as those born in more recent years, were at an increased risk for ASD. Last, a longer gestational period was associated with a decreased risk for ASD.

3.4.2. Relationships After Adjusting for the Other Covariates

When including all covariates (with maternal age) in the adjusted model, each of the covariates that were associated with ASD in the pairwise analyses (described above) remained significantly associated with the risk for ASD (Figure 3.11A). In addition, twin status became significantly associated with ASD.

A)


OR
LCL
UCL
p
Sex (ref=Male):
Female
0.23
0.21
0.26
<.0001
Twin Status (ref=Singleton):
Twin
0.71
0.51
0.97
0.0335
Birth Order (ref=First):
Second
0.94
0.82
1.06
0.2913
Third
0.73
0.62
0.86
0.0002
Fourth
0.60
0.47
0.77
<.0001
Fifth
0.47
0.32
0.70
0.0002
Sixth
0.33
0.21
0.52
<.0001
Birth Demographics:
Maternal Age (years)
1.02
1.01
1.03
0.0010
Birth Year (years)
1.01
1.00
1.02
0.0106
Other Birth Information:
Gestational Age (weeks)
0.96
0.93
0.99
0.0026
Birth Weight Percentile
1.00
0.99
1.00
0.2245
0.2
0.4
0.6
0.8
1
1.2
1.4
Odds Ratio

B)

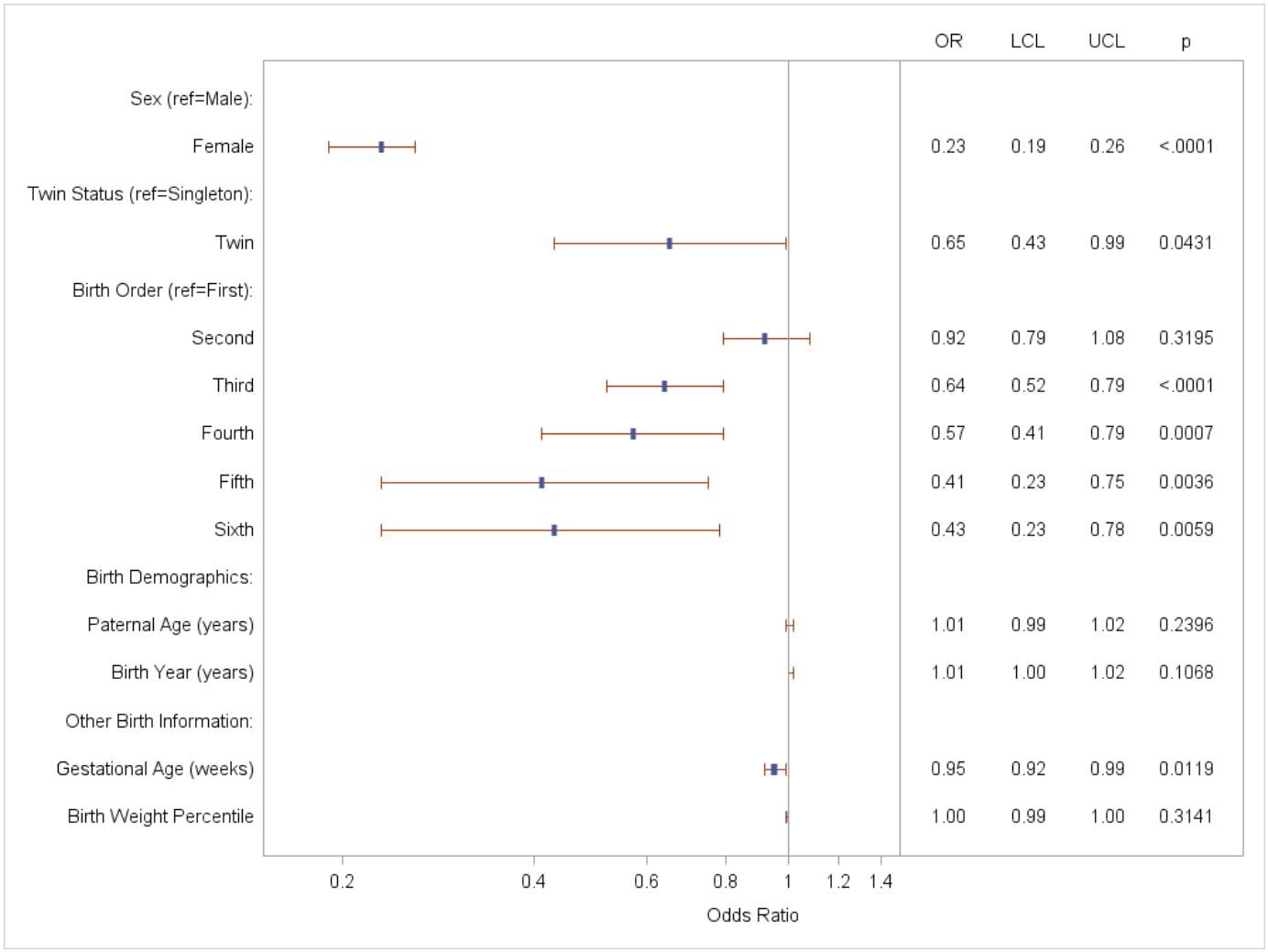


Figure 3.11. Relationships between ASD and each covariate, adjusted for all other covariates in the ASD families: A) the covariates include maternal age; B) the covariates include paternal age. An odds ratio >1 indicates that individuals are at an increased risk for ASD, while an odds ratio <1 indicates that individuals are at a decreased risk. OR=odds ratio; LCL=lower 95% confidence limit; UCL=upper 95% confidence limit; p=p-value.

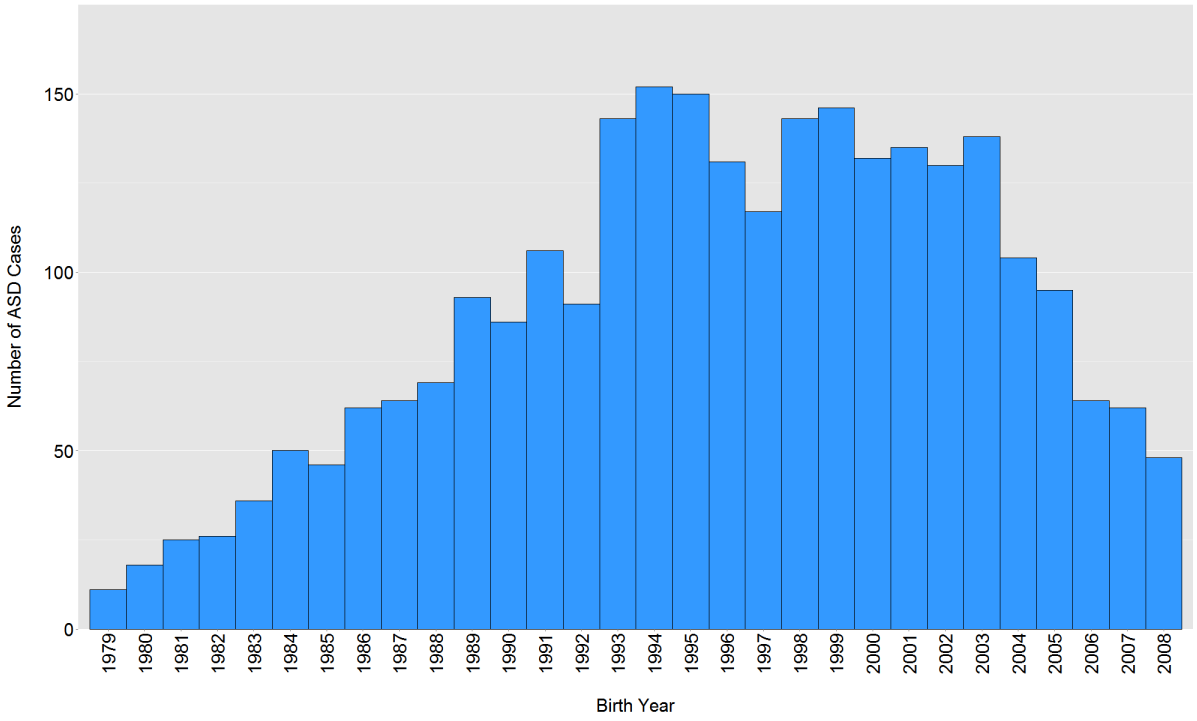
Third- and later-born children were at a decreased risk for ASD compared to their first-born siblings. The odds ratios ranged from 0.70 for the third-born children to 0.36 for the sixth- or later-born children; these estimates indicate that the odds of having ASD in children born third or later decrease by 30% to 64% compared to the first-born children. Alternatively, the odds were not significantly different between the first- and second-born children.

Every one-year increase in maternal age at the birth of an offspring was associated with a 2% increase in the odds of having ASD (p=0.001). Paternal age was not associated with the risk for ASD in the same models (Figure 3.11B; p=0.24), probably due to the small number of families for which paternal information was available. In the models with paternal age, the significance levels also decreased for the other covariates, probably due to the smaller sample sizes (e.g. from 2,385 families to 1,495 families when using individuals with any ASD claim).

Each one-year increase in birth year was associated with a 1% increase in the odds of having ASD (p=0.01). This was roughly reflected in the distribution of the ASD cases by birth year (Figure 3.12A). There was a gradual yearly increase in the number of cases born between 1979 and 1992, followed by a large jump in 1993, and similar numbers of cases up to and including 2003; between 2004 and 2008 there was a gradual decrease in the number of cases. This decrease was probably observed because the children born between 2004 and 2008 were only 5 to 9 years of age at the end of the study period and may not have received a diagnosis of ASD until a later age.

The numbers of ASD cases by the year of the first ASD claim also increased during the study period (Figure 3.12B). There was a gradual yearly increase in the number of cases with a claim between 1987 and 2002, followed by a large jump in 2003; the number of cases then remained stable up to 2012.

A)



B)

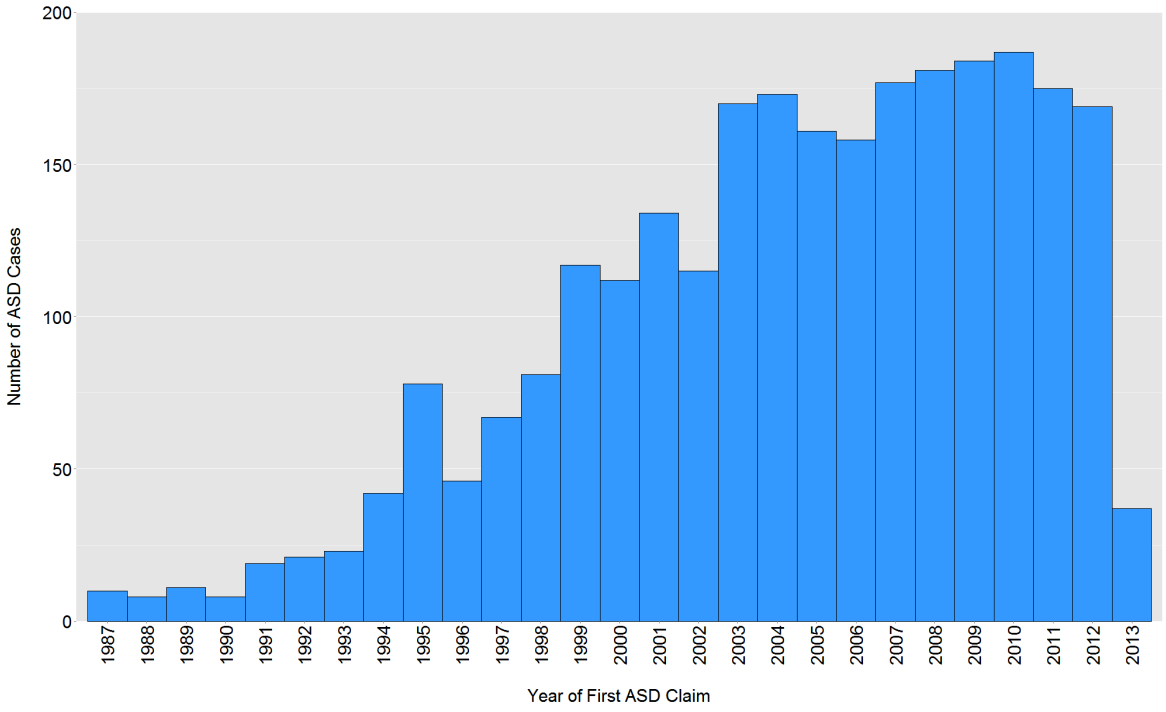


Figure 3.12. Distribution of the ASD cases in the ASD families by A) birth year and B) year of the first ASD claim from the hospital, medical, or education records

Increased gestational age was associated with a decreased risk for ASD, while birth weight percentile was not associated with ASD (p=0.24). A one-week increase in gestational age was associated with a 4% decrease in the risk for ASD (p=0.003).

Interestingly, being a twin became associated with a decreased risk for ASD (OR=0.71, 95% CI: 0.51-0.97, p=0.03). However, when the more stringent ASD case definitions were used, twin status was no longer significantly associated with ASD, although the trend of a decreased risk for ASD remained (Figure 3.13).

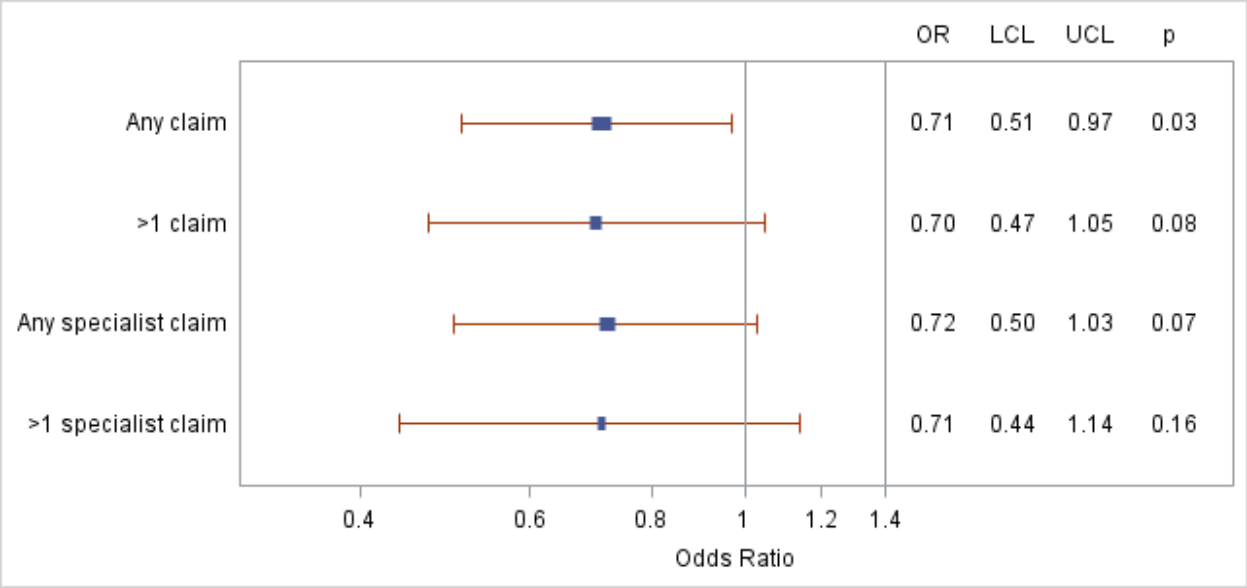


Figure 3.13. Relationships between ASD and twin status using each ASD case definition. The model was adjusted for all other covariates, including maternal age. An odds ratio >1 indicates that twins are at an increased risk for ASD, while an odds ratio <1 indicates that twins are at a decreased risk for ASD. The size of each square (in blue) reflects the sample size using that ASD case definition. OR=odds ratio; LCL=lower 95% confidence limit; UCL=upper 95% confidence limit; p=p-value.

3.4.3. Combined Effects of the Covariates

The Cragg-Uhler/Nagelkerke R-square values for each covariate are listed in Table 3.14. For all of the covariates, including maternal age, the R-square was 15.9%. The R-square value was 13.5% for sex alone, indicating that the majority of the liability in ASD was explained by sex. Birth order and maternal age each had R-squares greater than 1%, while the rest of the covariates had R-squares less than 1%. When including paternal age rather than maternal age, the total Cragg-Uhler/Nagelkerke R-square value was 16.1%.

Table 3.14. Pseudo R-square values for the covariates

Covariate	Cragg-Uhler/Nagelkerke R-square (%)
Twin status	0.02
Sex	13.5
Birth order	2.0
Maternal age	1.0
Paternal age	0.4
Birth year	0.7
Birth weight percentile	0.04
Gestational age	0.09
Total[a]	15.9
Total[b]	16.1

[a]For the total effects, the covariates are twin status, sex, birth order, maternal age, birth year, birth weight percentile, and gestational age.
[a]For the total effects, the covariates are twin status, sex, birth order, paternal age, birth year, birth weight percentile, and gestational age.

3.4.4. Relationships After Including Family Size

To investigate whether birth order was truly related to the risk for ASD, the frequencies of birth order for the ASD cases were tabulated by family size. As illustrated by Figure 3.14 and Table 3.15, 44.2% of the individuals with ASD in the ASD families were from a family with only two children and 75.7% were from a family with either two or three children. When grouped by

family size, the individuals with ASD were fairly evenly distributed across birth orders. Birth order was highly correlated with family size (Spearman rank correlation=0.48, $p<0.0001$), while birth order index and family size were not correlated (Spearman rank correlation=0.01, $p=0.32$).

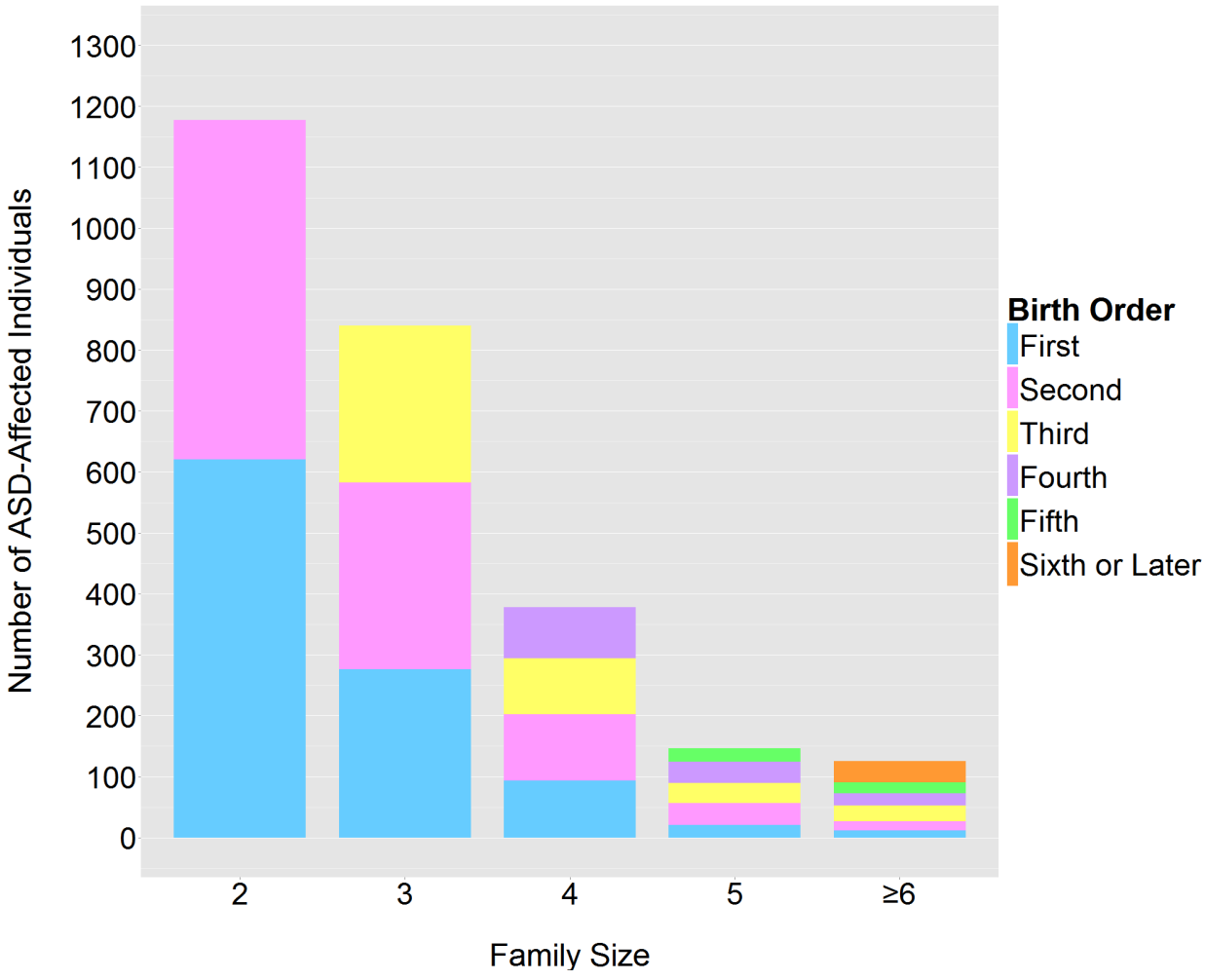


Figure 3.14. Distribution of birth order by family size among the individuals with ASD in the ASD families

Table 3.15. Frequency (%) of birth order by family size among the individuals with ASD in the ASD families

Frequency (%)		Birth order						Total
		First	Second	Third	Fourth	Fifth	Sixth or later	
Family size	2	620 (23.3)	557 (20.9)	—	—	—	—	1,177 (44.2)
	3	276 (10.4)	307 (11.5)	257 (9.6)	—	—	—	840 (31.5)
	4	94 (3.5)	108 (4.1)	92 (3.5)	84 (3.2)	—	—	378 (14.3)
	5	21 (0.8)	36 (1.4)	33 (1.2)	34 (1.3)	22 (0.8)	—	146 (5.5)
	6 or more	12 (0.5)	15 (0.6)	26 (1.0)	20 (0.8)	18 (0.7)	34 (1.3)	125 (4.9)
Total		1,023 (38.5)	1,023 (38.5)	408 (15.3)	138 (5.3)	40 (1.5)	34 (1.3)	2,666

In the pairwise analyses, larger family size was associated with a lower risk for ASD (OR=0.70; 95% CI: 0.67-0.73; p<0.0001), while birth order was not associated with ASD (p=0.53). In the adjusted model that included birth order index and family size, as well as the other covariates, birth order index was not significantly associated with the risk for ASD (p=0.35), while larger family size was associated with a decreased risk for ASD (OR=0.71; 95% CI=0.67-0.74; p<0.0001). Most of the other covariates (i.e. twin status, birth order index, maternal age, birth year, and birth weight percentile) were not significantly associated with ASD. However, females remained at a significantly lower risk for ASD than males (OR=0.23; 95% CI=0.21-0.26; p<0.0001) and shorter gestational ages remained associated with a higher risk for ASD (OR=0.96; 95% CI=0.94-0.99; p=0.005).

