

A THREE-GENERATION APPROACH IN BIODEMOGRAPHY IS BASED ON THE DEVELOPMENTAL PROFILES AND THE EPIGENETICS OF FEMALE GAMETES

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1. ABSTRACT

We suggest that there are three premises underlying the need for biodemographic analyses of three-generations: 1.) To describe the structure of the genome, we need to use (apart from mutations) other kinds of heritable changes such as those mediated by facultative elements (variations) and epigenetic alterations. 2.) There are many reasons to analyze individual development and its deviations, such as the biodemographic perspective of fertilization - but also including all long-term intra-generational events of oogenesis and meiosis (beginning with the embryogenesis of the individual's mother - or during the grandmother's pregnancy). 3.) We need to explore the reality that every fertilized egg links - physically and genetically - three successive generations. We focus on genetic and epigenetic events, which start during egg cell lineage determination in $F(n-2)$ gestation and which influence the developmental profile of $F(n)$ generation cohorts.

The three-generation approach in epidemiology and biodemography is important so that we might increase our understanding of the effects of environmental forces, such as viral epidemics, and of catastrophes, such as the Chernobyl accident. It is also important for evaluating the processes of senescence and the determinants of human disease.

2. INTRODUCTION

The question of when human development begins determines how we analyze the genotype/phenotype variability and health of cohorts over generations. The response is neither subjective nor academic.

Unlike males, whose spermatogonia are constantly dividing, females at birth are believed to have all the oocytes they will ever produce. From this perspective, development begins before fertilization. Modern reproductive biology describes a human oocyte using three perspectives: (i) a $2n$ -gamete arrested at the Meiosis II stage, (ii) a zygote after fertilization, and (iii) an egg cell controlling early development.

It necessarily follows that the determination of human development starts before fertilization. Development encompasses all gametogenesis and meiosis processes starting during the grandmother's fetal period and continuing during the mother's gestation.

We briefly describe two oogenesis-associated epigenetic processes: erasure of parental imprints (and subsequent epigenetic maternalization) and their hereditary implications. In humans, three oocyte-dependent reproduction processes (meiosis and gametogenesis, fertilization and early development) are error prone (1, 2). Thus, a study of the genetic determinants of aging must examine genetic variation over all three generations.

Environmental factors acting during $F(n-2)$ gestation on the oocyte meiosis machinery will be manifest in both the perinatal and postnatal period of $F(n)$ generations. Modern social processes might delay the age of pregnancy and therefore cause mutations in the gamete to increase. Alternately, the nutritional and environmental exposure states may affect the level of oxidation and other factors affecting DNA, which could alter the regulatory molecules involved in meiosis. There are diverse approaches how best to determine from $F(n-2)$ to $F(n)$ cohorts: a.) the genetic/demographic effect of viral epidemics resulting in outbreaks of insertional mutagenesis and b.) the trans-generational effects of chronic micro or macro nutritional deficiency or of diverse environmental catastrophes such as the Chernobyl radiation exposure or the 1984 chemical spill in Bhopal, India.

3. OOGENESIS LINKING THREE GENERATIONS

Primordial germ cells (PGC) appear in the 2-3 month old embryo; then they migrate into the gonadal ridge. Each PGC becomes an oogonium and enters into meiosis I, giving rise to the primary oocyte. At birth meiosis I is arrested for females in the diplotene stage and is dormant until puberty. A few hours before ovulation, the first meiotic block is removed. The oocyte, blocked on meiosis II metaphase, completes meiosis only after sperm

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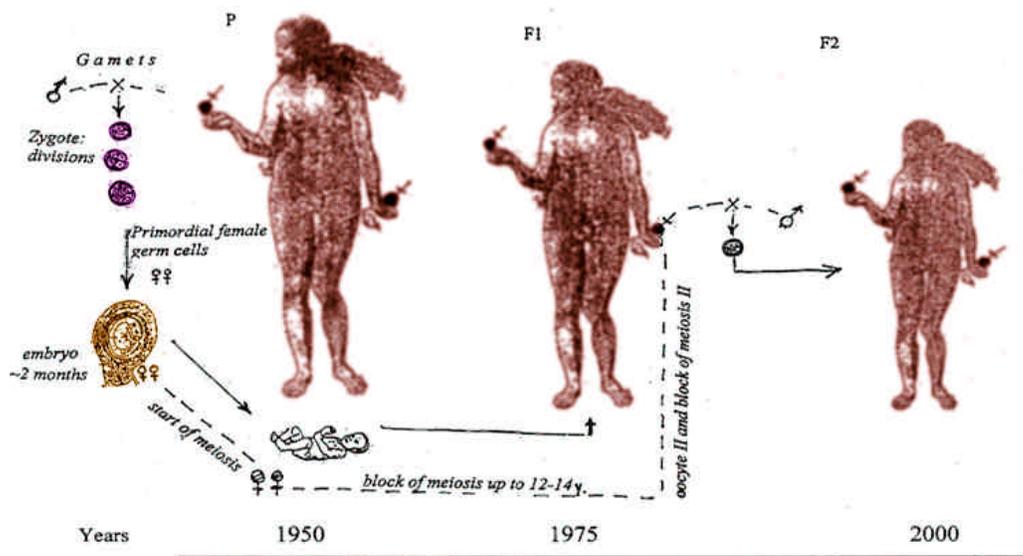


