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PERSPECTIVE

The Autism Birth Cohort: a paradigm for gene–environment–timing research

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The reported prevalence of autism spectrum disorders (ASDs) has increased by 5- to 10-fold over the past 20 years. Whether ASDs are truly more frequent is controversial; nonetheless, the burden is profound in human and economic terms. Although autism is among the most heritable of mental disorders, its pathogenesis remains obscure. Environmental factors are proposed; however, none is implicated. Furthermore, there are no biomarkers to screen for ASD or risk of ASD. The Autism Birth Cohort (ABC) was initiated to analyze *gene* × *environment* × *timing* interactions and enable early diagnosis. It uses a large, unselected birth cohort in which cases are prospectively ascertained through population screening. Samples collected serially through pregnancy and childhood include parental blood, maternal urine, cord blood, milk teeth and rectal swabs. More than 107 000 children are continuously screened through questionnaires, referral, and a national registry. Cases are compared with a control group from the same cohort in a 'nested case–control' design. Early screening and diagnostic assessments and re-assessments are designed to provide a rich view of longitudinal trajectory. Genetic, proteomic, immunologic, metagenomic and microbiological tools will be used to exploit unique biological samples. The ABC is a paradigm for analyzing the role of genetic and environmental factors in complex disorders.
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Introduction

For millennia, philosophers have debated the role of nature (genetics) and nurture (environment) in health and disease. This debate has intensified with advances in human genomics. Indeed, recent emphasis on metagenomics, epigenetics and systems biology reflects increasing appreciation for an integrated approach to medicine and biology. This is particularly true in developmental neuroscience in which an awareness of the vulnerability of the fetus and the child to environmental factors already influences public health investments, ranging from folate supplementation during pregnancy to preschool environmental enrichment programs. Studies linking drugs such as thalidomide and divalproate to fetal defects, and animal models in which gestational exposure to infectious agents results in anatomical and behavioral deficits, clearly show the importance of analyzing gene–environment interactions within their temporal context.[1–3] An opportunity to do this at the population level has arisen with the advent of large prospective pregnancy and birth cohorts.[4]

Autism is a neurodevelopmental disorder, defined by the presence of: (1) deficits in social interactions, (2) deficits in communication and (3) restricted, repetitive and stereotyped patterns of behavior, interests and activities.[5] The observation that these features may vary in severity and time of onset led to the concept of 'autism spectrum disorders' (ASDs), comprising primarily autistic disorder (childhood autism), Asperger's disorder and pervasive developmental disorder-not otherwise specified.

The reported prevalence of ASDs in continental Europe, the United Kingdom and the United States has increased by 5- to 10-fold over the past 20 years.[6] The extent to which the increase in reported prevalence represents a *bona fide* increase or is due

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to enhanced and earlier case detection and/or modification of diagnostic criteria is not known. Nonetheless, there is consensus that the burden in economic as well as social and individual terms is profound. In the United States alone, given a birth rate of 4 million and a prevalence of 1/150, almost 27 000 children at risk for ASD are born annually.[7] Autism is among the most heritable of mental disorders, but the genetic basis and pathogenesis of most cases remain obscure.[8] Environmental factors are proposed; however, none has been established as causal.[9] Furthermore, although these disorders may begin in prenatal life, there are no known biomarkers at birth that can be used for diagnosis.

The Autism Birth Cohort

The Autism Birth Cohort (ABC) was established to address the natural history of ASD, explore genetic and pre- or perinatal environmental factors in causation, as well as the interplay between genes and environment, and to facilitate discovery of biomarkers with potential to enable early recognition and treatment. Although not restricted to the following candidate environmental factors, the ABC was designed to focus on prenatal or postnatal infection, obstetric risk factors and dietary and/or environmental exposure to potential toxins during pregnancy and postnatal life. ABC resources include a serial collection of detailed questionnaires and biological samples for genetic, transcriptomic, proteomic, microbiologic and toxicologic analyses.