To further evaluate the effects of family size, the relationships between ASD and birth order were examined separately for families containing two children only, three children only, and four or more children. For the ASD families containing only two children, the second-born children were significantly less likely to have ASD than their first-born siblings, both in the pairwise analyses and after adjusting for the effects of the other covariates (adjusted analyses: OR=0.81; 95% CI=0.68-0.97; p=0.02). For the ASD families containing only three children, ASD was not significantly associated with birth order. For the ASD families containing four or more children, the second-born children were at an increased risk for ASD compared to their first-born siblings (OR=1.37; 95% CI=1.01-1.85; p=0.04) after adjusting for the effects of the other covariates, while there was no significant difference in the risk for ASD between the first-born children and their third- or later-born siblings.

The Cragg-Uhler/Nagelkerke R-square values for birth order index and family size were 0.04% and 5.2%, respectively. As such, family size accounted for a larger proportion of the liability in ASD than most of the other covariates, except for sex. For the model including sex, twin status, maternal age, birth year, birth order index, family size, gestational age, and birth weight percentile, the R-square was 18.2%. When including paternal age rather than maternal age, the R-square was 18.1%.

3.5. RELATIONSHIPS BETWEEN ASD AND PRENATAL AND PERINATAL COMPLICATIONS

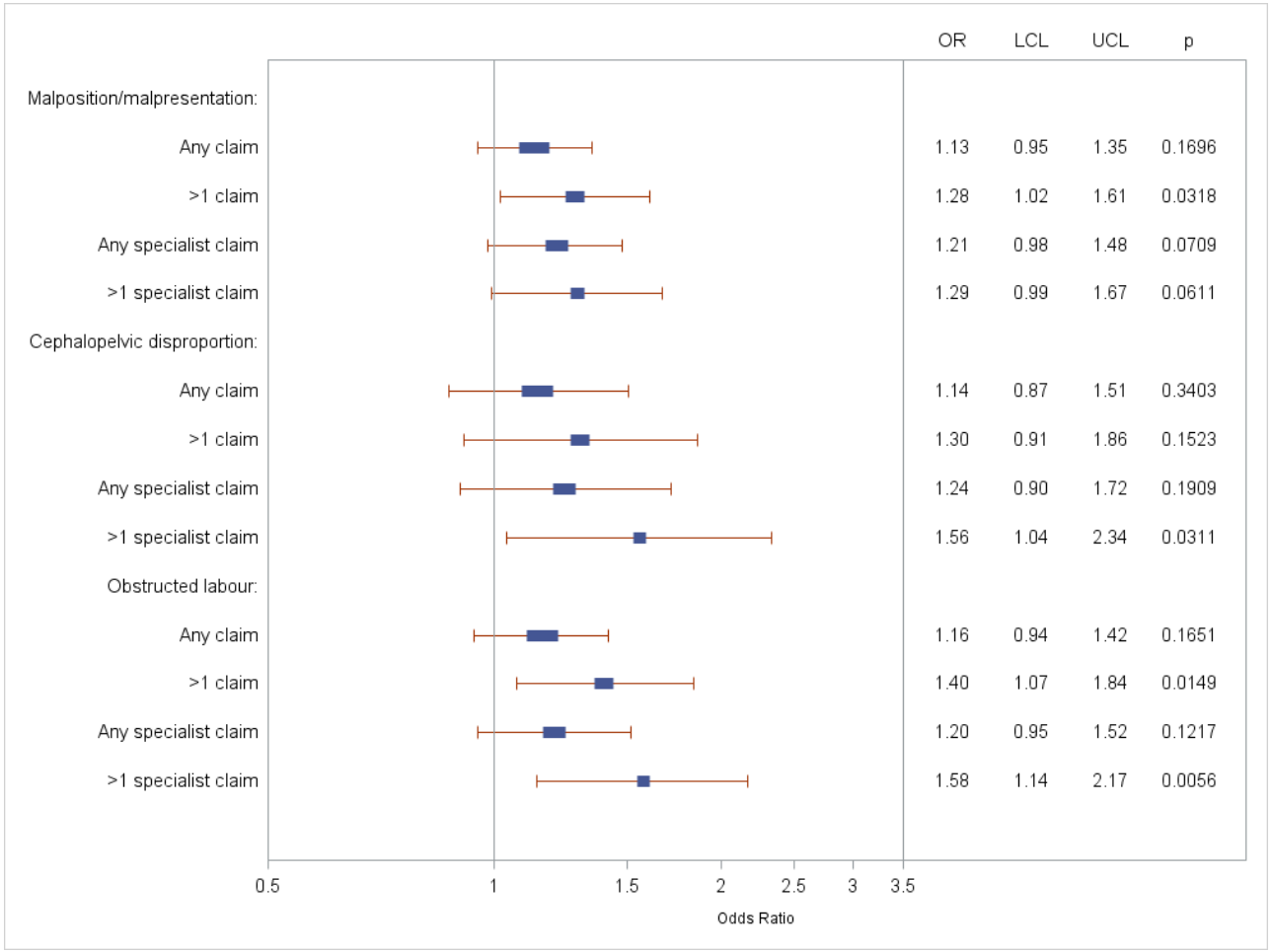
3.5.1. Pairwise Relationships

In the pairwise analyses, cephalopelvic disproportion (653), obstructed labour (660), respiratory distress syndrome (769), other respiratory conditions (770), foetal and neonatal haemorrhage (772), haematological disorders (776), and lower 1- and 5-minute Apgar scores were associated with an increased risk for ASD at the 5% significance level, regardless of the ASD case definition that was used (Appendices 16 to 19). When the ASD families were selected based on any ASD claim or any specialist claim, the individuals with ASD experienced more complications of the amniotic cavity and membranes (658) and perinatal infections (771) than their unaffected siblings. When the ASD families were selected by at least two ASD claims or at least two specialist claims, ASD was associated with endocrine and metabolic disturbances (775). When the ASD families were selected by at least two specialist claims, malposition and malpresentation of the foetus (652) was associated with an increased risk for ASD, while excessive vomiting in pregnancy (643) was related to a decreased risk.

3.5.2. Relationships After Adjusting for the Covariates

After adjusting for the effects of the covariates, the associations were less significant for most of the complications that were associated with ASD in the pairwise analyses (Appendices 16 to 19). None of the prenatal complications were consistently associated with ASD when different ASD case definitions were used. However, a few prenatal complications, including malposition and malpresentation of the foetus (652), cephalopelvic disproportion (653), complications of the amniotic cavity and membranes (658), obstructed labour (660), and umbilical cord complications (663), were associated with an increased risk for ASD with p-values less than 0.05 using one or two of the four ASD case definitions (Figure 3.15).

A)


OR
LCL
UCL
p
Malposition/malpresentation:
Any claim 1.13 0.95 1.35 0.1696
>1 claim 1.28 1.02 1.61 0.0318
Any specialist claim 1.21 0.98 1.48 0.0709
>1 specialist claim 1.29 0.99 1.67 0.0611
Cephalopelvic disproportion:
Any claim 1.14 0.87 1.51 0.3403
>1 claim 1.30 0.91 1.86 0.1523
Any specialist claim 1.24 0.90 1.72 0.1909
>1 specialist claim 1.56 1.04 2.34 0.0311
Obstructed labour:
Any claim 1.16 0.94 1.42 0.1651
>1 claim 1.40 1.07 1.84 0.0149
Any specialist claim 1.20 0.95 1.52 0.1217
>1 specialist claim 1.58 1.14 2.17 0.0056
0.5
1
1.5
2
2.5
3
3.5
Odds Ratio

B)

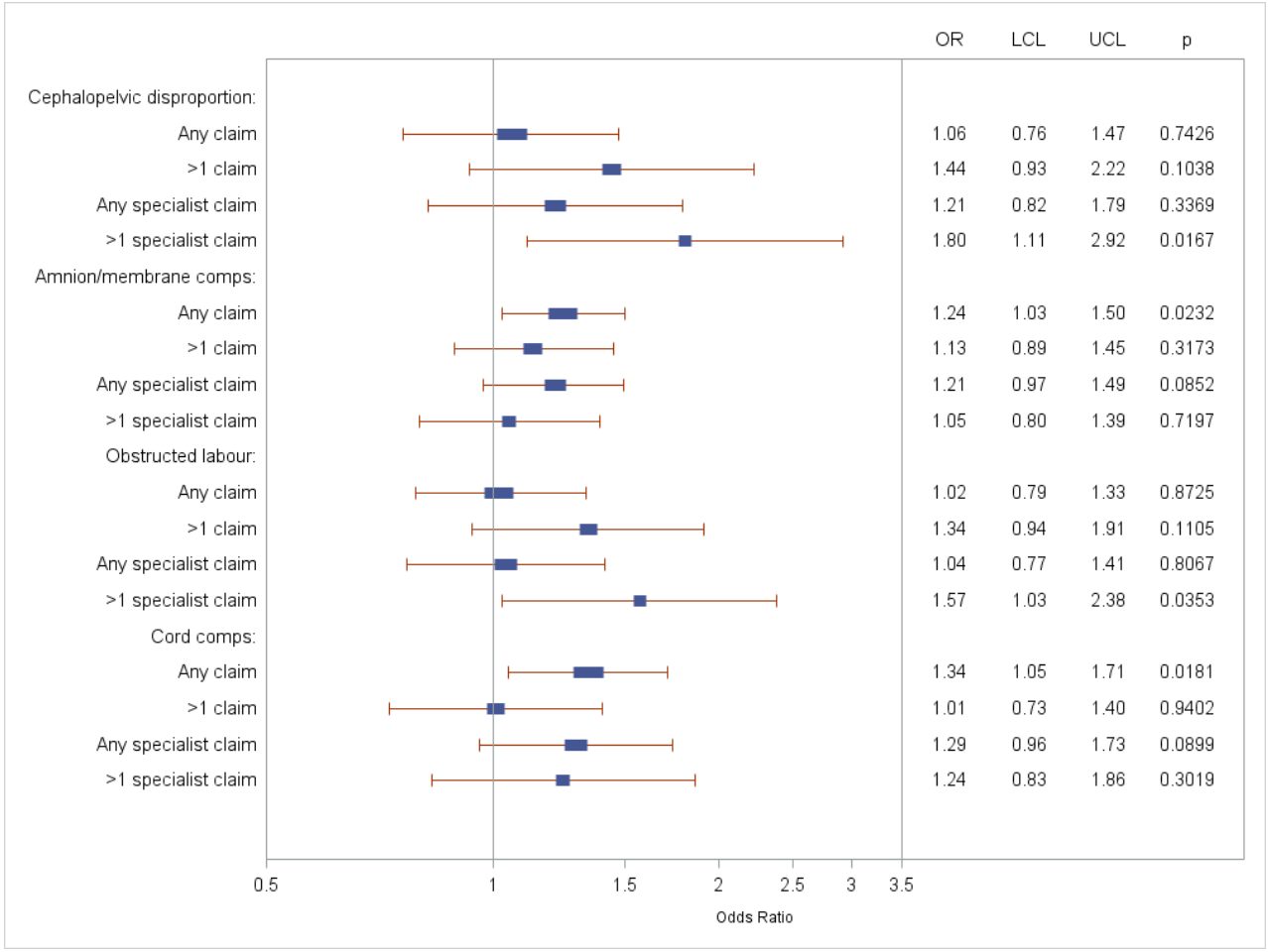
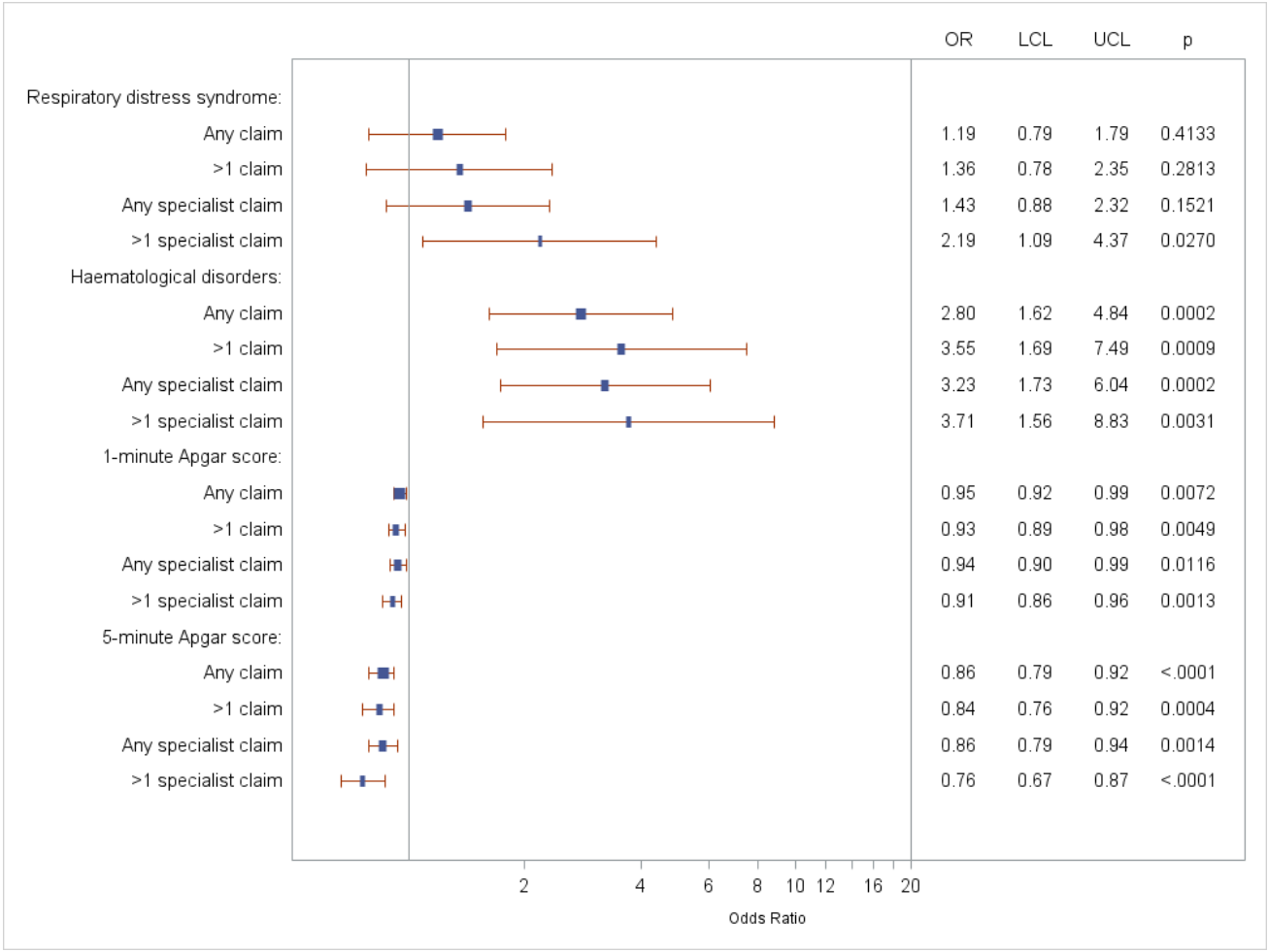


Figure 3.15. Significant associations between ASD and prenatal complications after adjusting for the covariates: A) the covariates include maternal age; B) the covariates include paternal age. The results using each ASD case definition are shown for each complication that was significant ($p<0.05$) using at least one definition. An odds ratio >1 indicates an increased risk for ASD in a child due to his/her mother's prenatal complication, while an odds ratio <1 indicates a decreased risk for ASD in a child. The size of each square (in blue) reflects the sample size using that ASD case definition. OR=odds ratio; LCL=lower 95% confidence limit; UCL=upper 95% confidence limit; p=p-value.

Of the perinatal complications, haematological disorders of the newborn (776) and 1- and 5-minute Apgar scores remained significantly associated with ASD, regardless of the ASD case definitions used and whether the model was adjusted for maternal or paternal age (Figure 3.16). In the models with maternal age, the odds ratios for haematological disorders ranged from 2.80 to 3.71 (depending on the case definition used), which indicate that the odds of having ASD for an individual with a haematological disorder are 2.8 to 3.7 times the odds of having ASD for an individual without this disorder. The odds ratio estimates for haematological disorders were larger in the models with paternal age, but the confidence intervals were also larger due to a smaller sample size.

A)


OR LCL UCL p
Respiratory distress syndrome:
Any claim 1.19 0.79 1.79 0.4133
>1 claim 1.36 0.78 2.35 0.2813
Any specialist claim 1.43 0.88 2.32 0.1521
>1 specialist claim 2.19 1.09 4.37 0.0270
Haematological disorders:
Any claim 2.80 1.62 4.84 0.0002
>1 claim 3.55 1.69 7.49 0.0009
Any specialist claim 3.23 1.73 6.04 0.0002
>1 specialist claim 3.71 1.56 8.83 0.0031
1-minute Apgar score:
Any claim 0.95 0.92 0.99 0.0072
>1 claim 0.93 0.89 0.98 0.0049
Any specialist claim 0.94 0.90 0.99 0.0116
>1 specialist claim 0.91 0.86 0.96 0.0013
5-minute Apgar score:
Any claim 0.86 0.79 0.92 <.0001
>1 claim 0.84 0.76 0.92 0.0004
Any specialist claim 0.86 0.79 0.94 0.0014
>1 specialist claim 0.76 0.67 0.87 <.0001
2 4 6 8 10 12 16 20
Odds Ratio

B)

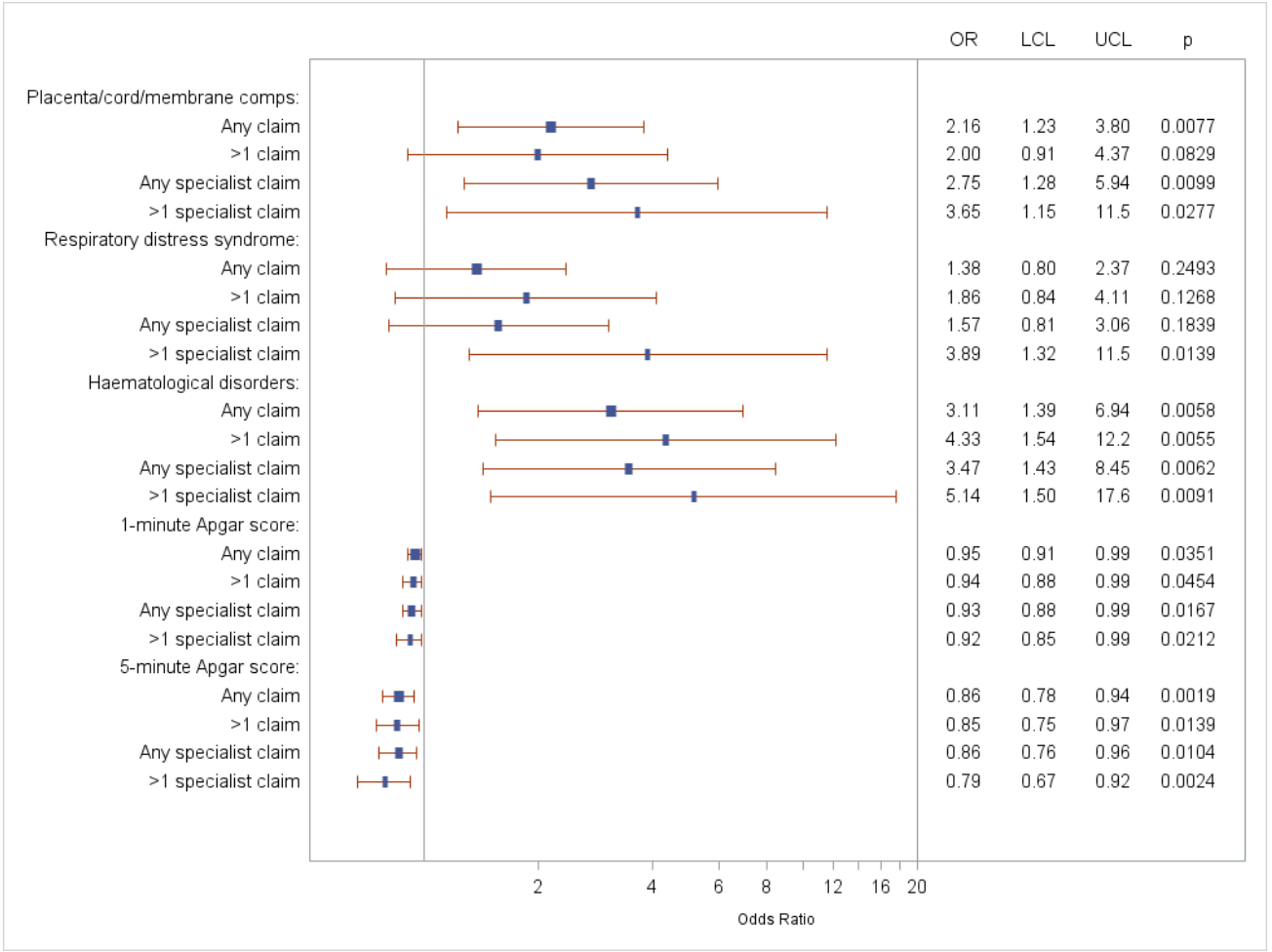


Figure 3.16. Significant associations between ASD and perinatal complications after adjusting for the covariates: A) the covariates include maternal age; B) the covariates include paternal age. The results using each ASD case definition are shown for each complication that was significant ($p<0.05$) using at least one definition. An odds ratio >1 indicates that the perinatal complication is associated with an increased risk for ASD, while an odds ratio <1 indicates that the complication is associated with a decreased risk for ASD. The size of each square (in blue) reflects the sample size using that ASD case definition. OR=odds ratio; LCL=lower 95% confidence limit; UCL=upper 95% confidence limit; p=p-value.