Figure 1. Female gametogenesis and oogenesis in the three-generation concept. The scheme shows how female oogenesis links each three successive female generations. The female cohorts are conditionally placed at three successive time periods: 1950, 1975 and 2000. Every individual from the F_n generation occurs from one F_{n-1} mother egg, which originated as a primary oocyte during first two months of the F_{n-2} grandmother, pregnancy. Thus the gestation conditions of the grandmother cohorts' influence on epigenetic egg reprogramming and MI recombination/repair processes might be intragenerational. Their impact is manifested on the pattern of gene expression and phenotype of the grandchild cohorts with evident biodemographic implications.

penetration. Every individual from a $F(n)$ generation develops from the mother's egg ($F(n-1)$ generation), which originated as a primary oocyte during the grandmother's pregnancy or $F(n-2)$ generation (see Figure 1). Every egg, physically and genetically, thus links three female generations. This is the cornerstone for more comprehensive biodemographic studies of fertility processes, the evolution of the genetic determinants of senescent process (e.g., longevity limits), and their disruption by environmental processes.

We focus on three events, which occur during the germ cell lineage PGC-oogonium-oocyte in $F(n-2)$ gestation and which have developmental and health implications for the next two generations: *i.*) start of meiosis *ii.*) epigenetic reprogramming and *iii.*) sensitivity of PGC/oocytes to cell stress stimulating activation of mobile elements (ME) and insertional mutagenesis.

Female and male gametogenesis and meiosis differ in their dynamics and control mechanisms. Male meiosis is a continuous process. It begins at puberty with spermatocytes progressing from prophase I through meiosis II in a week. Female meiosis includes two developmental blocks and lasts decades. Before a meiotic arrest, at least three key events will have occurred: DNA replication, synapses of homologous chromosomes, and meiotic recombination.

These events also influence chromosome disjunction and segregation. Between 10 and 25 percent of fetuses have aneuploidy (trisomy and monosomy are the most common forms of aneuploidy). Between 80 and 90 percent of aneuploids result from chromosome non-

dysjunction at maternal metaphase I (MI) (3). Female meiotic check points acting during pachytene and spindle assembly are missing or weakened, and mutations accumulate in genes whose products control double strand breaks, initiating meiotic recombination. Aneuploidy may also occur due to premature separation of the sister chromatid, which happens more frequently in the oocytes of older women (4). Environmental factors acting during $F(n-2)$ gestation on the oocyte meiosis machinery will be manifest in both the perinatal and postnatal period of $F(n)$ generations. As social processes delay the age of pregnancy (and therefore cause additional mutations), intra-generational demographic studies of problems of advanced age of the mother at birth are needed.

4. EPIGENETICS OF OOGENESIS

In addition to the transition of structural information via DNA/chromosome replication, the mammalian hereditary system encompasses dynamic or epigenetic information (e.g., gene/chromosome parental imprinting and X-inactivation). Epigenetic changes are connected with reversible modifications of DNA (methylation of CpG repeats in promoters or in differentially methylated regions). They also include feedback regulation of gene/chromatin states via DNA binding proteins or ribonucleic acid (RNA) intermediates. Maternal PGCs contain both parental genomic imprints. Transition from PGC to oocyte I is accompanied by two genome reprogramming events: Erasure of parental imprints and subsequent epigenetic maternalization (5-7). Erasure starts after PGC entry into the genital ridge and consists of rapid, genome-wide demethylation (including reactivation of the female X chromosome). Because this is where telomeres

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are restored to their “optimal” size to overcome senescence, erasure is crucial to human aging and longevity.

Understanding the biochemical and cellular processes by which telomere extension occurs is important for analyzing the processes of human senescence and of carcinogenesis. Epigenetic maternalization (or initiation of new imprints) continues in the $F(n-1)$ generation during maturation of growing oocytes. Maternally inherited oocyte proteins (DNMT1, HPI, EED, YY1) are accumulated and used to demethylate and activate the paternal genome after fertilization (5). A deficit of oocyte proteins prevents normal development. A conceptus that has all normal genes, but lacks maternal imprints, develops as if it had lost the maternal genome (8, 9). Discordance of monozygotic (MZ) twins on the X-inactivation-pattern or on the manifestation of imprinting-dependent diseases indicates that the establishment and maintenance of epigenetic states is a flexible and vulnerable process (10). If diverse environmental factors (such as pollution, nutrition, and lifestyle) influence the epigenetic dynamics of the oocyte in $F(n-2)$ and $F(n-1)$, they can cause genotype/phenotype changes in the $F(n)$ cohorts.

