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Transgenerational genetic effects of the paternal Y chromosome on daughters' phenotypes

Vicki R Nelson¹, Sabrina H Spezio¹, and Joseph H Nadeau[†]

¹Case Western Reserve University, School of Medicine, Cleveland, OH, USA

Abstract

Aims—Recent evidence suggests that transgenerational genetic effects contribute to phenotypic variation in complex traits. To test for the general occurrence of these effects and to estimate their strength, we took advantage of chromosome substitution strains (CSSs) of mice where the Y chromosome of the host strain has been replaced with the Y chromosome of the donor strain. Daughters of these CSS-Y males and host strain females are genetically identical and should be phenotypically indistinguishable in the absence of transgenerational genetic effects of the fathers' Y chromosome on daughters' phenotypes.

Materials & methods—Assay results for a broad panel of physiological traits and behaviors were compared for genetically identical daughters of CSS-Y males and host strain females from the B6-Chr^{A/J} and B6-Chr^{PWD} panels of CSSs. In addition, behavioral traits including specific tests for anxiety-related behaviors were tested in daughters of B6-Chr¹²⁹ and 129-Chr^{B6} CSS-Y males.

Results—Across a panel of 41 multigenic traits assayed in the B6-Chr^{A/J} panel of CSSs females and 21 multigenic traits in the B6-Chr^{PWD} panel females, the frequency and strength for transgenerational genetic effects were remarkably similar to those for conventional inheritance of substituted chromosomes. In addition, we found strong evidence that the Y chromosome from the 129 inbred strain significantly reduced anxiety levels among daughters of B6-Chr¹²⁹ CSS-Y males.

Conclusion—We found that transgenerational genetic effects rival conventional genetic effects in frequency and strength, we suggest that some phenotypic variation found in conventional studies of complex traits are attributable in part to the action of genetic variants in previous generations, and we propose that transgenerational genetic effects contribute to 'missing heritability'.

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[†]Author for correspondence: Department of Genetics, BRB731, CWRU, School of Medicine, 10900, Euclid Ave, Cleveland, OH 44106, USA, Tel.: +1 216 368 0581, Fax: +1 216 368 3832, jhn4@case.edu.

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Keywords

chromosome substitution strains; epigenetics; missing heritability; transgenerational effects; Y chromosome

In recent years, a greater understanding of phenotypic variation and human disease has been achieved through the characterization of the genetic architecture of complex traits and identification of complex trait genes (QTLs) [1,2]. However, despite unprecedented analytical and technological advances, genes underlying many common diseases have remained remarkably elusive. In most instances, the cumulative effects of known genetic variants account for an unexpectedly small portion of heritability [2–4]. As a result, many of the genetic determinants underlying phenotypic variation and disease risk have not yet been detected. Among the usual explanations for ‘missing heritability’ are overestimates of heritability, unexplored regions of the genome, untested classes of genetic variants, and the action of many rare genetic variants [3–5].

Inheritance of epigenetic changes could contribute to phenotypic variation in the absence of DNA sequence differences [6]. The molecular basis for these epigenetic effects could be DNA methylation [7–11], histone modification [12], or possibly small RNAs [13–15]. Many examples of transgenerational effects resulting from environmental exposures have been reported [7,8, 16–18]. Perhaps, what is more interesting from a genetic and evolutionary perspective are transgenerational genetic effects where variants in one generation affect phenotypes in subsequent generations without inheritance of the original genetic variant [6]. Under these conditions, traits still show heritability, but the association between genotype and phenotype in studied individuals is weakened or lost. Published mammalian examples that have overt phenotypic consequences include pigmentation defects [13], embryogenesis and adult growth [14], cardiac hypertrophy [15] and testicular cancer [19,20]. Background genetic variation, social influences and environmental factors greatly complicate the discovery of transgenerational genetic effects, especially in humans. With animal models, however, these complications can usually be precisely controlled, thereby enabling rigorous tests for genetic variants that have persistent phenotypic effects across generations.

Two major questions in studies of transgenerational genetic effects concern the frequency of affected traits and the strength of their phenotypic effects. In particular, are traits that show these unusual inheritance patterns common or rare, and are their phenotypic effects strong or weak, compared with QTLs that are inherited in the conventional manner?

Our test for the frequency and strength of transgenerational genetic effects is based on the observation that although the father’s Y chromosome is not transmitted to daughters, phenotypic effects might nevertheless be epigenetically inherited. To control genetic background so that transgenerational genetic effects can be attributed specifically to the Y chromosome, we used chromosome substitution strains (CSSs) [21,22]. A CSS is made by substituting a single chromosome from a donor strain on an inbred host strain background (Figure 1A). The resulting strain is identical to the original inbred host strain except for homozygosity (or hemizygosity) for the substituted chromosome. Daughters of these males do not inherit the substituted Y chromosome and are, therefore, genetically identical to

females from the host strain (Figure 1B). By controlling the influence of potentially confounding genetic, social and environmental factors, we found striking evidence for frequent and strong phenotypic changes in daughters that are attributable in a transgenerational genetic manner to the paternal Y chromosome.