After adjusting for the effects of maternal age and the other covariates, the odds ratios for 5-minute Apgar scores ranged from 0.76 to 0.86, indicating that the odds of having ASD decreased by 14% to 24% for every one-unit increase in the score. The odds ratios for 1-minute scores ranged from 0.91 to 0.95, meaning that every one-unit increase in the score was associated with a 5% to 9% decrease in the odds of having ASD. Upon including both haematological disorders of the newborn and the 5-minute Apgar score in the same model, both variables remained significantly associated with ASD (haematological disorders: OR=2.41, 95% CI=1.39-4.20, p=0.002; 5-minute Apgar scores: OR=0.87, 95% CI=0.81-0.94, p=0.0006). This indicates that haematological disorders and low 5-minute Apgar scores may contribute to the risk for ASD independently of one another. The Cragg-Uhler/Nagelkerke R-squares for haematological disorders, 1-minute Apgar scores, and 5-minute Apgar scores were quite small (i.e. less than 0.5%), suggesting that they may account for a small proportion of the liability in ASD (Table 3.16).

Table 3.16. Pseudo R-square values for the significant perinatal complications

Perinatal complication	Cragg-Uhler/Nagelkerke R-square
Haematological disorders	0.3%
1-minute Apgar score	0.3%
5-minute Apgar score	0.5%

In addition to these complications, respiratory distress syndrome (769) remained associated with an increased risk for ASD when using the individuals with ASD with at least two specialist claims (p=0.03 when using maternal age; p=0.01 when using paternal age). Using three of the four ASD case definitions, complications of the placenta, cord, and membranes (762) were also associated with ASD, though only when adjusting for paternal age.

3.5.3. Relationships After Including Birth Order Index and Family Size as Covariates

After adjusting for the effects of the covariates, including birth order index and family size, the associations between ASD and the prenatal and perinatal complications were similar to those observed when including birth order as a covariate instead (Table 3.17). Similarly, haematological disorders of the newborn (776) and 1- and 5-minute Apgar scores were the only complications that remained significantly associated with ASD, regardless of the ASD case definitions used.

Table 3.17. Significant associations between ASD and the prenatal and perinatal complications by the covariates included

Prenatal and perinatal complications	ASD case definition	Model A		Model B	
ICD-9-CM code: Medical condition[169]		Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Prenatal complications					
652: Malposition and malpresentation of foetus	≥2 claims	1.28 (1.02-1.61)	0.03	1.25 (1.00-1.57)	0.055
653: Cephalopelvic disproportion	≥2 specialist claims	1.56 (1.04-2.34)	0.03	1.42 (0.95-2.13)	0.09
660: Obstructed labour	≥2 claims	1.40 (1.07-1.84)	0.01	1.33 (1.01-1.75)	0.04
	≥2 specialist claims	1.58 (1.14-2.17)	0.006	1.47 (1.07-2.03)	0.02
Perinatal complications					
769: Respiratory distress syndrome	≥2 specialist claims	2.19 (1.09-4.37)	0.03	2.04 (1.02-4.08)	0.04
776: Haematological disorders	Any claim	2.80 (1.62-4.84)	0.0002	2.67 (1.54-4.65)	0.0005
	≥2 claims	3.55 (1.69-7.49)	0.0009	3.37 (1.60-7.09)	0.001
	Any specialist claim	3.23 (1.73-6.04)	0.0002	3.04 (1.62-5.69)	0.0005
	≥2 specialist claims	3.71 (1.56-8.83)	0.003	3.65 (1.53-8.70)	0.004
1-minute Apgar score	Any claim	0.95 (0.92-0.99)	0.007	0.95 (0.91-0.99)	0.007
	≥2 claims	0.93 (0.89-0.98)	0.005	0.93 (0.88-0.98)	0.004
	Any specialist claim	0.94 (0.90-0.99)	0.01	0.95 (0.91-0.99)	0.02
	≥2 specialist claims	0.91 (0.86-0.96)	0.001	0.90 (0.84-0.95)	0.0003
5-minute Agpar score	Any claim	0.86 (0.79-0.92)	<0.0001	0.86 (0.79-0.93)	<0.0001
	≥2 claims	0.84 (0.76-0.92)	0.0004	0.84 (0.76-0.93)	0.0008
	Any specialist claim	0.86 (0.79-0.94)	0.001	0.86 (0.79-0.95)	0.002
	≥2 specialist claims	0.76 (0.67-0.87)	<0.0001	0.76 (0.67-0.86)	<0.0001

Model A was adjusted for sex, maternal age, birth year, birth order, twin status, gestational age, and birth weight percentile
Model B was adjusted for sex, maternal age, birth year, birth order index, family size, twin status, gestational age, and birth weight percentile

3.5.4. Relationships After Removing Individuals with Congenital Conditions

A significant number of individuals (919 or 34.4% of the individuals with ASD and 1,061 or 24.8% of their unaffected siblings) from the ASD families had at least one claim for a congenital condition. Interestingly, a large proportion of individuals (45%) with haematological disorders of the newborn had at least one claim for other congenital anomalies of the circulatory system (ICD-9 code 747; e.g. congenital anomalies of the aorta, pulmonary artery, great veins, and peripheral vascular system). In fact, individuals with a haematological disorder were 11.6 times more likely to have a congenital condition than individuals without a haematological disorder (OR=11.6, 95% CI: 6.7-20.3, $p<0.0001$). Furthermore, a one-unit increase in the 5-minute Apgar score was associated with a 19% decrease in the risk of having a congenital anomaly (OR=0.81, 95% CI: 0.75-0.88, $p<0.0001$).

After removing the individuals with a claim for any congenital anomaly that was significantly different between the individuals with ASD and their unaffected siblings (Figure 3.6), the sample sizes were significantly reduced for each of the four ASD case definitions (e.g. from 6,943 to 4,221 individuals for the least stringent ASD definition [a 39% reduction]) (Table 3.18). Haematological disorders were no longer associated with ASD in either the pairwise analyses or the analyses with the covariates. After adjusting for the effects of the covariates, 5-minute Apgar scores were only significantly associated with ASD when using individuals with at least one specialist claim for ASD (OR=0.83, 95% CI: 0.71-0.97, $p=0.02$). The results were similar when including birth order index and family size as covariates (results not shown).

Table 3.18. Relationships between ASD and haematological disorders of the newborn and 5-minute Apgar scores by ASD case definition after removing individuals with congenital anomalies that were significantly different between the individuals with ASD and their unaffected siblings

OR (95% CI) p-value	Any ASD claim (n=4,221)	≥2 ASD claims (n=2,502)	Any specialist claim (n=3,055)	≥2 specialist claims (n=1,695)
	Pairwise			
Haematological disorders	1.11 (0.45-2.73) 0.82	1.98 (0.60-6.52) 0.26	0.98 (0.35-2.70) 0.96	2.71 (0.64-11.4) 0.17
5-minute Apgar scores	0.90 (0.82-0.98) **0.02**	0.89 (0.80-1.00) **0.049**	0.90 (0.81-1.00) 0.054	0.82 (0.71-0.94) **0.005**
	Adjusted[a]			
Haematological disorders	1.49 (0.54-4.10) 0.44	2.59 (0.70-9.59) 0.15	1.32 (0.42-4.11) 0.63	3.09 (0.65-14.6) 0.16
5-minute Apgar scores	0.91 (0.82-1.01) 0.06	0.90 (0.80-1.02) 0.11	0.92 (0.81-1.03) 0.15	0.83 (0.71-0.97) **0.02**

[a]Models were adjusted for sex, maternal age, birth year, birth order, twin status, gestational age, and birth weight percentile

3.5.5. ASD and Haematological Disorders of the Newborn

In this study, 71 of 82 individuals (86.6%) with claims for haematological disorders of the newborn had at least one claim in the hospital records. Haematological disorders of the newborn include a broad group of perinatal complications, such as haemorrhagic disease, transient neonatal thrombocytopenia, disseminated intravascular coagulation, other transient neonatal disorders of coagulation, polycythemia neonatorum, congenital anaemia, anaemia of prematurity, transient neonatal neutropenia, and other specified and unspecified haematological disorders. Among the haematological disorder diagnoses from the hospital records, 42 (58.3%) were anaemia of prematurity (776.6) (Table 3.19). Although transient neonatal thrombocytopenia (776.1), disseminated intravascular coagulation (776.2), polycythemia neonatorum (776.4), congenital anaemia (776.5), and other specified transient haematological disorders (776.8) were

also observed, the frequencies of these perinatal haematological disorders were much lower than that for neonatal anaemia.

Table 3.19. Frequency (%) of the individuals from the ASD families with a hospital claim for the subtypes of haematological disorders of the newborn (n=71)

ICD-9-CM code: Medical condition[169]	Frequency (%)
776.1: Transient neonatal thrombocytopenia	15 (21.1)
776.4: Polycythemia neonatorum	9 (12.7)
776.6: Anaemia of prematurity	42 (59.2)
Other[a]	6 (8.5)

[a]Includes 776.2: disseminated intravascular coagulation in newborn, 776.5: congenital anaemia, and 776.8: other specified transient haematological disorders

The subtypes of haematological disorders with a frequency of at least 10 (i.e. transient neonatal thrombocytopenia and anaemia of prematurity) were tested for associations with ASD using all of the ASD families. After adjusting for the covariates, both transient neonatal thrombocytopenia (OR: 3.74, 95% CI: 1.20-11.7, p=0.02) and anaemia of prematurity (OR: 2.50, 95% CI: 1.20-5.22, p=0.01) were associated with an increased risk for ASD (Table 3.20). The results were similar when birth order index and family size were included instead of birth order (results not shown).

Table 3.20. Relationships between ASD and the subtypes of haematological disorders of the newborn

ICD-9-CM code: Medical condition[169]	Odds ratio (95% CI)	p-value[a]
776.1: Transient neonatal thrombocytopenia	3.74 (1.20-11.7)	0.02
776.6: Anaemia of prematurity	2.50 (1.20-5.22)	0.01

[a]Models were adjusted for sex, twin status, maternal age, birth year, birth order, gestational age, and birth weight percentile

4. DISCUSSION

In the present study, after adjusting for the effects of the covariates, we found that the individuals with ASD experienced more haematological disorders of the newborn and had lower 1- and 5-minute Apgar scores than their unaffected siblings, which is in agreement with our hypothesis. Although twins were more likely to have haematological disorders and had lower Apgar scores than singletons, the prevalence of ASD was not significantly different between twins and their singleton siblings. The findings from this study underscore the importance of 1) accounting for the effects of multiple known confounders, 2) ASD case definitions, and 3) accounting for disorders that are comorbid with ASD, when examining the relationships between ASD and prenatal and perinatal complications using administrative data.

4.1. ASD AND PERINATAL COMPLICATIONS

4.1.1. Haematological Disorders

Haematological disorders of the newborn were consistently associated with ASD, irrespective of the ASD case definition that was used and whether maternal or paternal age was included as one of the covariates. To our knowledge, this is the first study to assess the relationship between ASD and the broad category of haematological disorders of the newborn, although several previous studies have investigated the effects of neonatal anaemia on the risk for ASD. Using administrative data for 924 ASD cases and 128,809 controls in Nova Scotia, Canada, Dodds *et al.* did not observe a significant association between neonatal anaemia and ASD, though the relationship was close to reaching statistical significance (RR: 1.50, 95% CI: 0.98-2.32)[104]. After combining the results from four previous studies, Gardener *et al.* observed that neonatal anaemia was associated with an increased risk for ASD. The summary effect estimate (calculated by

combining and weighting the relative risks from the previous studies) was 7.87, with a 95% confidence interval of 1.43 to 43.36[91]; this was one of the strongest effects observed in this meta-analysis study. In the present study, the confidence intervals for haematological disorders of the newborn (OR: 2.80; 95% CI: 1.62-4.84) and anaemia of prematurity (OR: 2.50; 95% CI: 1.50-5.22) were narrower than that in the meta-analysis mentioned above; this is likely due to the larger sample size that was used in the present study. To our knowledge, aside from neonatal anaemia, the other haematological disorders (e.g. transient neonatal thrombocytopenia) have not been widely investigated in the ASD population.

After birth, most infants experience a decrease in the amount of plasma erythropoietin (usually lasting until six to eight weeks after birth)[178], which suppresses erythropoiesis and results in a reduction in haemoglobin[179]. However, this phenomenon is more severe in preterm infants (due to lower erythropoietin production, a lower hematocrit, decreased survival of red blood cells, rapid growth, and iatrogenic blood loss, among several other factors) and leads to anaemia of prematurity[178,179]. As a result of increased survival rates among preterm and low birth weight infants, the incidence of anaemia of prematurity has increased[178]. In addition to anaemia, two related conditions, red blood cell transfusion and iron deficiency, have also been associated with ASD and neurodevelopment.

Red blood cell transfusion, which is the traditional treatment for anaemia of prematurity, has been hypothesized to affect neurodevelopment. The potential mechanisms include circulatory overload, excessive iron, impaired oxygen transport and delivery, abnormal immune and inflammatory responses, and infection (although the risk is low)[180,181]. Studies have investigated

whether there are differences in neurodevelopmental outcome between the use of liberal and restrictive guidelines for transfusions, but the results are inconsistent. By limiting the number of transfusions, the restrictive guidelines may reduce the number of donors and other risks to which an infant is exposed; on the other hand, allowing lower haemoglobin levels may pose a risk to infants[182]. Bell *et al.* documented a higher frequency of apnea and serious adverse brain events among infants that were assigned to the restrictive transfusion guidelines compared to those assigned to the liberal guidelines[183]. At 18 to 21 months of age, Whyte *et al.* observed diminished cognitive function in the restrictive group compared to the liberal group from a different clinical trial[184]. Alternatively, two later reports about the cohort used by Bell *et al.* (when the subjects were 8 to 15 years old) described more brain abnormalities and lower scores for intelligence, academic achievement, and neuropsychological assessments in the liberal group compared to the restrictive group[182,185]. To better elucidate the associations between neurodevelopment and red blood cell transfusions, further studies are needed.

Because a significant amount of iron is contained in haemoglobin, which is decreased after birth and in anaemia of prematurity[179], reduced iron levels in the neonate may be related to ASD. Iron deficiency may affect activities in the brain, including energy metabolism, metabolism of neurotransmitters, and myelin formation[186]. Studies have illustrated an association between iron deficiency and ASD, developmental delay, intellectual disability, and other psychiatric conditions in older individuals[186-188]. However, prospective studies from birth are needed to examine the causal relationships between iron deficiency and ASD. Furthermore, due to the correlations among anaemia, blood transfusion, and iron deficiency, more comprehensive studies

are needed to investigate the biological mechanism for the observed relationship between haematological disorders and ASD.

4.1.2. Apgar Scores

In this study, both 1- and 5-minute Apgar scores were associated with ASD using each of the four case definitions, though 5-minute scores exhibited a stronger association with ASD than 1-minute scores. In many previous studies, low 1- and/or 5-minute Apgar scores were associated with ASD. Of two meta-analyses, one observed a significant association between ASD and low 5-minute scores[91], while the other observed a significant association with both 1- and 5-minute scores[189]. Most previous studies categorized the Apgar scores as normal (7 to 10) or low (0 to 6) and examined the associations between ASD and low scores. When the Apgar scores were similarly categorized in the present study, both 1- and 5-minute scores less than 7 were associated with an increased risk for ASD (1-minute score: OR=1.30, 95% CI: 1.10-1.53, p=0.002; 5-minute score: OR=2.20, 95% CI: 1.37-3.53, p=0.001).

More broadly, previous studies have demonstrated that low Apgar scores are associated with a higher risk of neonatal mortality and various neurological conditions (e.g. cerebral palsy and cognitive impairment)[190]. Additionally, Mobaddemia *et al.* observed significant associations between several proxies of impaired gas exchange (i.e. hypoxia and hypercarbia), including low Apgar scores, and intellectual disability and ASD[189]. Impaired gas exchange can damage brain tissue and cells during the neonatal period, potentially leading to adverse neurological outcomes. However, they suggested that the observed association might be between impaired gas exchange

and intellectual disability (which tends to be comorbid with ASD), rather than between impaired gas exchange and ASD.

It is not surprising that a decrease in 5-minute Apgar scores had a stronger association with ASD than a decrease in 1-minute scores. The 1-minute Apgar score provides information about the condition of the newborn immediately after birth. Typically, if the Apgar score is low at 1 minutes after birth, resuscitative efforts will occur and the 5-minute Apgar score will reflect the infant's response to resuscitation. As such, when a child has a low 1-minute score, the 5-minute score will often be higher; this is evidenced by more newborns with scores in the "normal" range (7 to 10) at 5 minutes compared to 1 minute both in a previous cohort[191] and in the sample used in this study (Table 4.1). Additionally, the 5-minute score has been shown to predict neonatal morbidity and mortality better than the 1-minute score[191,192].

Table 4.1. Frequency (%) of 1- and 5-minute Apgar scores (by category) in the ASD families

Apgar score category	1-minute Apgar scores	5-minute Apgar scores
Low (1-3)	164 (2.5)	18 (0.3)
Moderately abnormal (4-6)	671 (10.0)	74 (1.1)
Normal (7-10)	5,865 (87.5)	6,619 (98.6)

Despite the associations between Apgar scores and neurological impairments that have been reported in the literature and were observed in the present study, it is not recommended to use Apgar scores to predict long-term health for several reasons[171]. The Apgar score was designed to assess the newborn's condition at birth, rather than to evaluate the effects of delivery on long-

term neurodevelopmental outcomes[193]. The Apgar score has also been shown to have a low inter-rater reliability, in addition to low sensitivity to measure the newborn's condition at birth and low specificity between scores. As such, it is recommended that the Apgar score be used in conjunction with other assessments (e.g. evaluating pH from an umbilical artery blood sample) to determine whether neurological damage occurred during delivery.

4.1.3. Removing Individuals with Congenital Conditions

In the present study, the prevalences of congenital anomalies (excluding chromosomal anomalies) were 34.4% and 24.8% in the individuals with ASD and their unaffected siblings, respectively, which were significantly higher than the rates that were reported in the literature (i.e. 5-10% for individuals with ASD[97,151-155] and 8% for unaffected siblings[153]). Of the 18 congenital anomalies, 14 occurred significantly more frequently in the individuals with ASD compared to their unaffected siblings, as has been consistently reported in the previous studies. The congenital anomalies that occurred the most frequently in the individuals with ASD were other anomalies of the heart, genital organs, musculoskeletal system, and limbs, with individual prevalences ranging from 5.3% to 8.6%. In previous studies, some of these anomalies were also observed to occur the most frequently among ASD cases, though the prevalence estimates were slightly lower (approximately 1.5% to 4.0%)[97,153].

There are several potential explanations for the discrepancies between the published rates of congenital anomalies and those observed in the present study. First, the claims for congenital anomalies in the provincial data repository have not been validated and may not be accurate. Second, many previous studies examined the presence of congenital anomalies that were

documented at an early age (e.g. during the first year of birth), while the present study captured anomalies that were documented at any age. Last, the definitions that were used to select the congenital anomalies may have differed between the present study and previous studies. For example, Timonen-Soivio *et al.* examined major and minor anomalies separately[155], while Brownell *et al.* only examined major congenital anomalies in a Manitoba study[13].

Individuals with a chromosomal anomaly (indicated by the ICD-9 code 758) were removed from the ASD families because these anomalies may be responsible for the ASD phenotype and the goal of this study was to identify non-genetic risk factors for ASD. However, there was not sufficient evidence to support removing individuals with other congenital conditions for the majority of the analyses. To our knowledge, no previous studies have reported the relationships between ASD and prenatal and perinatal complications after excluding individuals with congenital conditions. In fact, most of these studies[94,104,125,137,194,195] did not exclude individuals based on any other medical conditions, though some excluded individuals with known neurogenetic syndromes (e.g. tuberous sclerosis, Fragile X Syndrome, and Down Syndrome)[93,111] or conditions that were classified as a pervasive developmental disorder under the DSM-IV criteria but were not included in ASD in the DSM-5 (i.e. Rett's Syndrome and Childhood Disintegrative Disorder)[112,196]. Nonetheless, the relationships between ASD and the significant perinatal complications (i.e. haematological disorders of the newborn and 5-minute Apgar scores) were re-examined after removing individuals with any congenital anomaly that occurred significantly more frequently in the individuals with ASD compared to their unaffected siblings (Figure 3.6). In the adjusted analyses, haematological disorders were no longer associated with

ASD, while 5-minute Apgar scores were only associated with ASD when using individuals with more than one specialist claim (Table 3.12).

There are several possible explanations for these results. First, the prevalences of haematological disorders and low Apgar scores (i.e. less than 7) were relatively low in the ASD families. After removing approximately one-third of the families due to the presence of other congenital conditions, the sample sizes for these complications were even smaller. As such, there may not have been sufficient power to detect significant associations between ASD and these complications. Second, some congenital conditions may be confounders for the relationships between ASD and perinatal complications. For example, 45% of the individuals with haematological disorders of the newborn also had a claim for other congenital anomalies of the circulatory system (indicated by the ICD-9 code 747). If congenital anomalies of the circulatory system tend to be comorbid with ASD and haematological disorders of the newborn are associated with these anomalies, then the associations between ASD and haematological disorders may be due to confounding by congenital anomalies of the circulatory system. Most of the congenital conditions occurred significantly more frequently among the individuals with ASD than among their unaffected siblings (Figure 3.6); the majority of these conditions were also significantly more common among ASD cases compared to control individuals in at least one previous study[97,152,153,197] and may be comorbid with ASD. Although some studies have examined the association between ASD and congenital anomalies, among other prenatal and perinatal variables, none of these studies investigated the potential confounding effects of congenital anomalies on the relationships among prenatal and perinatal complications and ASD., which warrant further investigation.

4.2. ASD AND THE COVARIATES

In the pairwise analyses without the covariates, many prenatal and perinatal complications were associated with ASD. However, few of these associations remained significant after adjusting for the effects of the covariates (most of which were significantly associated with ASD, as expected), illustrating that false associations may be observed between ASD and prenatal and perinatal complications if the effects of known confounders are not incorporated in the statistical model.