Given that methylation patterns are erased early in embryogenesis and reestablished soon after implantation, it was suggested that the “waves” of methylation that initially establish its tissue-specific patterns does not stop at birth but actually evolves throughout life, albeit at a very slow rate (11). Several lines of evidence indicate that: i.) hypermethylation is initially a property of aging cells in normal tissues; ii.) normal individuals have substantial acquired variability in epigenetic silencing; and iii.) this age-related variability is likely to be the precursor for an aberrant methylation seen in cancer tissues (12). Studies of colon cancer show that aging in the human epithelium is accompanied by progressively increasing epigenetic mosaicism in colonic stem cells leading to the considerable crypt-to-crypt variability in gene expression patterns or epigenetic mosaicism. It is reasonable to assume that “age-related methylation is a fundamental predisposing event” to spontaneous age-related neoplastic transformation and that this methylation has potential implications for the pathogenesis of several age-related diseases (e.g., atherosclerotic vascular disease [11, 12]). We may speculate that due to variations of environmental exposure, nutrition, or lifestyle (e.g., behavioral risk factors such as smoking or alcohol consumption), the long-term trans-generational process of oocyte maturation will inevitably be accompanied by an increased level of epigenetic variability.

5. ACTIVATION OF MOBILE ELEMENTS AND VIRUSES IN EARLY EMBRYOGENESIS

Human (and other) species are under constant stress from viral pandemics and foreign DNA invasion. Viral DNA and RNA are mutagenic factors activating transpositions of mobile elements (MEs) (13). MEs constitute about 50% of human genome families (such as Alu, SINE, LINE). Retroviruses containing long terminal repeats (LTR) are now recognized as an ME that is capable

of being inserted into diverse sites of host chromosomes. LTR retroelements, including defective endogenous retroviruses, make up 8% of human genome (13,14). Some of these families entered the primate and human germ as infectious retroviruses and then amplified via retrotransposition. Experimental genetic studies of insertional mutagenesis show that the first cleavage divisions and germ cell lineage are sensitive targets for ME activation and transposition. As a result, clusters of similar mutations may appear in successive generations (15, 16). Viruses are also known to trigger the host DNA methylation processes in cellular infections. Such epigenetic alterations can sometimes spread to loci adjacent to the sites of viral/mobile elements integration (17).

6. MAJOR BIODEMOGRAPHIC IMPLICATIONS

Biodemographic implications of observed epigenetic variability are clear. For example, an evaluation of the pattern of methylation of selected loci in normal epithelium across age cohorts is needed to stratify human populations and to correlate this pattern with aging and age related diseases. Drug treatments and a preventative approach would vary in efficacy, depending on the underlying epigenetic state of the target loci (11, 12).

Therefore, we need to estimate the genetic/demographic effect of epidemics on $F(n-2)$ to $F(n)$ cohorts. For example, a comparison of the pandemic effect of the 1918 global influenza epidemics on the genotype/phenotype pattern of individuals from the $F(n)$ cohort that originated from grandmothers who were pregnant in 1918 with the similar cohorts that originated from grandmothers who were pregnant before (1917) and after (1919) needs to be analyzed. Such genetic/demographic analyses are also needed to understand environmental catastrophes such as the Chernobyl reactor accident, the release of ionizing radiation from the Mayak production facility in the South Ural area, or the chemical spill in Bhopal, India.

This will likely lead to novel and more powerful data collection designs for the study of innate and acquired genetic mutations in both healthy and diseased organisms. For example, in the Mayak production facility catchment area, data was collected on three generations (30,000 offspring) during 53 years of follow-up - starting with 30,000 individuals who were exposed to high levels of ionizing radiation (beginning in 1950). Such a multi-generation cohort study is necessary to identify genetic determinants of a complex phenotype such as senescence.

Our conclusion about the necessity of introducing the three-generation approach to analyzing genetic factors in biodemography is based on two premises: 1.) In classical embryological and modern cell biology/molecular data, oogenesis is a fundamental process for embryogenesis (18); and 2.) Data on genome organization and variability differentiate among three kinds of heritable changes: mutations, variations and epigenetic alterations (19). The latter two sources of genetic variability are not a feature of classical genetic models and require a dynamic, stochastic formulation of the genetic transmission of traits (19).

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7. ACKNOWLEDGEMENTS

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