The Norwegian Mother and Child Cohort

The ASD cases in the ABC study are identified from Norwegian Mother and Child Cohort (MoBa) participants. The MoBa is a nationwide population-based pregnancy cohort initiated in 1999.[10,11] At termination of recruitment in December 2008, 90 700 mothers, 72 100 fathers and 108 500 children were enrolled. The last child to be included was born in 2009 (Supplementary Figure 1).

Information is obtained from questionnaires, biological materials, sub-studies and linkage to registries. Mothers complete questionnaires during pregnancy and at intervals after birth (Supplementary Table 1). Fathers complete one questionnaire during pregnancy. The questionnaires query about health, dietary intake, socio-economic status, child development and behavior, and psychosocial and emotional status of the mother, father and child. Blood samples are obtained from both parents during pregnancy and from mothers at birth. A urine sample is also taken from the mother during pregnancy. From the child, a blood sample is taken from the umbilical cord directly after birth. Plasma, RNA and DNA are collected from blood. Pilot analyses of aliquots of blood samples retrieved from the MoBa biobank using oligonucleotide microarrays, quantitative real-time PCR, Luminex technology (Austin, TX, USA) and matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry indicate the viability of these materials for genetic and expression profiling, proteomics, microbiology and toxicology.

The MoBa 18-month questionnaire includes the Early Screening of Autistic Traits and the Modified Checklist for Autism in Toddlers.[12,13] The 36-month questionnaire includes the Social Communication Questionnaire (SCQ) and selected Modified Checklist for Autism in Toddlers items.[14–16] Information on both cases and controls with respect to signs and symptoms of ASD provides the basis for describing the natural history of ASD and defining endophenotypes that may provide insights into the pathogenesis of ASD.

Identification of children with ASD and selection of controls

Potential ASD cases within the MoBa cohort are identified through four mechanisms (Figure 1): (1) screening at 36 months, (2) professional referrals by the healthcare system, (3) self-referrals from parents and (4) linkage with the Autism Database, which is coordinated by the Norwegian Institute of Public Health and funded by the Research Council of Norway. The Autism Database includes MoBa children diagnosed with ASD in the Norwegian healthcare system (hospitals and outpatient clinics). From 2008 onwards, data are collected through the nationwide Norwegian Patient Registry. To enhance capture of potential cases that elude identification by screening at the age of 3 years, new MoBa questionnaires have been designed for 5- and 7-year-old children that include specific questions about autism, autistic traits and Asperger's syndrome. We will also identify cases through referral and a national patient registry. ABC controls are selected randomly among MoBa participants, matched to potential cases by birth date (± 14 days).

Screening criteria

The ABC screening mechanism includes the SCQ in addition to other selected items.[14–16] All 40 SCQ items are included in the 36-month questionnaire, but only those 33 items that do not require language to be present (SCQ-33) are used in the ABC screening algorithm. The screening criteria are outlined in Table 1.

Clinical assessments

Screen-positive children are assessed clinically at 36–42 months of age to collect detailed neurobehavioral and developmental information and to generate a diagnosis of ASD or associated disorders, using standardized and validated diagnostic instruments (Table 2). Core diagnostic instruments are the Autism Diagnostic Interview–Revised and the Autism Diagnostic Observation Schedule.[17,18] The ASD subgroups