4.2.1. Twin Status

Twins experienced more of the prenatal and perinatal complications than their singleton siblings (Figure 3.10 in the twin families and Appendices 12 to 14 in the ASD families), as expected. These complications included haematological disorders and lower Apgar scores, which were also significantly associated with an increased risk for ASD. As such, it was expected that twins would be at an increased risk for ASD, due to having a higher frequency of these complications. Interestingly, after adjusting for the effects of the covariates, being a twin was significantly associated with a decreased risk for ASD in the families with any ASD claim. However, the odds ratio and p-value were marginal (OR=0.71; 95% CI=0.51-0.97; p=0.03). Additionally, when using the more stringent ASD case definitions, the association between twin status and ASD did not remain significant, even though the trend remained. To our knowledge, no other studies have reported a significantly lower risk for ASD in twins compared to singletons. However, the lack of association that was observed when using the more stringent case definitions is consistent with several large population-based studies, which observed that twins or other multiples were not at an increased risk for ASD[104,124,125].

There are several possible explanations for these observations. First, although information was collected for all of the twins born in Manitoba during the study period, the selected ASD families only contained 236 twins; of these, only 213 twins (of which 76 were ASD cases) were included in the final models with the covariates. Therefore, the sample size was quite small to compare the prevalence of ASD between the twins and their singleton siblings. Furthermore, among the 213 twins included in the final models, only 13 twins (6.1%) had a claim for a haematological disorder and 6 twins (2.8%) had a 5-minute Apgar score less than 7. Second, the effect of prenatal and perinatal complications on the risk for ASD may be negligible. Although moderate odds ratios were observed for the relationships between haematological disorders and ASD, the risk for ASD in the general population is relatively low. The pseudo R-square estimates also suggested that haematological disorders and 1- and 5-minute Apgar scores account for only a small proportion of the liability in ASD (Cragg-Uhler/Nagelkerke R-square estimates: 0.3%, 0.3%, and 0.5%, respectively). Last, the p-values were not corrected for multiple testing; the significant association between ASD and twin status in the families with any ASD claim ($p=0.03$) would not withstand correction for multiple testing if such a correction were applied.

If twin status were truly associated with a decreased risk for ASD, this would indicate that twinning is protective against ASD. It has been reported that few natural twin pregnancies reach term and result in the birth of twins[198]. As such, it has been hypothesized that twin pairs who survive to birth may have better fitness than singletons, although this remains largely unknown. We are planning to test this hypothesis for ASD, as described in the Future Directions (section 4.5.2) below.

4.2.2. Birth Order and Family Size

In the present study, first-born children were at a higher risk for ASD compared to their third- or later-born siblings. These results are in agreement with those from two previous studies[90,137]. However, upon accounting for the confounding effect of family size, birth order (in the form of birth order index) was not associated with ASD. When the relationships between ASD and birth order were examined separately for different family sizes, second-born children were at a lower risk for ASD than their first-born siblings in families with only two children, while second-born children were at a higher risk than their first-born siblings in families with four or more children. Taken together, the results from the present study illustrate the complexity of the relationship between ASD and birth order; furthermore, this study demonstrates the potential to observe false associations between birth order and the outcome of interest if family size is not taken into account. Most previous studies did not account for family size when examining the relationships between ASD and birth order, which may explain some of the heterogeneity among previous results for the relationship between ASD and birth order, as described in the Introduction (section 1.4.1.2). Recently, a few groups have attempted to examine the relationships among family size, birth order, and ASD, as described in the Introduction, though they yielded discrepant results[139,140]. It is unclear whether these research groups considered collinearity between birth order and family size, which may have resulted in false associations. The relationships between family size and ASD are discrepant between these and the present study, however this may be related to methodological differences between the studies (e.g. including families with only one child or accounting for the collinearity among family size and birth order).

4.2.3. Combined Effects of the Covariates

To our knowledge, this is the first study to report pseudo R-square values for prenatal and perinatal complications associated with ASD. The Cragg-Uhler/Nagelkerke R-square values were 15.9% for the adjusted model that included birth order and 18.2% for the model that included birth order index and family size. This indicates that other factors explain approximately 80% to 85% of the liability in ASD, which is expected because ASD is a complex disorder that is attributed to a host of genetic, epigenetic, and environmental risk factors.

Among the covariates included in the adjusted models, sex explained the largest proportion of the liability in ASD (13.5%), while most of the other covariates each accounted for <1% of the liability. This result is not surprising because the 4:1 male to female ratio is a well-documented characteristic of ASD. Family size accounted for the second largest proportion of the liability in ASD (5.2%), which further illustrates the importance of this covariate.

The pseudo R-square values were relatively small for the rest of the variables, indicating that they explained a limited proportion of the liability in ASD. For example, the R-square values were 0.3% and 0.5% for haematological disorders of the newborn and 5-minute Apgar scores, respectively. However, because few individuals had a haematological disorder (1.2%) or an Apgar score less than 7 (1.4%), it was not expected that these complications would explain a large proportion of the liability for ASD. However, their effect sizes were relatively large for these complications, especially compared to the effect sizes for common genetic variants, which typically have odds ratios less than 1.2[51]. The odds ratios for haematological disorders of the newborn ranged from 2.80 to 3.71, while the odds ratios for 5-minute Apgar scores ranged from

0.76 to 0.86 (inverse ratios=1.16 to 1.32). Similarly, the odds ratios were also relatively large for sex (odds ratio=0.23; inverse ratio=4.35) and family size (odds ratio=0.71; inverse ratio=1.41). Despite the small R-square values that were observed for many of the covariates and significant predictors, the moderate effect sizes indicate that they are likely important non-genetic risk factors for ASD.

4.2.4. Genetic Versus Non-Genetic Effects

Some of the "non-genetic" risk factors that were identified for ASD (e.g. gestational age) are complex traits, which are affected by both genetic and environmental factors. Additionally, the genetic risk factors for some of these complex traits may overlap with one another, leading to positive associations between these variables. As such, the potential impact of genetic factors on the relationships between these "non-genetic" risk factors and ASD should be examined in future studies.

4.3. ASD CASE SELECTION

4.3.1. Algorithms to Identify ASD Cases

Haematological disorders of the newborn and low 1- and 5-minute Apgar scores were the only complications that were consistently associated with ASD, regardless of the case definition that was used. In addition to haematological disorders and Apgar scores, some other complications were also associated with ASD, though only when using more stringent case definitions. This may indicate that such complications were truly associated with ASD, but had small effect sizes and could only be observed in a sample with more stringent ASD diagnoses. On the other hand, some complications were only associated with ASD when using the least stringent case

definition (i.e. any ASD claim). Because these complications were not associated with ASD when using the more stringent case definitions, these may be false associations that were observed due to false positives among the ASD cases. In the absence of validation for the ASD claims, other characteristics of the ASD families (e.g. concordance rates among twins and sibling recurrence rates) should be compared to published values in order to discern which ASD case definition most accurately captures the ASD population in Manitoba.

4.3.2. Presence of Other Mental and Behavioural Disorders

The majority of the individuals with ASD (87.9%) had a claim for at least one other mental or behavioural disorder. Based on previous reports, it was expected that most individuals with ASD would have at least one comorbid psychiatric disorder, though the prevalence was higher than expected (i.e. 88% versus approximately 70%)[156,157]; this may be because some disorders that were included in our analysis (e.g. developmental delay) were not included in the previous studies. The disorders that occurred the most frequently among the individuals with ASD were neurotic disorders (which includes generalized anxiety disorder, panic disorder, phobic disorders, and obsessive compulsive disorder, among others), conduct disorder or oppositional defiant disorder, hyperkinetic syndrome of childhood or attention deficit hyperactivity disorder, and developmental delay. All of these disorders (with the exception of developmental delay) were also observed to occur the most frequently in previous studies at rates comparable to those observed in the present study (i.e. approximately 30% to 45%)[156,157].

The temporal order of the claims for ASD and other mental and behavioural disorders was examined among the ASD cases and compared between the four case definitions that were used.

Of the individuals with ASD, 12.1% did not have any claims for other mental or behavioural disorders (Figure 3.8). As such, we may be more confident that these children truly have ASD compared to the children with claims for other disorders. However, the mean age of these children was significantly younger than the mean age among those with claims for other disorders, suggesting that these individuals may not have been old enough to receive another diagnosis before the end of the study period. Alternatively, this observation may indicate that the group of individuals with claims for multiple mental and behavioural disorders have more clinical complexity than those who only had claims for ASD.

When comparing the temporal order of claims for ASD and other mental and behavioural disorders across ASD case definitions, a smaller proportion of individuals had a claim for another disorder after the most recent ASD claim in the families with more than one ASD claim or more than one specialist claim than in the families selected based on any ASD claim or any specialist claim (Table 3.6). This may suggest that the more stringent case definitions contain more individuals who truly have ASD and fewer cases of diagnostic substitution than the less stringent case definitions. However, there are also other explanations for these observations (e.g. comorbidity between ASD and the other mental and behavioural disorders). As such, chart reviews and/or clinical evaluations of the study subjects are required to accurately interpret the temporal order of administrative claims for ASD and other mental and behavioural disorders and use this information to discern which case definition most accurately captures ASD cases.

4.4. LIMITATIONS

4.4.1. Paternal Information

In this study, siblings were identified using mother's information but not father's information because prenatal complications were some of the main predictors in this study. As such, some siblings may have been half-siblings. For example, in 2.2% of the ASD families, children born to the same mother had different males associated with their health care registration; because it was not possible to determine the biological father for these children, paternal information was set to missing for these families.

Although paternal age is highly correlated with maternal age, it is beneficial to examine the effects of both parental ages on the risk for ASD because the underlying biological mechanisms conferring risk may differ between the parents. As described above, less paternal information was available compared to maternal information in the present study. Consequently, the statistical power was lower to examine the relationship between ASD and paternal age compared to that for the relationship between ASD and maternal age.

4.4.2. Other Variables

Many other variables that were not examined in this study may be associated with ASD and/or prenatal and perinatal complications and are potential confounders. Some examples are maternal immunological conditions, socioeconomic status, and parental education. Because this study compared prenatal and perinatal complications between siblings, the subjects were matched for family information and parental characteristics. Nonetheless, these variables likely differed between ASD families and may have conferred a higher risk for ASD in some of the families.

4.4.3. Differences Between Hospital and Medical Claims

The claims from the hospital discharge abstracts were more specific than the medical services claims. The medical services claims only included the first three digits of the ICD code (e.g. the ICD-9 code 299 for pervasive developmental disorders), while the claims from the hospital discharge abstracts included the fourth- and fifth-digit subclassifications, when available (see Table 2.2 for the ICD-9-CM codes for ASD). Due to these differences, it was not possible to examine the relationships between an ASD subtype (e.g. autistic disorder) and a potential risk factor because few ASD claims were from the hospital records (Figure 3.1); thus, it was not possible to draw conclusions about the detailed relationships between them as in some previous studies[125,196,199]. However, because 86.6% of the claims for haematological disorders of the newborn were from the hospital records, the relationships between ASD and two subtypes of haematological disorders were examined.

4.4.4. Validation of ASD Diagnoses

The ASD claims in the provincial data repository have not been validated. However, we attempted to reduce the number of truly unaffected individuals who were identified as ASD cases by using four different ASD definitions. In a related study, our group identified 50 twin pairs with at least one twin diagnosed with ASD using the ICD-9 code 299 from the provincial data repository; of these, six twin pairs were recruited. From the six twin pairs (12 individuals), eight twins indicated that they had received an ASD diagnosis; seven of the eight twins had a claim for ASD in the provincial data repository. Through a medical chart review at the Health Sciences Centre in Winnipeg, Manitoba, the ASD diagnoses were confirmed for five of the eight twins. For the remaining three twins, information about their ASD diagnoses was from other institutes

in Manitoba. Currently, we are working to obtain their medical charts for confirmation of their ASD diagnosis. These results will provide us with a general idea of the accuracy of the ASD claims in the data repository, though the sample only represents a small subset of the individuals with ASD in Manitoba. Currently, another Canadian group is examining the validity of the ASD claims from the same data repository[200], but the results are not yet available.

4.4.5. Multiple Testing

In this study, statistical tests were performed for 29 prenatal and perinatal complications using four ASD case definitions and two sets of covariates. The results were not corrected for multiple testing because each test was not strictly independent from all of the other tests. For example, some of the prenatal and perinatal factors, such as intrauterine hypoxia and birth asphyxia, respiratory distress syndrome, and other respiratory conditions, represented similar conditions and were highly correlated with one another (Appendices 9 to 11). Furthermore, the samples that were selected using the more stringent ASD case definitions (i.e. case definition numbers 2 to 4) were subsets of the sample that was selected using the least stringent criteria (i.e. case definition number 1). Nonetheless, the p-values for the relationships between ASD and haematological disorders of the newborn and 1- and 5-minute Apgar scores were very small (0.003 to <0.0001) and would remain significant after the most stringent correction for multiple testing; therefore, these are likely to be true associations at the 5% significance level.

5. FUTURE DIRECTIONS

5.1. Validation of the Major Findings Using Control Families

In this study, we identified significant relationships between ASD and some perinatal conditions and covariates by comparing ASD cases to their unaffected siblings. Although the sibling comparison design has some advantages over the case-control study design, previous studies have reported that it may be biased under certain conditions (e.g. non-shared confounders are less similar than predictors among siblings, or random measurement error exists for the predictors)[201]. To validate the findings from the present study, control families that do not have any individuals with ASD were selected by matching to the ASD families using a propensity score model. Different from the present study, the ASD cases from families that did not have an unaffected sibling will also be included. Both the individuals with ASD and their unaffected siblings will be compared to the control subjects.

5.2. Is Being a Twin Protective Against ASD?

In this study, the relationships between ASD and twin status yielded interesting results that should be further investigated. Based on these results, we hypothesize that being a twin is protective against ASD, which means that twins with ASD may carry more genetic liability for ASD than singletons with ASD. To test this hypothesis, the prevalence of ASD among the singleton siblings of twins with ASD will be compared to the prevalence of ASD in the general population. If this hypothesis is correct, the prevalence of ASD is expected to be higher among the singleton siblings of twins with ASD than in the general population.

5.3. Gene Mapping for ASD Using an Epigenetic Twin Study Design

Our group is currently conducting an epigenetic twin study (Figure 5.1). Using the provincial data repository, 50 same-sex twin pairs with at least one twin diagnosed with ASD were identified. These twin pairs were contacted through a blind mail-out and were invited to return a questionnaire if they were interested in participating in the study. Of the 50 twin pairs, eight returned the questionnaire, corresponding to a response rate of 16%. Of the eight respondent twin pairs, six met the inclusion and exclusion criteria.

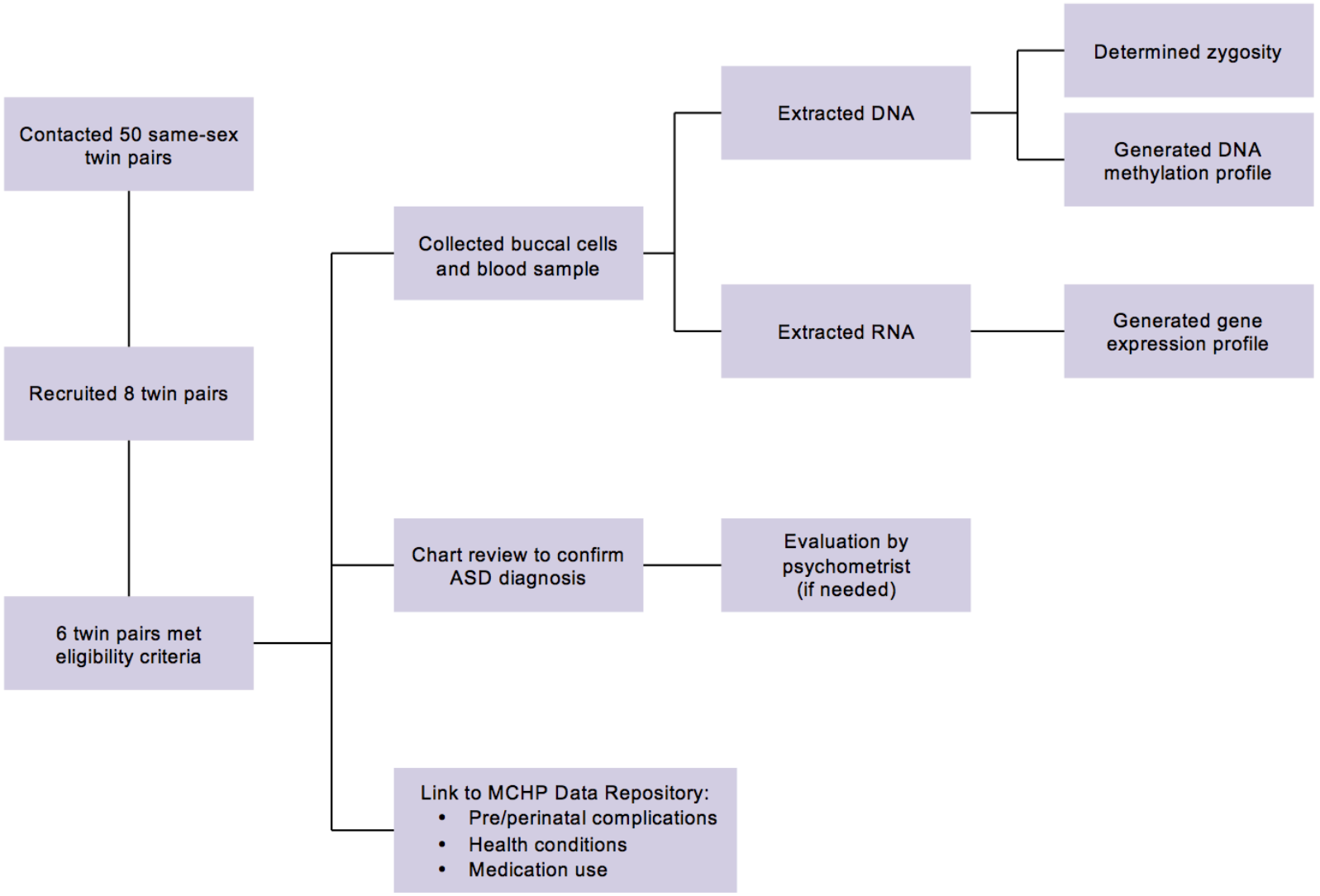


Figure 5.1. Flowchart of epigenetic twin study design

Our research nurses collected buccal epithelial cell samples and a blood sample from each twin. DNA was extracted from the buccal cells; DNA and RNA were also extracted from the peripheral blood mononuclear cells (PBMCs), which were isolated from the whole blood sample. The twin type of each twin pair was determined using 16 microsatellite markers. Using the HumanMethylation450 BeadChip, separate DNA methylation profiles were generated for the buccal cells and the PBMCs from each twin. A gene expression profile was also generated for each of the twins using RNA-sequencing. The global and site-specific DNA methylation and gene expression levels will be compared between the twins with and without ASD. The correlations between the DNA methylation and gene expression levels will also be examined.

As described in the Limitations (section 4.4.4), we have confirmed the ASD diagnosis for five of the eight twins with ASD and will continue our chart review for the remaining twins. If the information from the medical records is not sufficient, a trained psychometrist will evaluate each affected twin to confirm his/her ASD diagnosis. Last, Manitoba Health linked each twin to the provincial data repository using his/her PHIN. This linkage will be used to determine if any of the twins had prenatal or perinatal complications, long-term health problems, or medication use that may have affected his/her epigenetic profile. Ideally, this study will identify genetic loci where DNA methylation and gene expression levels are associated with ASD status.

6. SIGNIFICANCE

6.1. Identifying "High-Risk" Individuals

Elucidating both genetic and non-genetic risk factors for ASD may allow us to identify individuals who are at a higher risk for ASD than the general population. This strategy would be ideal if the identified risk factors constituted a large proportion of the liability for ASD. However, our study demonstrates that this is not the case except for sex. Nonetheless, the combined effects of the identified risk factors can be used to identify "high-risk" individuals in the future, especially in combination with genetic risk factors.

Following "high-risk" individuals may facilitate early assessment and diagnosis, leading to the implementation of intervention strategies at an early age. In recent years, extensive research has focused on early intervention strategies for ASD, such as intensive behavioural intervention (EIBI), a lengthy and intensive intervention program. Four out of five published meta-analyses concluded that EIBI was an effective intervention strategy for ASD[202]. More recently, a study also reported that ASD cases receiving EIBI had faster IQ and adaptive behaviour learning rate improvement compared to control ASD cases[203]. This intervention strategy, in addition to others, demonstrates the positive effects that early treatment can have on the developmental trajectories of individuals with ASD.

The results from this study show an increase in the number of ASD diagnoses by birth year in Manitoba. More work is required to ensure that resources are accessible to all families. Identifying individuals who are at a higher risk for ASD represents one approach by which to ensure that the needs of these individuals and their families are met.

6.2. Subgrouping Cohorts for Genetic Studies of ASD

To date, over 700 genes have been associated with ASD[204]. In order to accurately map genetic variants that contribute to the liability for ASD, it is imperative that individuals with ASD are grouped according to known causal factors. Based on the results from this study, researchers conducting genetic studies could identify the group of individuals with ASD who had a combination of risk factors, including a haematological disorder of the newborn or a low Apgar score at birth. At this stage, it is not clear whether genes play an important role in the aetiology of ASD in this group of individuals. However, this approach may allow researchers to investigate more homogeneous groups of individuals with ASD in genetic studies and as such, will help to disentangle genetic heterogeneity in ASD.

7. SUMMARY

In this study, haematological disorders of the newborn and low Apgar scores were consistently associated with ASD and had moderate odds ratios. However, after removing individuals with congenital conditions, haematological disorders were no longer associated with ASD, indicating that congenital anomalies may be another important confounder for the relationships between prenatal and perinatal complications and ASD. Taken together, the results from the present study indicate the importance of confounders, comorbid conditions with ASD, and different ASD case definitions in the relationships between prenatal and perinatal complications and ASD.

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APPENDICES

Appendix 1. ICD-9-CM codes for congenital anomalies

ICD-9-CM code[169]	Congenital anomaly
740	Anencephalus and similar anomalies
741	Spina bifida
742	Other congenital anomalies of nervous system
743	Congenital anomalies of eye
744	Congenital anomalies of ear, face, and neck
745	Bulbus cordis anomalies and anomalies of cardiac septal closure
746	Other congenital anomalies of heart
747	Other congenital anomalies of circulatory system
748	Congenital anomalies of respiratory system
749	Cleft palate and cleft lip
750	Other congenital anomalies of upper alimentary tract
751	Other congenital anomalies of digestive system
752	Congenital anomalies of genital organs
753	Congenital anomalies of urinary system
754	Certain congenital musculoskeletal deformities
755	Other congenital anomalies of limbs
756	Other congenital musculoskeletal anomalies
757	Congenital anomalies of the integument
758	Chromosomal anomalies
759	Other and unspecified congenital anomalies

Appendix 2. ICD-9-CM codes for mental and behavioural disorders

ICD-9-CM code[169]	Mental or behavioural disorder
290	Dementias
291	Alcohol-induced mental disorders
292	Drug-induced mental disorders
293	Transient mental disorders due to conditions classified elsewhere
294	Persistent mental disorders due to conditions classified elsewhere
295	Schizophrenic disorders
296	Episodic mood disorders
297	Delusional disorders
298	Other nonorganic psychoses
299	Pervasive developmental disorders
300	Anxiety, dissociative and somatoform disorders
301	Personality disorders
302	Sexual and gender identity disorders
303	Alcohol dependence syndrome
304	Drug dependence
305	Nondependent abuse of drugs
306	Physiological malfunction arising from mental factors
307	Special symptoms or syndromes, not elsewhere classified
308	Acute reaction to stress
309	Adjustment reaction
310	Specific nonpsychotic mental disorders due to brain damage
311	Depressive disorder, not elsewhere classified
312	Disturbance of conduct, not elsewhere classified
313	Disturbance of emotions specific to childhood and adolescence
314	Hyperkinetic syndrome of childhood
315	Specific delays in development
316	Psychic factors associated with diseases classified elsewhere
317	Mild intellectual disabilities
318	Other specified intellectual disabilities
319	Unspecified intellectual disabilities

Appendix 3. Prenatal and perinatal complications and the exclusions

ICD-9-CM code: Medical condition[169]	Exclusions[a]	Reason for exclusion	Frequency (%) in ASD families[b]	Frequency (%) in twin families[c]
Prenatal complications				
640: Haemorrhage in early pregnancy	—	—	377 (5.5)	732 (6.3)
641: Antepartum haemorrhage, abruption placentae, placenta previa	—	—	334 (4.9)	652 (5.6)
642: Hypertension complicating pregnancy, childbirth, puerperium	642.x2 642.x4	Postpartum complications	592 (8.7)	1,131 (9.7)
643: Excessive vomiting in pregnancy	—	—	169 (2.5)	316 (2.7)
644: Early or threatened labour	—	—	1,434 (21.0)	3,592 (30.9)
645: Late pregnancy	All	Gestational age was selected as a covariate	—	—
646: Other complications of pregnancy, not elsewhere classified	All	Encompasses a variety of conditions	—	—
647: Infectious and parasitic conditions in mother classifiable elsewhere, but complicating pregnancy, childbirth, puerperium	647.x2 647.x4	Postpartum complications	177 (2.6)	222 (1.9)
648: Other current conditions in mother classified elsewhere, but complicating pregnancy, childbirth, puerperium	All	Encompasses a variety of conditions	—	—
649: Other conditions or status of the mother complicating pregnancy, childbirth, puerperium	All	Encompasses a variety of conditions	—	—
650: Normal delivery	All	Not a complication	—	—
651: Multiple gestation	All	Bias for twins	—	—
652: Malposition and malpresentation of foetus	—	—	724 (10.6)	2,898 (24.9)
653: Cephalopelvic disproportion	—	—	259 (3.8)	274 (2.4)
654: Abnormality of organs and soft tissues of pelvis	654.2 654.x2 654.x4	Postpartum complications	615 (9.0)	1,081 (9.3)
655: Known or suspected foetal abnormality affecting management of mother	—	—	664 (9.7)	1,192 (10.2)

656: Other known or suspected foetal and placental problems affecting management of mother	All	Encompasses a variety of conditions	—	—
657: Polyhydramnions	—	—	93 (1.4)	241 (2.1)
658: Other problems associated with amniotic cavity and membranes	—	—	1,162 (17.0)	2,216 (19.1)
659: Other indications for care or intervention related to labour and delivery, not elsewhere classified	All	Encompasses a variety of conditions	—	—
660: Obstructed labour	660.5	Locked twins (bias for twins)	499 (7.3)	1,045 (9.0)
661: Abnormality of forces of labour	—	—	816 (12.0)	1,342 (11.5)
662: Long labour	662.3	Delayed delivery of second twin (bias for twins)	777 (11.4)	1,380 (11.9)
663: Umbilical cord complications	—	—	600 (8.8)	1,015 (8.7)
664: Trauma to perineum and vulva during delivery	All	Complications affect mother only	—	—
665: Other obstetrical trauma	All	Complications affect mother only	—	—
666: Postpartum haemorrhage	All	Postpartum complications	—	—
667: Retained placenta without haemorrhage	All	Postpartum complications	—	—
668: Complications of administration of anaesthetic or other sedation in labour and delivery	All	Complications affect mother only	—	—
669: Other complications of labour and delivery, not elsewhere classified	All	Encompasses a variety of conditions	—	—
670: Major puerperal infection	All	Postpartum complications	—	—
671: Venous complications in pregnancy and puerperium	All	Prevalence <1%	23 (3.4)	33 (0.3)
672: Pyrexia of unknown origin during puerperium	All	Postpartum complications	—	—
673: Obstetrical pulmonary embolism	All	Prevalence <1%	*	*

674: Other and unspecified complications of puerperium, not elsewhere classified	All	Postpartum complications	—	—
675: Infections of the breast and nipple associated with childbirth	All	Complications affect mother only	—	—
676: Other disorders of the breast associated with childbirth and disorders of lactation	All	Complications affect mother only	—	—
677: Late effect of complication of pregnancy, childbirth, puerperium	All	Postpartum complications	—	—
678: Other foetal conditions	All	Encompasses a variety of conditions	—	—
679: Complications of in utero procedures	All	Encompasses both maternal and foetal complications	—	—
Perinatal complications				
760: Foetus/newborn affected by maternal conditions which may be unrelated to present pregnancy	All	Prevalence <1%	42 (0.6)	121 (0.8)
761: Foetus/newborn affected by maternal complications of pregnancy	All	Prevalence <1%	13 (0.2)	163 (1.0)
762: Foetus/newborn affected by complications of placenta, cord, membranes	762.3	Placental transfusion syndrome (bias for twins)	134 (1.9)	229 (1.5)
763: Foetus or newborn affected by other complications of labour and delivery	All	Encompasses a variety of conditions	—	—
764: Slow foetal growth and foetal malnutrition	All	Birth weight and gestational age were selected as covariates	—	—
765: Disorders relating to short gestation and low birth weight	All	Birth weight and gestational age were selected as covariates	—	—
766: Disorders relating to long gestation and high birth weight	All	Birth weight and gestational age were selected as covariates	—	—

767: Birth trauma	—	—	128 (1.8)	174 (1.1)
768: Intrauterine hypoxia and birth asphyxia	—	—	352 (5.1)	840 (5.4)
769: Respiratory distress syndrome	—	—	165 (2.4)	881 (5.7)
770: Other respiratory conditions of foetus and newborn	—	—	729 (10.5)	2,579 (16.6)
771: Infections specific to perinatal period	—	—	308 (4.4)	697 (4.5)
772: Foetal and neonatal haemorrhage	—	—	148 (2.1)	449 (2.9)
773: Hemolytic disease of fetus/newborn, due to isoimmunization	All	Prevalence <1%	25 (0.4)	85 (0.5)
774: Other perinatal jaundice	—	—	1,001 (14.4)	2,802 (18.0)
775: Endocrine and metabolic disturbances specific to foetus and newborn	—	—	311 (4.5)	897 (5.8)
776: Haematological disorders of newborn	—	—	82 (1.2)	349 (2.2)
777: Perinatal disorders of digestive system	All	Prevalence <1%	38 (0.5)	149 (1.0)
778: Conditions involving integument and temperature regulation of foetus and newborn	—	—	142 (2.0)	234 (1.5)
779: Other and ill-defined conditions originating in the perinatal period	All	Encompasses a variety of conditions	—	—

*Frequency was less than six pregnancies and could not be presented due to privacy restrictions

[a]x indicates that any complication with a fifth-digit subclassification of 2 or 4 was excluded, regardless of the fourth-digit subclassification. For example, the ICD-9-CM code 642.64 indicates postpartum eclampsia; exclusions 642.x2 and 642.x4 indicate that all postpartum hypertension complications were excluded.

[b]The sample sizes were 6,826 pregnancies for the prenatal complications and 6,943 individuals for the perinatal complications.

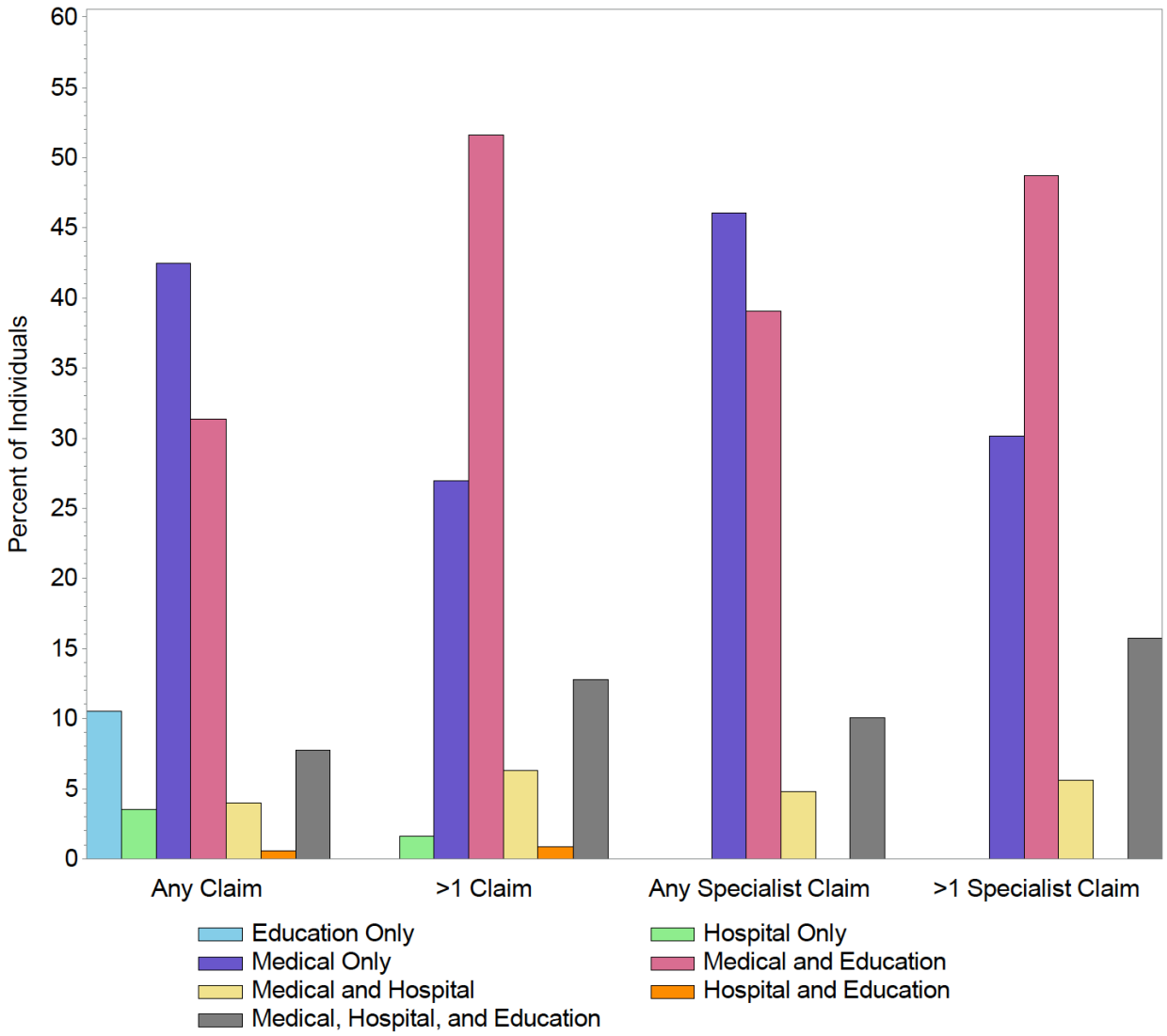
[c]The sample sizes were 11,632 pregnancies for the prenatal complications and 15,579 individuals for the perinatal complications.

Appendix 4. ICD-9-CM codes for multiple gestation or delivery

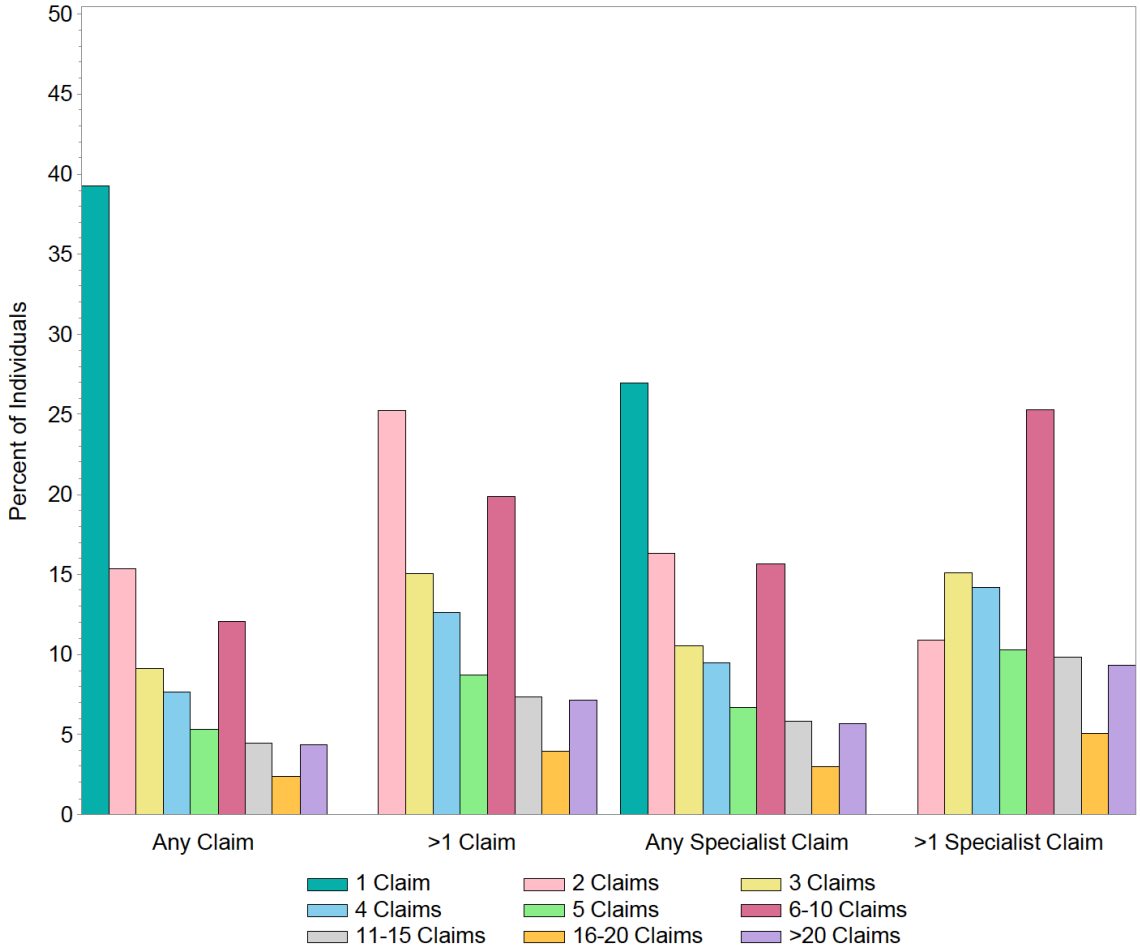
ICD-9-CM code[169]	Code description
651	Multiple gestation
651.00, 651.01, 651.03	Twin pregnancy
651.10, 651.11, 651.13	Triplet pregnancy
651.20, 651.21, 651.23	Quadruplet pregnancy
651.30, 651.31, 651.33	Twin pregnancy with foetal loss and retention of one foetus
651.40, 651.41, 651.43	Triplet pregnancy with foetal loss and retention of one or more foetus(es)
651.50, 651.51, 651.53	Quadruplet pregnancy with foetal loss and retention of one or more foetus(es)
651.60, 651.61, 651.63	Other multiple pregnancy with foetal loss and retention of one of more foetus(es)
651.70, 651.71, 651.73	Multiple gestation following (elective) foetal reduction
651.80, 651.81, 651.83	Other specified multiple gestation
651.90, 651.91, 651.93	Unspecified multiple gestation
V27	Outcome of delivery
V27.2	Twins, both live born
V27.3	Twins, one live born and one stillborn
V27.4	Twins, both stillborn
V27.5	Other multiple birth, all live born
V27.6	Other multiple birth, some live born
V27.7	Other multiple birth, all stillborn
V31	Twin, mate live born
V32	Twin, mate stillborn
V33	Twin, unspecified
V34	Other multiple, all mates live born
V35	Other multiple, all mates stillborn
V36	Other multiple, mates live- and stillborn
V37	Other multiple, unspecified

Appendix 5. Number of children from the ASD families by parental age groups

Frequency (%)		Paternal age (years)			**Total**
		<30	30-34	≥35	
Maternal age (years)	<30	665 (37.1)	296 (16.5)	108 (6.0)	**1,069 (59.6)**
	30-34	42 (2.3)	262 (14.6)	190 (10.6)	**494 (27.5)**
	≥35	7 (0.4)	35 (2.0)	189 (10.5)	**231 (12.9)**
Total		**714 (39.8)**	**593 (33.1)**	**487 (27.1)**	**1,794**

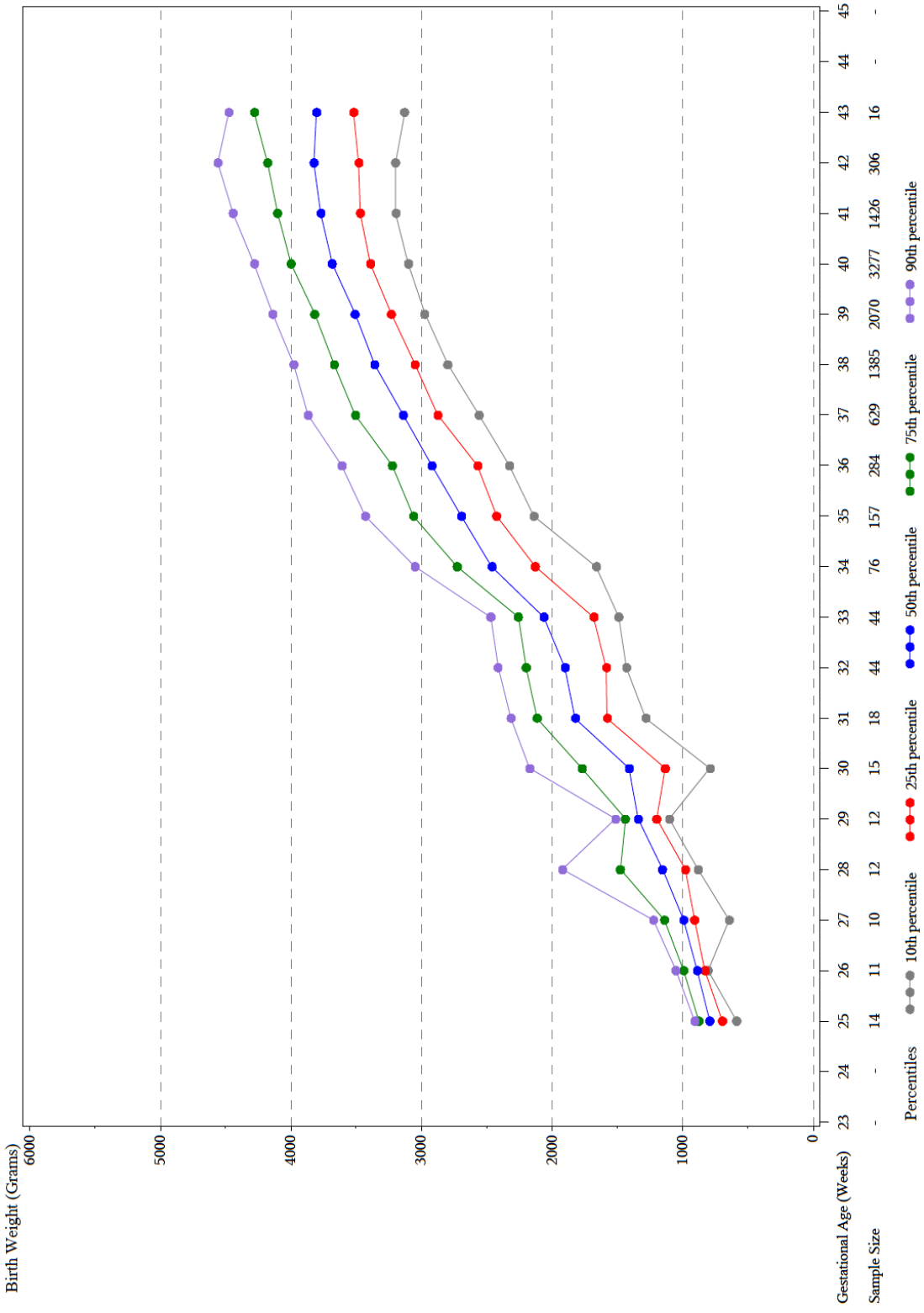


Appendix 6. Proportion of the individuals with ASD with claims from each data source (i.e. medical service claims, hospital discharge abstracts, or special needs funding applications) or combination of sources using four different case definitions. Specialist claims were from the medical service database only.