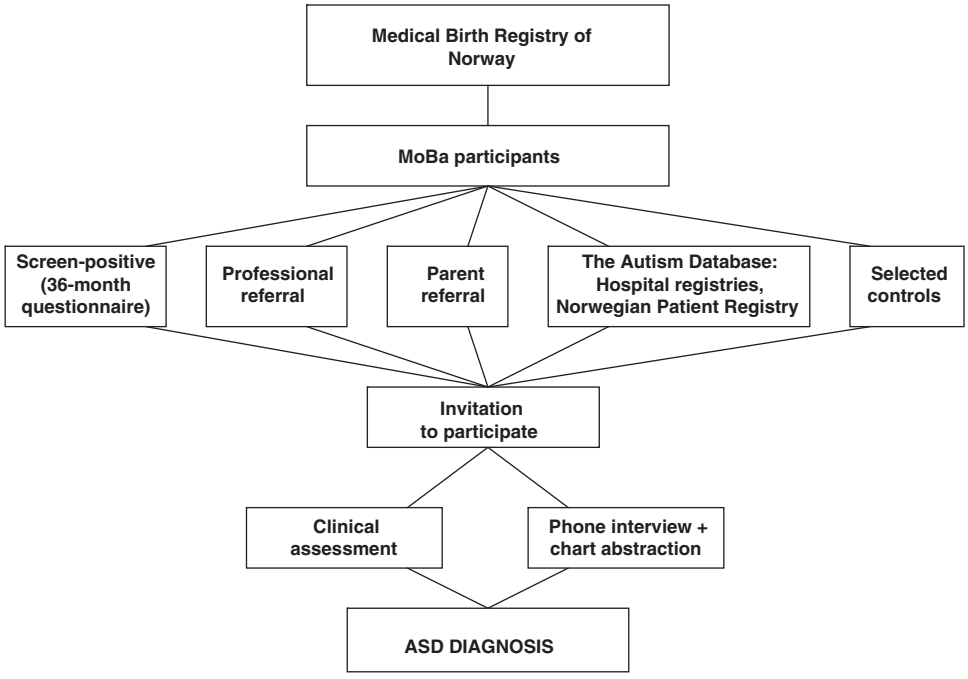

Figure 1 Strategy for identifying children with autism spectrum disorder (ASD) in the Autism Birth Cohort (ABC). The ABC is nested within the Norwegian Mother and Child Cohort Study (MoBa).

Table 1 The ABC study: screening criteria at age 36 months

Screening criteria based on the SCQ and other selected items in the MoBa questionnaire at 36 months[a]	
1	SCQ-33 score $\geqslant 12$
2	Repetitive behavior sub-domain score on SCQ-33 = 9
3	Parent reports language delay and child has been referred to a specialist for it
4	Parent reports autism/autistic trait and/or reports that child has been referred to a specialist for it
5	Parent reports worry that child shows little interest in playing with other children
6	Parent reports that others (family, day-care staff, well-baby nurse) have expressed concern for the child's development
Children are defined as screen-positive when:	
	Meeting one or more of criteria 1, 2, 3 or 5 and meeting criterion 6
	Meeting criterion 4

Abbreviations: ABC, Autism Birth Cohort; ASD, autism spectrum disorder; MoBa[10], The Norwegian Mother and Child Cohort Study; SCQ, Social Communication Questionnaire.
Note that all 40 SCQ items are included in the 36-month questionnaire, but only those 33 items that do not require language to be present (SCQ-33) are scored.
[a]Screening was implemented for MoBa children born on or after 1 February 2002; thus, the oldest 6500 MoBa participants were not screened for ASD.

included are (with or without concurrent mental retardation): Autistic Disorder, (DSM-IV 299.00), Asperger's Disorder (DSM-IV 299.80) and pervasive developmental disorder-not otherwise specified (DSM-IV 299.80).

Expected number of cases

On the basis of an evaluation of recent reports, we assume a prevalence of ASD of 6 per 1000 in the MoBa cohort; thus, the ABC has the potential to identify approximately 600 ASD cases.[19–22] All screen-positive potential cases are invited to participate in clinical assessments. We anticipate assessing a minimum of 2000 children. Blood and stool samples are taken at the time of assessment to enable molecular, serological and microbiological analyses.