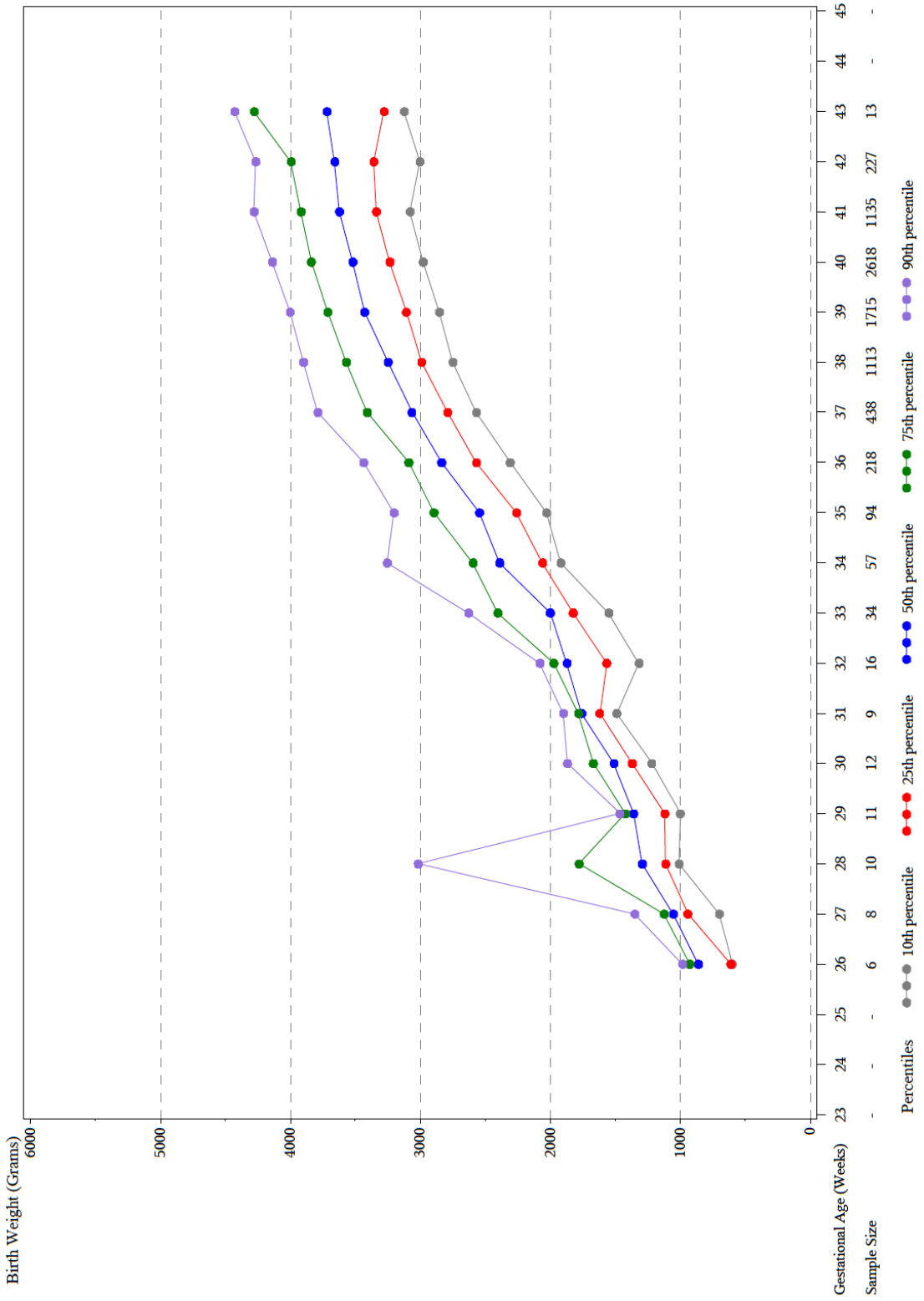


Appendix 7. Proportion of the individuals with ASD by the number of ASD claims from the hospital, medical, and education records using four different case definitions. Specialist claims were from the medical service database only.

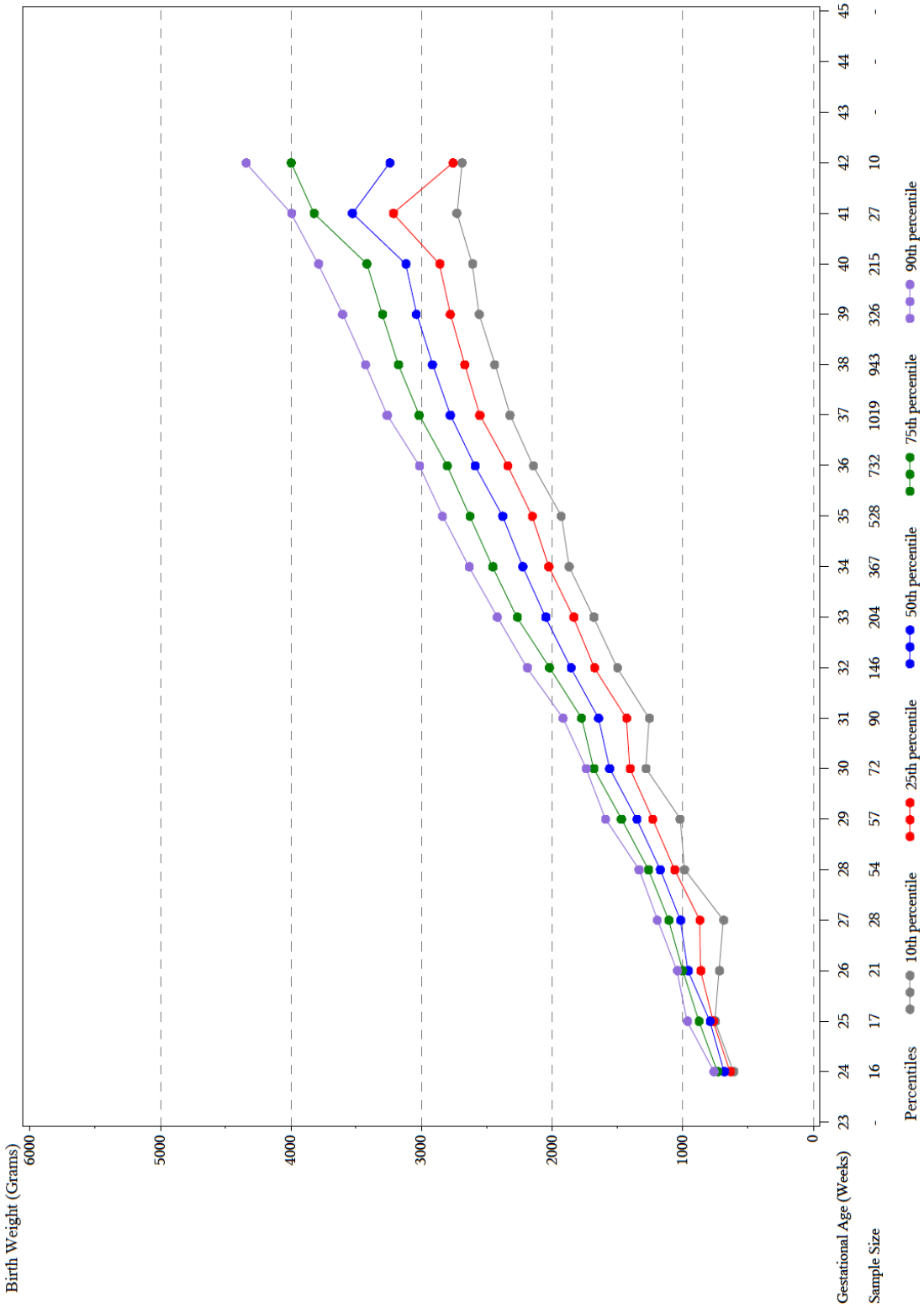
A)


Birth Weight (Grams)
6000
5000
4000
3000
2000
1000
0
Gestational Age (Weeks)
23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Sample Size
- - 14 11 10 12 12 15 18 44 44 76 157 284 629 1385 2070 3277 1426 306 16 - -
Percentiles
10th percentile
25th percentile
50th percentile
75th percentile
90th percentile

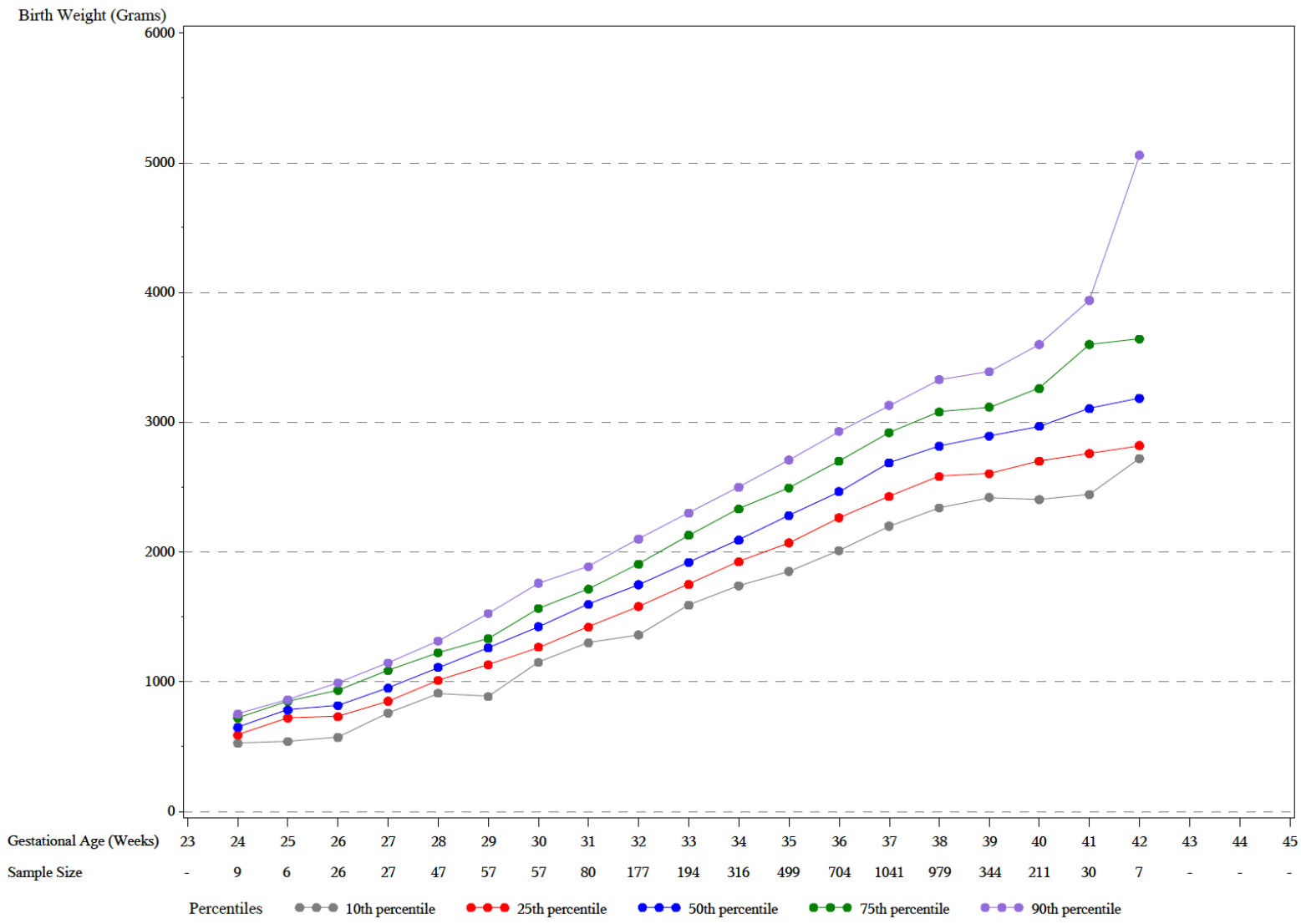
B)


Birth Weight (Grams)
6000
5000
4000
3000
2000
1000
0
Gestational Age (Weeks)
23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Sample Size
- - - 6 8 10 11 12 9 16 34 57 94 218 438 1113 1715 2618 1135 227 13 - -
Percentiles
10th percentile
25th percentile
50th percentile
75th percentile
90th percentile

C)


Birth Weight (Grams)
6000
5000
4000
3000
2000
1000
0
Gestational Age (Weeks)
23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Sample Size
- 16 17 21 28 54 57 72 90 146 204 367 528 732 1019 943 326 215 27 10 - - -
Percentiles
10th percentile
25th percentile
50th percentile
75th percentile
90th percentile

D)



Appendix 8. Birth weight percentiles by gestational age in A) male singletons, B) female singletons, C) male twins, and D) female twins

Appendix 9. Pairwise relationships among the prenatal complications

OR Lower CL Upper CL p-value	Prenatal complications (Predictor)															
Prenatal complications (Outcome)	640	641	642	643	644	647	652	653	654	655	657	658	660	661	662	663
640 Haemorrhage		4.63 3.27 6.56 ****	1.58 1.11 2.25 *	1.28 0.67 2.44	2.20 1.73 2.80 ****	1.58 0.86 2.88	1.00 0.70 1.44	1.03 0.58 1.85	1.81 1.29 2.53 ***	1.55 1.10 2.18 *	0.94 0.35 2.54	1.79 1.37 2.34 ****	1.28 0.86 1.91	0.92 0.64 1.31	1.27 0.91 1.77	0.70 0.45 1.09
641 Placental complications			0.94 0.61 1.43	1.13 0.54 2.33	2.16 1.69 2.77 ****	1.15 0.57 2.29	1.17 0.82 1.67	0.79 0.40 1.54	1.32 0.91 1.93	1.54 1.08 2.18 *	0.84 0.29 2.46	1.44 1.08 1.91 *	0.45 0.25 0.83 **	0.61 0.40 0.93 *	0.52 0.33 0.82 **	0.60 0.37 0.98 *
642 Hypertension				0.98 0.47 2.04	0.91 0.68 1.22	1.10 0.53 2.29	1.73 1.24 2.41 **	1.41 0.79 2.51	0.76 0.50 1.16	1.40 0.97 2.01	1.80 0.76 4.26	0.92 0.68 1.25	1.84 1.22 2.77 **	1.04 0.73 1.49	0.97 0.68 1.39	0.95 0.63 1.41
643 Vomiting					1.79 1.19 2.70 **	1.05 0.33 3.33	1.05 0.58 1.89	0.46 0.12 1.71	1.28 0.68 2.40	0.89 0.48 1.66	1.33 0.31 5.80	1.15 0.72 1.84	1.49 0.78 2.85	1.64 0.99 2.72	1.84 1.11 3.05 *	0.98 0.51 1.89
644 Early/threatened labour						1.49 0.98 2.25	1.22 0.98 1.52	0.64 0.42 0.96 *	0.88 0.69 1.14	1.54 1.24 1.91 ****	1.95 1.13 3.34 *	1.78 1.50 2.11 ****	0.94 0.72 1.22	1.09 0.89 1.35	0.97 0.78 1.20	0.92 0.71 1.17
647 Infections/parasitic conditions							0.63 0.34 1.18	0.57 0.18 1.76	0.86 0.46 1.60	1.43 0.84 2.44	1.75 0.51 6.07	1.16 0.74 1.81	0.66 0.31 1.39	0.50 0.26 0.97 *	0.40 0.19 0.81 *	2.32 1.42 3.78 ***

652 Malposition								1.53 1.04 2.25 *	1.42 1.09 1.85 **	1.08 0.82 1.42	2.88 1.65 5.03 ***	1.01 0.81 1.26	3.18 2.47 4.09 ****	0.83 0.63 1.09	1.18 0.92 1.52	1.12 0.84 1.49
653 Disproportion									0.99 0.57 1.70	1.14 0.70 1.88	2.29 0.83 6.31	1.69 1.16 2.47 **	24.2 15.3 38.1 ****	2.64 1.78 3.93 ****	2.41 1.60 3.62 ****	0.71 0.39 1.28
654 Abnormal organ/pelvic tissue										2.04 1.48 2.82 ****	1.14 0.49 2.63	0.42 0.30 0.58 ****	0.49 0.32 0.76 **	0.25 0.16 0.38 ****	0.42 0.28 0.62 ****	0.57 0.37 0.88 *
655 Foetal abnormality											1.56 0.77 3.17	1.39 1.11 1.75 **	1.12 0.79 1.57	0.92 0.69 1.23	1.21 0.92 1.59	0.49 0.33 0.74 ***
657 Polyhydramnios												2.59 1.63 4.12 ****	1.96 1.02 3.74 *	0.98 0.51 1.89	1.54 0.86 2.77	2.08 1.15 3.77 *
658 Amniotic cavity/ membrane complication													1.70 1.33 2.17 ****	2.15 1.78 2.61 ****	1.93 1.58 2.36 ****	1.16 0.91 1.48
660 Obstructed labour														1.97 1.51 2.58 ****	4.05 3.15 5.20 ****	0.54 0.35 0.83 **
661 Abnormal forces of labour															2.57 2.09 3.16 ****	1.00 0.76 1.32

662 Long labour																0.91 0.69 1.19
663 Umbilical cord complications																

* $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $0.0001 < p \leq 0.001$; **** $p \leq 0.0001$

Appendix 10. Pairwise relationships among the perinatal complications

Odds Ratio Lower CL Upper CL p-value	Perinatal complications (Predictor)												
Perinatal complications (Outcome)	762	767	768	769	770	771	772	774	775	776	778	1-min Apgar	5-min Apgar
762 Placenta/cord/membrane complications		1.75 0.56 5.53	5.27 3.08 9.01 ****	6.00 2.75 13.0 ****	2.27 1.37 3.78 **	2.63 1.34 5.18 **	5.06 2.13 12.0 ***	1.95 1.22 3.12 **	1.66 0.76 3.66	10.8 4.11 28.2 ****	2.44 0.85 7.00	0.67 0.61 0.74 ****	0.64 0.54 0.75 ****
767 Birth trauma			2.36 1.33 4.17 **	0.99 0.31 3.18	1.32 0.78 2.22	1.06 0.46 2.44	3.69 1.79 7.61 ***	1.94 1.28 2.94 **	1.84 0.95 3.58	3.63 1.39 9.49 **	3.81 1.87 7.77 ***	0.85 0.77 0.94 **	0.69 0.59 0.80 ****
768 Hypoxia/ asphyxia				2.42 1.24 4.72 **	3.83 2.78 5.27 ****	1.93 1.17 3.20 *	2.12 1.03 4.39 *	2.19 1.61 2.98 ****	1.06 0.58 1.92	2.90 1.20 7.00 *	3.01 1.56 5.82 **	0.53 0.49 0.58 ****	0.43 0.37 0.50 ****
769 Respiratory distress syndrome					31.3 20.0 48.9 ****	9.61 5.69 16.2 ****	104 47.8 227 ****	15.1 9.89 23.2 ****	6.07 3.39 10.9 ****	424 153 >999 ****	1.53 0.50 4.65	0.52 0.46 0.58 ****	0.32 0.26 0.39 ****
770 Respiratory conditions						4.54 3.36 6.12 ****	10.4 6.93 15.7 ****	4.04 3.33 4.91 ****	4.50 3.29 6.14 ****	37.9 20.7 69.5 ****	2.88 1.80 4.60 ****	0.59 0.56 0.62 ****	0.35 0.31 0.39 ****
771 Infections							5.75 3.59 9.21 ****	2.28 1.73 3.00 ****	2.07 1.33 3.24 **	13.1 7.70 22.3 ****	1.91 0.98 3.71	0.81 0.76 0.87 ****	0.71 0.64 0.80 ****

772 Haemorrhage								5.87 3.97 8.67 ****	4.86 2.74 8.61 ****	69.9 31.9 153 ****	4.37 1.87 10.2 ***	0.64 0.58 0.70 ****	0.47 0.40 0.55 ****
774 Jaundice									3.90 2.84 5.36 ****	19.2 10.5 35.2 ****	1.85 1.13 3.03 *	0.77 0.73 0.81 ****	0.65 0.59 0.72 ****
775 Endocrine/metabolic disturbances										21.5 9.03 50.9 ****	2.49 1.07 5.82 *	0.73 0.67 0.79 ****	0.56 0.48 0.66 ****
776 Haematological disorders											1.16 0.22 6.13	0.49 0.43 0.57 ****	0.30 0.22 0.39 ****
778 Integument/temperature regulation conditions												0.86 0.77 0.95 **	0.82 0.67 0.99 *
1-minute Apgar score													
5-minute Agpar score													

* $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $0.0001 < p \leq 0.001$; **** $p \leq 0.0001$

Appendix 11. Pairwise relationships between the prenatal and perinatal complications

Odds Ratio Lower CL Upper CL p-value	Perinatal complications (Predictor)												
Prenatal complications (Outcome)	762	767	768	769	770	771	772	774	775	776	778	1-min Apgar	5-min Apgar
640	1.09 0.49 2.44	0.77 0.31 1.90	0.81 0.46 1.42	3.43 2.02 5.83 ****	1.39 0.99 1.95	1.11 0.66 1.88	3.51 2.01 6.15 ****	1.38 1.02 1.87 *	2.35 1.52 3.63 ***	2.78 1.25 6.17 *	2.03 1.05 3.95 *	0.95 0.89 1.03	0.97 0.83 1.12
641	2.87 1.53 5.38 **	0.44 0.13 1.48	1.65 1.04 2.61 *	5.48 3.35 8.96 ****	2.36 1.73 3.20 ****	1.73 1.08 2.78 *	2.50 1.37 4.55 **	1.37 1.01 1.86 *	1.57 0.96 2.57	4.44 2.23 8.88 ****	0.25 0.06 1.07	0.84 0.79 0.90 ****	0.78 0.69 0.89 ****
642	1.27 0.57 2.87	1.56 0.72 3.40	1.18 0.70 1.97	1.57 0.80 3.06	1.55 1.10 2.18 *	1.17 0.69 2.00	1.29 0.61 2.73	1.54 1.13 2.09 **	1.98 1.21 3.26 **	1.86 0.74 4.64	1.16 0.54 2.48	0.89 0.82 0.96 **	0.77 0.67 0.89 ***
643	0.78 0.18 3.39	N/A	0.76 0.29 1.97	2.84 1.13 7.18 *	1.23 0.69 2.19	0.56 0.21 1.53	2.02 0.69 5.87	1.52 0.93 2.49	0.55 0.18 1.67	0.33 0.03 3.23	0.58 0.12 2.93	1.02 0.89 1.16	1.02 0.77 1.35
644	0.99 0.59 1.65	0.69 0.40 1.20	0.89 0.64 1.24	8.89 5.86 13.5 ****	2.59 2.11 3.17 ****	2.22 1.64 2.99 ****	3.07 2.01 4.70 ****	2.51 2.08 3.01 ****	1.91 1.40 2.62 ****	7.16 4.00 12.8 ****	1.62 1.02 2.57 *	0.88 0.84 0.92 ****	0.77 0.71 0.85 ****
647	1.50 0.48 4.64	0.58 0.12 2.80	0.20 0.04 0.87 *	1.25 0.43 3.68	1.55 0.93 2.60	2.46 1.28 4.71 **	0.94 0.28 3.12	0.98 0.60 1.61	1.29 0.59 2.84	0.99 0.20 5.03	0.77 0.21 2.93	0.89 0.80 0.99 *	0.81 0.66 0.99 *

652	1.33 0.73 2.39	2.12 1.23 3.66 **	2.19 1.56 3.07 ****	4.30 2.78 6.64 ****	2.53 2.00 3.20 ****	1.21 0.81 1.81	2.88 1.78 4.67 ****	2.19 1.76 2.73 ****	1.72 1.19 2.50 **	3.96 2.14 7.30 ****	0.90 0.49 1.68	0.80 0.76 0.84 ****	0.71 0.64 0.78 ****
653	0.81 0.24 2.68	2.91 1.26 6.75 *	2.79 1.62 4.82 ***	0.40 0.11 1.53	1.15 0.72 1.83	0.88 0.41 1.89	0.12 0.01 1.05	1.14 0.74 1.75	1.04 0.51 2.15	1.07 0.26 4.46	2.82 1.24 6.39 *	0.95 0.86 1.06	0.98 0.79 1.21
654	0.55 0.22 1.41	0.29 0.10 0.86 *	0.82 0.51 1.33	1.41 0.75 2.64	1.32 0.96 1.82	0.74 0.43 1.29	1.46 0.75 2.85	0.96 0.70 1.30	1.67 1.04 2.70 *	1.41 0.61 3.30	0.66 0.29 1.51	1.07 0.99 1.15	1.03 0.89 1.20
655	0.62 0.27 1.41	0.33 0.12 0.89 *	0.31 0.16 0.59 ***	1.46 0.83 2.57	1.33 1.00 1.78	1.18 0.77 1.82	0.68 0.33 1.40	1.10 0.84 1.43	1.96 1.31 2.92 **	1.61 0.73 3.54	1.15 0.60 2.19	1.00 0.94 1.07	0.86 0.77 0.97 *
657	0.54 0.06 4.79	0.55 0.06 4.86	0.48 0.13 1.81	5.28 2.10 13.3 ***	2.89 1.64 5.12 ***	1.63 0.66 4.02	2.46 0.77 7.87	2.25 1.29 3.93 **	3.46 1.54 7.75 **	5.45 1.61 18.4 **	2.02 0.57 7.21	0.85 0.75 0.97 *	0.70 0.55 0.88 **
658	0.97 0.57 1.64	1.56 0.97 2.52	1.01 0.72 1.40	2.77 1.86 4.11 ****	1.64 1.33 2.03 ****	1.62 1.19 2.21 **	2.16 1.41 3.30 ***	1.76 1.46 2.13 ****	1.22 0.88 1.70	2.25 1.27 4.01 **	1.24 0.77 2.00	0.90 0.86 0.95 ****	0.79 0.72 0.86 ****
660	0.59 0.25 1.40	5.76 3.38 9.81 ****	1.87 1.24 2.81 **	0.55 0.25 1.21	1.12 0.81 1.54	1.01 0.62 1.65	2.34 1.32 4.15 **	1.32 1.00 1.74	1.71 1.10 2.64 *	1.23 0.51 2.97	1.51 0.80 2.85	0.82 0.77 0.87 ****	0.78 0.69 0.88 ****
661	1.06 0.60 1.87	1.23 0.71 2.14	1.11 0.78 1.58	0.59 0.31 1.11	0.98 0.76 1.27	0.52 0.33 0.84 **	0.52 0.26 1.04	0.96 0.76 1.21	0.89 0.60 1.33	0.27 0.08 0.93 *	1.53 0.93 2.51	1.03 0.98 1.09	0.99 0.88 1.10

662	0.65 0.32 1.29	2.41 1.49 3.90 ***	1.61 1.16 2.25 **	0.43 0.21 0.89 *	1.02 0.78 1.32	1.28 0.89 1.85	1.10 0.63 1.90	0.98 0.78 1.23	1.02 0.69 1.51	0.26 0.08 0.89 *	1.69 1.03 2.77 *	0.96 0.91 1.01	0.88 0.80 0.98 *
663	57.7 34.5 96.4 ****	1.87 1.07 3.26 *	2.73 1.98 3.76 ****	0.99 0.54 1.81	1.61 1.23 2.10 ***	1.09 0.71 1.67	1.60 0.92 2.81	1.00 0.77 1.30	0.41 0.23 0.75 **	1.15 0.50 2.61	1.60 0.91 2.80	0.74 0.70 0.78 ****	0.68 0.61 0.75 ****

* $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $0.0001 < p \leq 0.001$; **** $p \leq 0.0001$
N/A indicates no occurrences of both complications in the same individual (OR < 0.001)

Appendix 12. Pairwise relationships between the prenatal complications and the covariates

OR Lower CL Upper CL p-value	Prenatal complications (Outcome)															
Covariates (Predictor)	640	641	642	643	644	647	652	653	654	655	657	658	660	661	662	663
Sex	1.04 0.83 1.31	1.04 0.82 1.31	1.07 0.86 1.35	1.35 0.93 1.95	0.95 0.82 1.09	0.60 0.42 0.87 **	0.98 0.82 1.17	0.79 0.58 1.08	1.22 0.99 1.51	0.79 0.65 0.95 *	0.81 0.51 1.29	0.85 0.74 0.98 *	0.81 0.66 0.99 *	1.12 0.95 1.31	0.94 0.79 1.10	1.02 0.85 1.23
Twin	1.09 0.47 2.54	1.05 0.44 2.54	2.48 1.22 5.02 *	2.95 1.01 8.64 *	6.92 4.37 11.0 ****	1.26 0.38 4.20	25.4 15.7 41.0 ****	0.16 0.02 1.44	1.05 0.48 2.29	2.27 1.26 4.07 **	9.48 4.39 20.5 ****	1.23 0.74 2.04	2.11 1.14 3.92 *	0.43 0.19 0.96 *	0.71 0.37 1.37	0.47 0.18 1.20
Maternal Age	1.00 0.98 1.03	1.03 1.01 1.05 **	1.01 0.98 1.03	0.94 0.90 0.97 ***	0.97 0.95 0.98 ****	0.98 0.95 1.01	1.03 1.02 1.04 ****	1.02 0.99 1.05	1.18 1.15 1.22 ****	1.12 1.10 1.14 ****	1.03 0.99 1.07	0.99 0.98 0.99 *	1.01 0.99 1.03	1.01 0.99 1.02	1.01 0.99 1.02	0.97 0.96 0.99 **
Paternal Age	1.02 0.99 1.04	1.02 0.99 1.04	1.00 0.97 1.03	0.97 0.92 1.02	0.99 0.98 1.91	1.00 0.95 1.04	1.01 0.99 1.03	0.98 0.95 1.01	1.13 1.10 1.17 ****	1.09 1.06 1.12 ****	0.99 0.95 1.03	0.99 0.97 1.01	0.99 0.97 1.02	0.99 0.98 1.01	1.00 0.99 1.02	0.97 0.95 0.99 **
Birth Year	1.06 1.04 1.09 ****	1.01 0.99 1.03	0.99 0.96 1.02	1.08 1.04 1.12 ****	1.04 1.03 1.05 ****	1.01 0.98 1.04	1.00 0.99 1.02	0.97 0.93 1.00	1.11 1.09 1.14 ****	1.20 1.17 1.23 ****	0.97 0.91 1.04	1.03 1.02 1.04 ****	1.01 1.00 1.03	1.06 1.04 1.08 ****	1.05 1.03 1.07 ****	0.96 0.93 1.00
Birth Order																
Second	1.25 0.96 1.62	1.29 0.98 1.70	0.42 0.33 0.54 ****	0.78 0.52 1.16	1.10 0.94 1.28	1.21 0.82 1.78	0.74 0.61 0.90 **	0.33 0.23 0.46 ****	28.8 17.9 46.1 ****	2.04 1.62 2.56 ****	0.68 0.42 1.09	0.53 0.45 0.62 ****	0.38 0.30 0.48 ****	0.67 0.56 0.80 ****	0.40 0.33 0.48 ****	0.94 0.76 1.15

Third	0.98 0.70 1.38	1.19 0.84 1.68	0.40 0.28 0.55 ****	0.65 0.38 1.13	1.17 0.97 1.42	0.99 0.60 1.63	0.80 0.63 1.02	0.30 0.19 0.47 ****	34.8 20.3 59.5 ****	2.41 1.83 3.19 ****	0.55 0.28 1.06	0.48 0.39 0.59 ****	0.37 0.27 0.51 ****	0.84 0.67 1.05	0.41 0.32 0.52 ****	0.95 0.73 1.23
Fourth	1.06 0.65 1.73	1.57 1.00 2.47	0.48 0.30 0.78 **	1.18 0.59 2.36	1.19 0.90 1.57	1.00 0.48 2.06	0.72 0.50 1.03	0.11 0.04 0.32 ****	30.3 15.4 59.7 ****	2.64 1.79 3.89 ****	0.71 0.30 1.73	0.60 0.45 0.81 ***	0.38 0.24 0.62 ****	0.73 0.52 1.03	0.32 0.22 0.48 ****	0.72 0.48 1.08
Fifth	2.20 1.18 4.09 *	1.95 1.03 3.69 *	0.30 0.13 0.73 **	0.82 0.25 2.65	1.64 1.09 2.46 *	0.93 0.30 2.91	0.80 0.46 1.38	0.29 0.08 1.04	45.8 19.3 109 ****	3.58 2.05 6.27 ****	0.31 0.04 2.29	0.51 0.31 0.82 **	0.23 0.09 0.60 **	1.20 0.75 1.91	0.32 0.17 0.61 ***	0.98 0.55 1.73
Sixth or Later	1.93 0.95 3.91	1.13 0.49 2.61	0.63 0.27 1.45	1.47 0.46 4.65	1.48 0.94 2.32	2.16 0.76 6.09	0.85 0.47 1.52	N/A	28.9 10.2 82.0 ****	3.76 2.01 7.03 ****	N/A	0.62 0.37 1.03	0.38 0.16 0.88 *	0.86 0.49 1.52	0.29 0.14 0.60 ***	0.70 0.36 1.38
Gestational Age	0.88 0.85 0.93 ****	0.82 0.78 0.85 ****	0.87 0.83 0.93 ****	1.02 0.93 1.13	0.70 0.68 0.73 ****	0.95 0.88 1.04	0.85 0.82 0.89 ****	1.20 1.09 1.34 ***	0.87 0.83 0.92 ****	0.88 0.85 0.92 ****	0.83 0.78 0.89 ****	0.85 0.82 0.87 ****	1.05 0.99 1.12	1.09 1.04 1.14 ***	1.12 1.07 1.18 ****	1.07 1.02 1.13 **
Birth Weight (100 grams)	0.98 0.96 0.99 *	0.94 0.93 0.96 ****	0.96 0.94 0.98 ****	1.01 0.98 1.05	0.91 0.90 0.92 ****	0.99 0.96 1.02	0.95 0.93 0.96 ****	1.10 1.07 1.14 ****	1.01 0.99 1.03	0.98 0.97 0.99 *	0.94 0.91 0.98 ***	0.95 0.94 0.96 ****	1.07 1.05 1.09 ****	1.03 1.02 1.05 ****	1.04 1.03 1.06 ****	0.99 0.98 1.01

* $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $0.0001 < p \leq 0.001$; **** $p \leq 0.0001$
N/A indicates no occurrences of complication in sixth or later pregnancy (OR < 0.001)

Appendix 13. Pairwise relationships between the perinatal complications and the covariates

Odds Ratio Lower CL Upper CL p-value	Perinatal complications (Outcome)										
Covariates (Predictor)	762	767	768	769	770	771	772	774	775	776	778
Sex	1.13 0.77 1.65	0.73 0.50 1.06	0.77 0.60 0.99 *	0.82 0.57 1.19	0.78 0.66 0.93 **	1.02 0.81 1.30	0.63 0.43 0.92 *	0.70 0.60 0.83 ****	0.77 0.57 1.03	1.02 0.62 1.67	0.49 0.33 0.73 ***
Twin	0.20 0.03 1.59	0.68 0.21 2.15	1.39 0.72 2.70	15.2 8.24 28.2 ****	5.60 3.93 7.97 ****	1.75 1.02 3.00 *	6.61 3.45 12.7 ****	3.03 2.07 4.42 ****	1.86 0.91 3.80	8.34 3.74 18.6 ****	0.58 0.17 1.94
Maternal Age	0.97 0.94 1.01	0.96 0.93 0.99 *	0.96 0.94 0.98 **	1.03 0.99 1.06	1.00 0.99 1.02	0.98 0.97 0.99 *	0.99 0.95 1.02	0.98 0.96 0.99 **	1.04 1.01 1.07 *	1.04 0.99 1.09	0.99 0.96 1.03
Paternal Age	1.02 0.98 1.07	0.95 0.91 0.99 *	0.95 0.92 0.98 ***	1.01 0.96 1.05	0.99 0.97 1.01	0.99 0.97 1.02	0.98 0.94 1.02	0.98 0.97 1.00	1.03 1.00 1.07	1.07 1.01 1.13 *	1.00 0.96 1.03
Birth Year	1.01 0.92 1.11	0.97 0.92 1.03	0.90 0.88 0.92 ****	1.02 0.99 1.05	1.00 0.98 1.01	1.00 0.98 1.02	0.99 0.91 1.09	0.97 0.94 1.01	1.02 0.99 1.04	1.03 0.98 1.07	1.00 0.87 1.16
Birth Order											
Second	1.26 0.79 2.03	0.37 0.24 0.56 ****	0.56 0.42 0.74 ****	0.68 0.45 1.02	0.72 0.59 0.87 ***	0.74 0.56 0.98 *	0.81 0.54 1.21	0.64 0.54 0.76 ****	0.81 0.58 1.13	0.31 0.15 0.61 ***	0.84 0.56 1.24

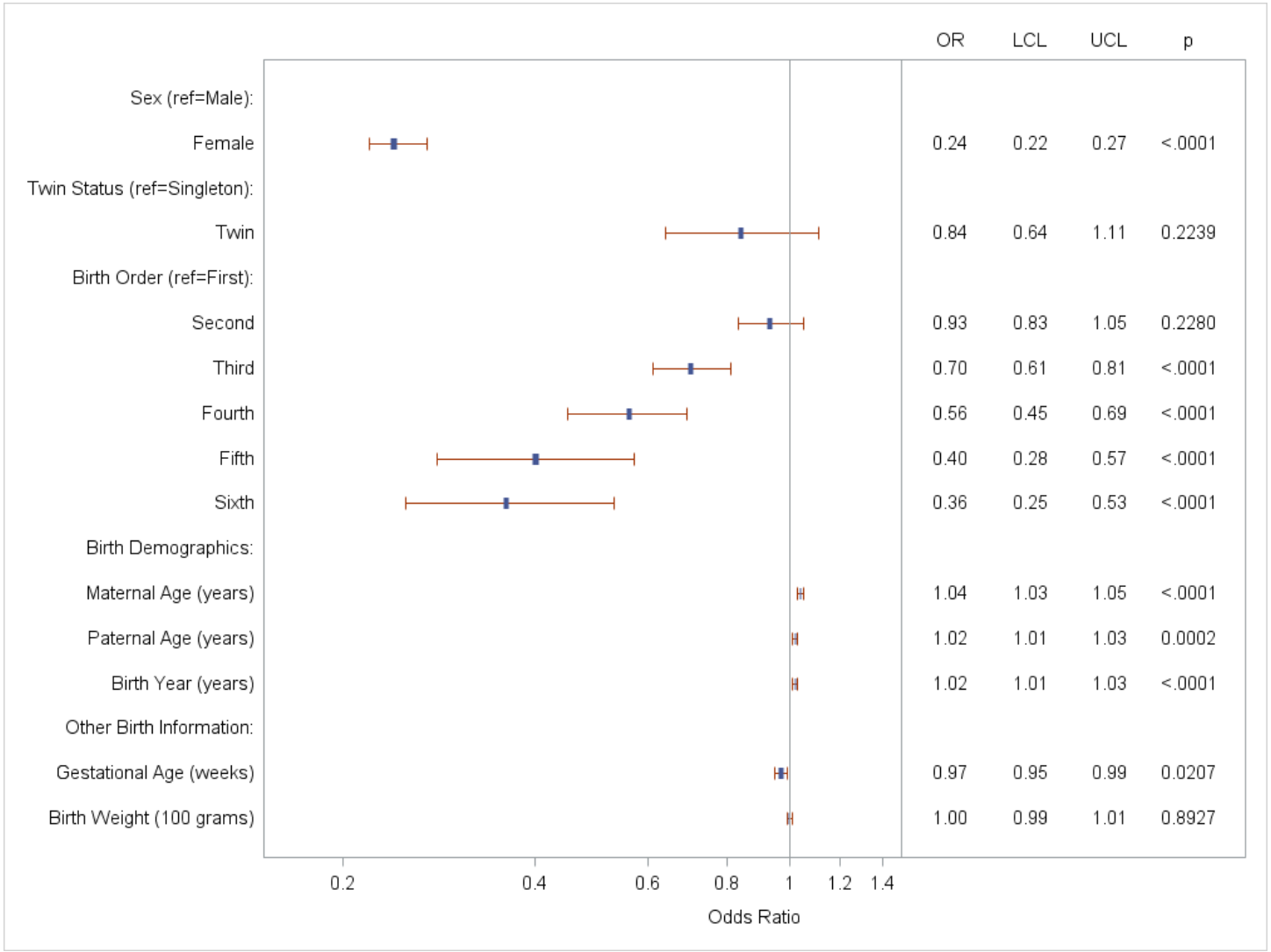
Third	2.32 1.39 3.89 **	0.36 0.20 0.64 ***	0.57 0.40 0.82 **	0.67 0.39 1.16	0.83 0.65 1.05	0.89 0.64 1.24	0.69 0.40 1.18	0.67 0.54 0.84 ***	1.21 0.81 1.81	0.99 0.50 1.94	0.95 0.59 1.54
Fourth	1.24 0.55 2.79	0.32 0.13 0.81 *	0.66 0.40 1.10	0.97 0.46 2.04	0.54 0.36 0.80 **	0.76 0.46 1.27	0.66 0.30 1.48	0.49 0.34 0.69 ****	1.04 0.57 1.90	1.43 0.58 3.55	0.35 0.12 0.99 *
Fifth	2.34 0.86 6.34	0.17 0.02 1.24	0.66 0.30 1.46	2.06 0.80 5.33	0.74 0.42 1.30	0.77 0.35 1.71	1.82 0.74 4.46	0.84 0.52 1.35	2.18 0.99 4.80	4.48 1.55 12.9 **	0.47 0.11 2.00
Sixth	1.89 0.58 6.18	0.19 0.03 1.37	0.30 0.09 0.93 *	1.96 0.63 6.13	0.99 0.54 1.80	0.74 0.31 1.77	0.82 0.22 3.06	1.00 0.59 1.68	6.05 2.72 13.5 ****	0.59 0.07 5.30	0.53 0.12 2.33
Gestational Age	0.94 0.86 1.02	1.09 0.98 1.23	1.08 1.00 1.16 *	0.49 0.44 0.55 ****	0.69 0.66 0.71 ****	0.82 0.79 0.85 ****	0.67 0.63 0.72 ****	0.66 0.64 0.69 ****	0.73 0.69 0.77 ****	0.57 0.51 0.64 ****	0.99 0.91 1.07
Birth Weight (100 grams)	0.97 0.94 1.00	1.06 1.03 1.09 ***	1.00 0.98 1.02	0.77 0.75 0.79 ****	0.91 0.89 0.92 ****	0.93 0.92 0.95 ****	0.87 0.85 0.89 ****	0.90 0.89 0.92 ****	0.95 0.92 0.97 ****	0.78 0.76 0.81 ****	1.03 1.00 1.06

* $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $0.0001 < p \leq 0.001$; **** $p \leq 0.0001$

Appendix 14. Pairwise relationships between 1- and 5-minute Apgar scores and the covariates

Test statistic (df) p-value	Apgar scores (Outcome)	
Covariates (Predictor)	1-min Apgar	5-min Apgar
Sex	1.89 (4,102)	1.92 (4,123)
Maternal Age	1.98 (4,086) *	-2.23 (4,107) *
Paternal Age	1.37 (2,632)	-0.69 (2,645)
Birth Year	5.25 (4,102) ****	-1.13 (4,123)
Birth Order		
Second	5.13 (4,082) ****	3.76 (4,103) ***
Third	5.62 (4,082) ****	1.78 (4,103)
Fourth	4.10 (4,082) ****	2.02 (4,103) *
Fifth	0.02 (4,082)	-1.36 (4,103)
Sixth	2.07 (4,082) *	0.56 (4,103)
Gestational Age	15.3 (4,082) ****	19.2 (4,103) ****
Birth Weight (100 grams)	11.3 (4,044) ****	12.6 (4,065) ****

* $0.01 < p \leq 0.05$; ** $0.001 < p \leq 0.01$; *** $0.0001 < p \leq 0.001$; **** $p \leq 0.0001$


OR
LCL
UCL
p
Sex (ref=Male):
Female
0.24
0.22
0.27
<.0001
Twin Status (ref=Singleton):
Twin
0.84
0.64
1.11
0.2239
Birth Order (ref=First):
Second
0.93
0.83
1.05
0.2280
Third
0.70
0.61
0.81
<.0001
Fourth
0.56
0.45
0.69
<.0001
Fifth
0.40
0.28
0.57
<.0001
Sixth
0.36
0.25
0.53
<.0001
Birth Demographics:
Maternal Age (years)
1.04
1.03
1.05
<.0001
Paternal Age (years)
1.02
1.01
1.03
0.0002
Birth Year (years)
1.02
1.01
1.03
<.0001
Other Birth Information:
Gestational Age (weeks)
0.97
0.95
0.99
0.0207
Birth Weight (100 grams)
1.00
0.99
1.01
0.8927
0.2
0.4
0.6
0.8
1
1.2
1.4
Odds Ratio

Appendix 15. Pairwise relationships between ASD and the covariates in the ASD families. An odds ratio >1 indicates that individuals are at an increased risk for ASD, while an odds ratio <1 indicates that individuals are at a decreased risk. OR=odds ratio; LCL=lower 95% confidence limit; UCL=upper 95% confidence limit; p=p-value.