Follow-up

MoBa records provide information from early pregnancy onwards, through questionnaires and registry linkages. The MoBa database is linked to the Medical Birth Registry of Norway (www.fhi.no) and other national health registries. Linkage can also be established

Table 2 Clinical assessments in the ABC study

Clinical exam components
ADOS (video-taped)
Psychometric testing (video-taped): Stanford-Binet Intelligence Scales 5th edn[a], Mullen Scales of Early Learning[b]
Physical examination (video-taped)
Anthropometric measurements, photo
Maternal/parental interview
ADI-R (video/audio-taped)
PAPA (video/audio-taped)[c]
Vineland Adaptive Behavior Scales[d] (video/audio-taped)
Child and family psychiatric and medical history
ABC-specific biological samples
At the clinical assessment, a new blood sample (plasma, full blood, DNA, RNA) is collected from the child
If blood from mother and/or father is lacking in the MoBa biobank, blood is also collected from the parent(s)

Abbreviations: ABC, Autism Birth Cohort; ADI-R[17], Autism Diagnostic Interview–Revised; ADOS[18], Autism Diagnostic Observation Schedule; MoBa, Norwegian Mother and Child Cohort Study; PAPA, Preschool Age Psychiatric Assessment.
[a]Stanford-Binet[24]: From 2005 through 2008, full version. From 2009 onward, shortened version, 5 out of 10 subscales.
[b]Mullen[25]: From 2005 through 2008, fine motor and gross motor subscales for all. Full version of Mullen if child too low-functioning for SB5. From 2009 onward, selected items only from gross motor subscale, otherwise unchanged.
[c]PAPA[26] Used on 500 children, 2005 through 2008. Omitted from 2009 onwards.
[d]Vineland[27]: From 2005 through 2008, full version. From 2009 onward, communication sub-domain only.

with socioeconomic and demographic data from Statistics Norway (www.ssb.no).

Several other MoBa sub-studies intersect with the ABC. At present, these include sub-studies of attention deficit/hyperactivity disorder, language delay, preterm birth, *de novo* mutations and epigenetic events, and one-carbon metabolism and related single-nucleotide polymorphisms. All data emerging from MoBa sub-studies are collected into the central MoBa database to enable recognition of common themes and outcomes.

Attrition and characteristics of participants

Subject attrition is a substantial concern in longitudinal studies. The participation rate of invited mothers in MoBa is approximately 40%. Fathers are invited only if the mother participates. Father participation rate is approximately 83%. Among participants, response rates are approximately 95% for the early questionnaires, and then they decline (Supplementary Table 2). The 36-month questionnaire, which is the basis for the autism screening, has a 61% response rate. Approximately 50% of potential cases and controls invited to the ABC clinical assessments accept the invitation.

Comparisons of the MoBa cohort to the general Norwegian population indicate that the participants on average have a higher socio-economic status, with a higher proportion of parents having completed higher education, a lower proportion of single mothers, and a lower proportion of smoking mothers when compared with the population at large.[23] Similar selection biases are found when responders to the 36-month questionnaire are compared with non-responders.

Advantages of MoBa and the ABC

The MoBa includes >100 000 children and their parents, and is the only comprehensive population-based prospective cohort with the data required to analyze gene–environment–timing interactions and follow the trajectories of neurodevelopmental disorders such as ASD. Information and samples are collected from all children and both of their parents before, and independent of, diagnosis and severity of disease. Biological samples are optimized for genetic, transcriptomic, proteomic, microbiological and toxicological analyses. Thus, a wide range of exposures and outcomes can be studied in the cohort as a whole and in each participant. Sibships and twins represent an added value to studies of the contributions of genes and gene–environment interactions in disease development. Linkage of the cohort to nationwide health registries enables extensive longitudinal follow-up of the cohort at low costs.

As with any longitudinal population-based cohort, challenges include retaining participants, and the continuous investment required to establish and maintain the program until results can be achieved. However, the ABC has advantages that enhance the probability of success. Emigration is less common in Norway than in some other industrialized nations. Socialized medicine and national registries facilitate follow-up and case capture. Last, there is national recognition of the cohort as an important contribution to science and public health.

We view the ABC as an international scientific resource. We welcome input into models and platforms that can be used to extract information from data and sample sets. Please post comments and inquiries to Columbia University (e-mail:

abc@columbia.edu) or the Norwegian Institute of Public Health (e-mail: abc.coordinator@fhi.no).

Conflict of interest

The authors declare no conflict of interest.

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