Appendix 16. Relationships between ASD and the prenatal and perinatal complications with and without adjustments for the covariates using all the selected ASD families

Prenatal and perinatal complications	Estimates without adjustment		Estimates with adjustment[a]		Estimates with adjustment[b]	
ICD-9-CM code: Medical condition[169]	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Prenatal complications						
640: Haemorrhage in early pregnancy	1.15 (0.93-1.42)	0.19	1.11 (0.88-1.39)	0.40	0.95 (0.69-1.29)	0.73
641: Antepartum haemorrhage, abruptio placentae, placenta previa	1.11 (0.89-1.39)	0.35	1.16 (0.90-1.48)	0.25	1.26 (0.90-1.75)	0.18
642: Hypertension	1.03 (0.87-1.23)	0.70	0.97 (0.80-1.17)	0.75	0.94 (0.74-1.19)	0.60
643: Excessive vomiting	0.85 (0.62-1.17)	0.32	0.84 (0.59-1.20)	0.35	0.67 (0.38-1.17)	0.16
644: Early or threatened labour	0.93 (0.83-1.05)	0.23	0.89 (0.77-1.02)	0.10	0.96 (0.80-1.16)	0.66
647: Infectious and parasitic conditions in mother	1.02 (0.75-1.38)	0.91	0.90 (0.65-1.25)	0.52	1.12 (0.71-1.75)	0.64
652: Malposition and malpresentation of foetus	1.07 (0.92-1.25)	0.35	1.13 (0.95-1.35)	0.17	0.96 (0.77-1.20)	0.74
653: Cephalopelvic disproportion	1.32 (1.03-1.70)	0.03	1.14 (0.87-1.51)	0.34	1.06 (0.76-1.47)	0.74
654: Abnormality of organs and soft tissues of pelvis	0.92 (0.78-1.09)	0.33	0.92 (0.76-1.12)	0.41	0.87 (0.68-1.11)	0.26
655: Known or suspected foetal abnormality affecting mother	1.13 (0.96-1.33)	0.13	0.99 (0.83-1.20)	0.95	1.10 (0.86-1.41)	0.45
657: Polyhydramnios	1.22 (0.83-1.81)	0.31	1.19 (0.78-1.83)	0.42	1.12 (0.66-1.90)	0.67
658: Other problems associated with amniotic cavity and membranes	1.23 (1.09-1.40)	0.001	1.12 (0.97-1.29)	0.13	1.24 (1.03-1.50)	0.02
660: Obstructed labour	1.26 (1.05-1.51)	0.01	1.16 (0.94-1.42)	0.17	1.02 (0.79-1.33)	0.87
661: Abnormality of forces of	1.06 (0.91-1.23)	0.44	1.06 (0.90-1.25)	0.49	1.10 (0.89-1.37)	0.38

labour						
662: Long labour	1.09 (0.94-1.27)	0.28	0.96 (0.81-1.14)	0.63	0.88 (0.71-1.10)	0.28
663: Umbilical cord complications	1.01 (0.85-1.20)	0.92	1.06 (0.87-1.28)	0.58	1.34 (1.05-1.71)	0.02
Perinatal complications						
762: Foetus/newborn affected by complications of placenta, cord, membranes	1.12 (0.79-1.58)	0.54	1.28 (0.87-1.88)	0.22	2.16 (1.23-3.80)	0.008
767: Birth trauma	0.96 (0.67-1.37)	0.81	0.85 (0.57-1.25)	0.40	0.89 (0.54-1.47)	0.65
768: Intrauterine hypoxia and birth asphyxia	1.18 (0.95-1.47)	0.13	1.15 (0.90-1.47)	0.26	0.97 (0.72-1.31)	0.84
769: Respiratory distress syndrome	1.45 (1.06-1.97)	0.02	1.19 (0.79-1.79)	0.41	1.38 (0.80-2.37)	0.25
770: Other respiratory conditions	1.29 (1.11-1.51)	0.001	1.10 (0.91-1.32)	0.32	1.16 (0.92-1.47)	0.22
771: Perinatal infections	1.28 (1.01-1.61)	0.04	1.19 (0.91-1.54)	0.20	1.02 (0.72-1.44)	0.92
772: Foetal and neonatal haemorrhage	1.66 (1.20-2.30)	0.002	1.29 (0.88-1.88)	0.20	0.95 (0.57-1.57)	0.83
774: Perinatal jaundice	1.08 (0.94-1.24)	0.27	0.91 (0.77-1.07)	0.26	0.82 (0.67-1.02)	0.07
775: Endocrine, metabolic disturbances	1.14 (0.90-1.44)	0.27	1.07 (0.82-1.39)	0.64	1.05 (0.73-1.50)	0.81
776: Haematological disorders	2.28 (1.46-3.55)	0.0003	2.80 (1.62-4.84)	0.0002	3.11 (1.39-6.94)	0.006
778: Conditions involving integument and temperature regulation	1.21 (0.86-1.69)	0.27	0.95 (0.66-1.36)	0.77	0.99 (0.62-1.57)	0.95
1-minute Apgar score	0.94 (0.91-0.97)	0.0001	0.95 (0.92-0.99)	0.007	0.95 (0.91-0.99)	0.04
5-minute Apgar score	0.84 (0.78-0.90)	<0.0001	0.86 (0.79-0.92)	<0.0001	0.86 (0.78-0.94)	0.002

[a]Models were adjusted for sex, maternal age, birth year, birth order, twin status, gestational age, and birth weight percentile
[b]Models were adjusted for sex, paternal age, birth year, birth order, twin status, gestational age, and birth weight percentile

Appendix 17. Relationships between ASD and the prenatal and perinatal complications with and without adjustments for the covariates using the ASD families with at least one ASD-affected individual having ≥2 ASD claims

Prenatal and perinatal complications	Estimates without adjustment		Estimates with adjustment[a]		Estimates with adjustment[b]	
ICD-9-CM code: Medical condition[169]	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Prenatal complications						
640: Haemorrhage in early pregnancy	1.30 (0.98-1.72)	0.06	1.36 (0.99-1.87)	0.06	1.29 (0.84-1.98)	0.24
641: Antepartum haemorrhage, abruptio placentae, placenta previa	1.02 (0.76-1.36)	0.90	1.11 (0.80-1.54)	0.55	1.19 (0.78-1.83)	0.41
642: Hypertension	1.01 (0.81-1.26)	0.90	0.98 (0.76-1.25)	0.86	0.99 (0.73-1.34)	0.95
643: Excessive vomiting	0.78 (0.52-1.17)	0.23	0.84 (0.53-1.34)	0.46	0.71 (0.35-1.47)	0.36
644: Early or threatened labour	0.94 (0.81-1.10)	0.45	0.88 (0.74-1.06)	0.17	0.89 (0.70-1.13)	0.34
647: Infectious and parasitic conditions in mother	1.07 (0.72-1.58)	0.75	0.94 (0.61-1.45)	0.77	1.27 (0.70-2.29)	0.43
652: Malposition and malpresentation of foetus	1.19 (0.98-1.44)	0.07	1.28 (1.02-1.61)	0.03	1.05 (0.79-1.40)	0.73
653: Cephalopelvic disproportion	1.47 (1.07-2.01)	0.02	1.30 (0.91-1.86)	0.15	1.44 (0.93-2.22)	0.10
654: Abnormality of organs and soft tissues of pelvis	0.98 (0.78-1.22)	0.84	0.98 (0.76-1.27)	0.88	0.98 (0.71-1.34)	0.88
655: Known or suspected foetal abnormality affecting mother	1.15 (0.94-1.42)	0.18	1.02 (0.80-1.29)	0.90	1.08 (0.79-1.49)	0.63
657: Polyhydramnios	1.18 (0.72-1.93)	0.51	1.09 (0.63-1.88)	0.76	1.04 (0.53-2.06)	0.90
658: Other problems associated with amniotic cavity and membranes	1.16 (0.99-1.37)	0.08	1.04 (0.86-1.25)	0.69	1.13 (0.89-1.45)	0.32
660: Obstructed labour	1.48 (1.17-1.88)	0.001	1.40 (1.07-1.84)	0.01	1.34 (0.94-1.91)	0.11
661: Abnormality of forces of labour	0.89 (0.73-1.08)	0.22	0.91 (0.74-1.13)	0.41	0.97 (0.73-1.29)	0.85
662: Long labour	1.10 (0.91-1.34)	0.33	0.97 (0.78-1.22)	0.81	1.03 (0.78-1.38)	0.82

663: Umbilical cord complications	0.90 (0.72-1.13)	0.37	0.93 (0.72-1.20)	0.57	1.01 (0.73-1.40)	0.94
Perinatal complications						
762: Foetus/newborn affected by complications of placenta, cord, membranes	1.19 (0.75-1.89)	0.46	1.32 (0.78-2.22)	0.30	2.00 (0.91-4.37)	0.08
767: Birth trauma	0.86 (0.54-1.36)	0.52	0.78 (0.46-1.30)	0.34	0.72 (0.37-1.42)	0.34
768: Intrauterine hypoxia and birth asphyxia	1.06 (0.80-1.41)	0.67	1.08 (0.78-1.48)	0.64	0.97 (0.64-1.46)	0.87
769: Respiratory distress syndrome	1.73 (1.13-2.65)	0.01	1.36 (0.78-2.35)	0.28	1.86 (0.84-4.11)	0.13
770: Other respiratory conditions	1.44 (1.18-1.77)	0.0004	1.26 (0.99-1.62)	0.06	1.26 (0.92-1.74)	0.15
771: Perinatal infections	1.20 (0.89-1.63)	0.24	1.14 (0.80-1.61)	0.47	0.78 (0.49-1.24)	0.29
772: Foetal and neonatal haemorrhage	1.93 (1.27-2.92)	0.002	1.16 (0.72-1.88)	0.55	0.91 (0.49-1.69)	0.77
774: Perinatal jaundice	1.15 (0.96-1.37)	0.13	0.99 (0.80-1.22)	0.90	0.94 (0.71-1.23)	0.64
775: Endocrine, metabolic disturbances	1.47 (1.08-1.98)	0.01	1.24 (0.88-1.76)	0.23	1.19 (0.74-1.93)	0.47
776: Haematological disorders	2.85 (1.57-5.19)	0.0006	3.55 (1.69-7.49)	0.0009	4.33 (1.54-12.2)	0.006
778: Conditions involving integument and temperature regulation	1.23 (0.79-1.91)	0.36	0.94 (0.59-1.50)	0.79	1.29 (0.69-2.38)	0.42
1-minute Apgar score	0.92 (0.88-0.96)	0.0001	0.93 (0.89-0.98)	0.005	0.94 (0.88-0.99)	0.045
5-minute Apgar score	0.82 (0.75-0.89)	<0.0001	0.84 (0.76-0.92)	0.0004	0.85 (0.75-0.97)	0.01

[a]Models were adjusted for sex, maternal age, birth year, birth order, twin status, gestational age, and birth weight percentile
[b]Models were adjusted for sex, paternal age, birth year, birth order, twin status, gestational age, and birth weight percentile

Appendix 18. Relationships between ASD and the prenatal and perinatal complications with and without adjustments for the covariates using the ASD families with at least one ASD-affected individual having an ASD claim made by a selected specialist (i.e. geneticist, neurologist, paediatrician, or psychiatrist)

Prenatal and perinatal complications	Estimates without adjustment		Estimates with adjustment[a]		Estimates with adjustment[b]	
ICD-9-CM code: Medical condition[169]	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Prenatal complications						
640: Haemorrhage in early pregnancy	1.07 (0.83-1.36)	0.62	1.06 (0.80-1.40)	0.70	1.04 (0.72-1.51)	0.84
641: Antepartum haemorrhage, abruptio placentae, placenta previa	0.93 (0.72-1.21)	0.60	1.00 (0.74-1.33)	0.97	1.10 (0.74-1.63)	0.63
642: Hypertension	0.96 (0.79-1.17)	0.67	0.89 (0.71-1.11)	0.29	0.89 (0.68-1.17)	0.40
643: Excessive vomiting	0.78 (0.54-1.11)	0.16	0.75 (0.50-1.12)	0.16	0.61 (0.32-1.15)	0.13
644: Early or threatened labour	0.89 (0.78-1.03)	0.11	0.89 (0.76-1.05)	0.16	0.92 (0.74-1.15)	0.47
647: Infectious and parasitic conditions in mother	0.86 (0.60-1.23)	0.41	0.77 (0.52-1.15)	0.21	1.09 (0.64-1.86)	0.75
652: Malposition and malpresentation of foetus	1.12 (0.94-1.34)	0.19	1.21 (0.98-1.48)	0.07	1.05 (0.82-1.35)	0.70
653: Cephalopelvic disproportion	1.35 (1.01-1.80)	0.04	1.24 (0.90-1.72)	0.19	1.21 (0.82-1.79)	0.34
654: Abnormality of organs and soft tissues of pelvis	0.97 (0.79-1.17)	0.72	0.95 (0.76-1.19)	0.65	0.94 (0.71-1.25)	0.68
655: Known or suspected foetal abnormality affecting mother	1.12 (0.93-1.34)	0.23	0.95 (0.77-1.16)	0.59	1.05 (0.79-1.38)	0.76
657: Polyhydramnios	1.02 (0.65-1.61)	0.93	0.90 (0.55-1.48)	0.67	0.89 (0.49-1.64)	0.72
658: Other problems associated with amniotic cavity and membranes	1.20 (1.04-1.39)	0.02	1.12 (0.95-1.32)	0.19	1.21 (0.97-1.49)	0.09
660: Obstructed labour	1.30 (1.05-1.60)	0.01	1.20 (0.95-1.52)	0.12	1.04 (0.77-1.41)	0.81
661: Abnormality of forces of labour	0.98 (0.83-1.17)	0.83	1.00 (0.83-1.22)	0.97	1.01 (0.78-1.29)	0.96

662: Long labour	1.11 (0.93-1.32)	0.26	1.00 (0.82-1.22)	0.99	0.93 (0.71-1.20)	0.56
663: Umbilical cord complications	0.96 (0.79-1.18)	0.73	1.01 (0.80-1.26)	0.96	1.29 (0.96-1.73)	0.09
Perinatal complications						
762: Foetus/newborn affected by complications of placenta, cord, membranes	1.34 (0.87-2.07)	0.18	1.56 (0.96-2.53)	0.07	2.75 (1.28-5.94)	0.01
767: Birth trauma	1.00 (0.66-1.54)	0.99	0.99 (0.62-1.58)	0.96	0.96 (0.53-1.75)	0.89
768: Intrauterine hypoxia and birth asphyxia	1.00 (0.76-1.32)	0.995	0.94 (0.69-1.28)	0.69	0.86 (0.59-1.25)	0.42
769: Respiratory distress syndrome	1.49 (1.03-2.15)	0.04	1.43 (0.88-2.32)	0.15	1.57 (0.81-3.06)	0.18
770: Other respiratory conditions	1.29 (1.07-1.55)	0.007	1.12 (0.90-1.40)	0.32	1.11 (0.84-1.48)	0.46
771: Perinatal infections	1.37 (1.05-1.80)	0.02	1.28 (0.95-1.74)	0.11	1.07 (0.72-1.60)	0.73
772: Foetal and neonatal haemorrhage	1.65 (1.14-2.41)	0.009	1.25 (0.81-1.94)	0.31	0.91 (0.52-1.59)	0.73
774: Perinatal jaundice	1.06 (0.90-1.24)	0.49	0.91 (0.75-1.10)	0.31	0.86 (0.67-1.10)	0.24
775: Endocrine, metabolic disturbances	1.12 (0.86-1.47)	0.39	1.03 (0.76-1.39)	0.87	1.05 (0.69-1.59)	0.82
776: Haematological disorders	2.27 (1.37-3.76)	0.002	3.23 (1.73-6.04)	0.0002	3.47 (1.43-8.45)	0.006
778: Conditions involving integument and temperature regulation	1.19 (0.80-1.78)	0.38	1.00 (0.65-1.55)	0.99	0.94 (0.54-1.66)	0.84
1-minute Apgar score	0.93 (0.89-0.97)	0.0003	0.94 (0.90-0.99)	0.01	0.93 (0.88-0.99)	0.02
5-minute Apgar score	0.84 (0.78-0.91)	<0.0001	0.86 (0.79-0.94)	0.001	0.86 (0.76-0.96)	0.01

[a]Models were adjusted for sex, maternal age, birth year, birth order, twin status, gestational age, and birth weight percentile
[b]Models were adjusted for sex, paternal age, birth year, birth order, twin status, gestational age, and birth weight percentile

Appendix 19. Relationships between ASD and the prenatal and perinatal complications with and without adjustments for the covariates using the ASD families with at least one ASD-affected individual having ≥2 ASD claims made by a selected specialist (i.e. geneticist, neurologist, paediatrician, psychiatrist)

Prenatal and perinatal complications	Estimates without adjustment		Estimates with adjustment[a]		Estimates with adjustment[b]	
ICD-9-CM code: Medical condition[169]	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Prenatal complications						
640: Haemorrhage in early pregnancy	1.20 (0.85-1.69)	0.30	1.19 (0.80-1.75)	0.39	1.03 (0.62-1.73)	0.90
641: Antepartum haemorrhage, abruptio placentae, placenta previa	0.94 (0.67-1.32)	0.73	0.94 (0.64-1.36)	0.73	1.05 (0.65-1.70)	0.83
642: Hypertension	1.02 (0.79-1.33)	0.86	0.98 (0.73-1.32)	0.90	1.00 (0.70-1.43)	0.99
643: Excessive vomiting	0.59 (0.36-0.98)	0.04	0.62 (0.35-1.10)	0.10	0.54 (0.22-1.33)	0.18
644: Early or threatened labour	0.97 (0.81-1.16)	0.71	0.91 (0.73-1.13)	0.37	0.84 (0.64-1.11)	0.22
647: Infectious and parasitic conditions in mother	0.88 (0.54-1.43)	0.61	0.84 (0.49-1.44)	0.52	0.96 (0.47-2.00)	0.92
652: Malposition and malpresentation of foetus	1.26 (1.01-1.58)	0.04	1.29 (0.99-1.67)	0.06	1.19 (0.86-1.64)	0.29
653: Cephalopelvic disproportion	1.62 (1.13-2.34)	0.009	1.56 (1.04-2.34)	0.03	1.80 (1.11-2.92)	0.02
654: Abnormality of organs and soft tissues of pelvis	1.05 (0.82-1.36)	0.68	0.99 (0.73-1.32)	0.92	0.95 (0.67-1.36)	0.78
655: Known or suspected foetal abnormality affecting mother	1.03 (0.81-1.31)	0.80	0.88 (0.66-1.16)	0.36	0.93 (0.65-1.35)	0.71
657: Polyhydramnios	1.13 (0.66-1.94)	0.67	0.97 (0.54-1.76)	0.92	1.01 (0.49-2.09)	0.97
658: Other problems associated with amniotic cavity and membranes	1.14 (0.94-1.38)	0.18	1.01 (0.81-1.25)	0.96	1.05 (0.80-1.39)	0.72
660: Obstructed labour	1.54 (1.16-2.04)	0.003	1.58 (1.14-2.17)	0.006	1.57 (1.03-2.38)	0.04
661: Abnormality of forces of labour	0.92 (0.73-1.16)	0.46	0.95 (0.74-1.23)	0.72	1.02 (0.73-1.42)	0.91

662: Long labour	1.12 (0.88-1.41)	0.36	1.04 (0.80-1.35)	0.76	1.14 (0.81-1.60)	0.45
663: Umbilical cord complications	0.99 (0.74-1.31)	0.93	1.05 (0.76-1.44)	0.77	1.24 (0.83-1.86)	0.30
Perinatal complications						
762: Foetus/newborn affected by complications of placenta, cord, membranes	1.58 (0.86-2.90)	0.14	1.68 (0.85-3.31)	0.14	3.65 (1.15-11.5)	0.03
767: Birth trauma	0.64 (0.36-1.14)	0.13	0.70 (0.37-1.31)	0.26	0.69 (0.31-1.54)	0.36
768: Intrauterine hypoxia and birth asphyxia	1.01 (0.71-1.45)	0.96	0.96 (0.64-1.43)	0.84	0.90 (0.55-1.47)	0.67
769: Respiratory distress syndrome	2.74 (1.59-4.71)	0.0003	2.19 (1.09-4.37)	0.03	3.89 (1.32-11.5)	0.01
770: Other respiratory conditions	1.55 (1.22-1.96)	0.0003	1.29 (0.97-1.71)	0.08	1.27 (0.88-1.83)	0.20
771: Perinatal infections	1.29 (0.91-1.84)	0.16	1.18 (0.78-1.77)	0.44	0.84 (0.49-1.43)	0.53
772: Foetal and neonatal haemorrhage	1.83 (1.14-2.94)	0.01	0.99 (0.57-1.72)	0.97	0.84 (0.43-1.66)	0.62
774: Perinatal jaundice	1.13 (0.92-1.39)	0.24	0.93 (0.72-1.19)	0.54	0.85 (0.62-1.17)	0.32
775: Endocrine, metabolic disturbances	1.65 (1.15-2.36)	0.006	1.28 (0.85-1.94)	0.24	1.16 (0.67-2.03)	0.59
776: Haematological disorders	3.98 (1.90-8.32)	0.0002	3.71 (1.56-8.83)	0.003	5.14 (1.50-17.6)	0.009
778: Conditions involving integument and temperature regulation	1.53 (0.91-2.56)	0.11	1.25 (0.72-2.18)	0.43	1.57 (0.77-3.22)	0.22
1-minute Apgar score	0.90 (0.85-0.95)	<0.0001	0.91 (0.86-0.96)	0.001	0.92 (0.85-0.99)	0.02
5-minute Apgar score	0.75 (0.67-0.83)	<0.0001	0.76 (0.67-0.87)	<0.0001	0.79 (0.67-0.92)	0.002

[a]Models were adjusted for sex, maternal age, birth year, birth order, twin status, gestational age, and birth weight percentile
[b]Models were adjusted for sex, paternal age, birth year, birth order, twin status, gestational age, and birth weight percentile