Materials & methods

Mice consisted of the following: C57BL/6J-Chr Y<129S1/SvImJ> (B6-Y¹²⁹, B6 males with their Y chromosome derived from 129, JR #005547) and 129S1/SvImJ-Chr Y<C57BL/6J> (129-Y^{B6}, 129 males with their Y chromosome derived from B6, JR #005548) were derived as described previously [23,24] and maintained with repeated backcrossing to C57BL/6J (B6, JR #000664) and 129S1/SvImJ (129, JR #002448) strain females, respectively, to control for new mutations and genetic drift among inbred strains (Jackson Laboratory, ME, USA). We refer to these males generically as CSS-Y and to the daughters of CSS-Y males versus host females as 'B6' versus B6, and '129' versus 129. Control females for these studies were obtained from our independent C57BL/6J and 129S1/SvImJ colonies. We note that 'B6' and '129' test females are genetically identical to B6 and 129 control females, respectively.

Other strains included in this study were: C57BL/6J-Chr Y <PwD> (B6-Y^{PwD}, B6 males with their Y chromosome derived from the PwD inbred strain, JR #064660), C57BL/6JChr Y <A/J> (B6-Y^{A/J}, B6 males with their Y chromosome derived from the A/J inbred strain, JR #000646), C57BL/6J-Chr X <A/J> (B6-X^{A/J}, B6 mice with their X chromosome derived from the A/J inbred strain, JR #000646), C57BL/6JChr A <A/J> (B6-A^{A/J}, B6 mice with their autosomal chromosome derived from the A/J inbred strain, JR #000646), C57BL/6J-Chr Y <B6> (B6-Y^{B6}, B6 males with their Y chromosome derived from B6, JR #005548).

To test for substitution of the pseudo-autosomal region of the X chromosome in CSS-Y daughters, a SNP (rs30590889) was identified that distinguishes the pseudoautosomal region of the X and Y chromosomes from the host and donor strains. This SNP introduces an *AciI* restriction site at base 166428342 (NCBI Build 37). The region was PCR amplified (forward: CCCATGTGTTTGTTCCT; reverse: CGGGGTGACAGAGGAGAAT) and digested with *AciI*. CSS-Y daughters were homozygous for the host strain allele, indicating that the inherited paternal X chromosome did not carry material derived from the substituted Y chromosome, as expected given obligate recombination between the pseudoautosomal region of the X and Y chromosomes [25,26] and repeated backcrossing to the host strain.

Phenotype analysis

Data were obtained for 120 traits tested in the C57BL/6J-Chr^{A/J} CSS panel [22] and 38 traits tested in the C57BL/6J-Chr^{PwD} CSS panel [27] from the Mouse Phenome Database [101]. Mitochondrial CSSs were excluded from this analysis. In addition, data for the B6-Chr^{MSM} CSS panel [28,29] were also excluded in this study because the single multigenic trait that differed significantly between daughters of B6-ChrY^{MSM} males and C57BL/6J females was insufficient for a rigorous analysis.

Frequency of traits with significant phenotypic effects—The first step was to identify CSSs, for each trait, that differed significantly from the host strain ($p < 0.05$ after Bonferroni correction for multiple testing), then multigenic traits were selected where three or more CSSs showed such differences (excluding ‘B6’ in this calculation). Next, among these multigenic traits the frequency (percentage) of traits that showed significant phenotypic variation relative to the host strain was calculated separately for each CSS and for ‘B6’, and finally the average percentage across specific strains or combinations of strains was calculated (Table 1).

Average phenotypic effect—For each CSS, the strength of the phenotypic effect for each multigenic trait was calculated as the difference between the trait value for that CSS and the parental host strain, and was expressed as a function (percentage) of the difference between the corresponding inbred host and donor strains [30]. Then, separately for each CSS and ‘B6’, the mean phenotypic effect was calculated by averaging the individual phenotypic effects for all multigenic traits differing significantly between that CSS or ‘B6’ and the host strain. Finally, the average percentage across specific combinations of strains was calculated (Table 1).

Behavioral testing

Behavioral testing was performed by the Case Western Reserve University School of Medicine Rodent Behavior Core [102]. Mice were housed in the testing facility and handled by Rodent Behavior Core staff 7–10 days prior to testing. Females were 2–3 months of age at the beginning of testing and were age-matched such that the age difference between the test and control cohorts was no greater than 2 days. To control for social effects that may result from housing females with test or control males, male parents were removed from mating cages prior to birth and male siblings removed prior to postnatal day 3. Females included in the study were raised with at least one female sibling prior to weaning. After weaning, females were housed in groups of four, one of each 129, ‘129’, B6 and ‘B6’, to control for estrus cycle and social influences (Figure 1C). A total of 16 replicates of these groups of four were tested, for a total of 64 females in the study. These females were derived from 23 different males to minimize impact of possible parent-specific effects within the four test and control strains. Finally, cages were coded and sent for behavioral testing with the genotype removed to prevent bias during testing.

Phenotyping screen

Phenotyping for a panel of 42 behavioral traits was performed using a modified SmithKline, Beecham Pharmaceuticals, Harwell, Imperial College, Royal London Hospital, Phenotype Assessment (SHIRPA) protocol [31], which included indicators for gross defects in motor function, sensory and autonomic function, mood and behavior. A complete list of traits is provided in Online Supplementary Table 1 (see www.futuremedicine.com/doi/suppl/10.2217/epi.10.26).

Open-field test

Mice were placed in an open field for 15 min. Anxiety-prone behavior was measured by determining the exploratory pattern of the mouse over a 15-min test.

Elevated plus maze

Mice were placed in the center of an elevated four-arm maze with two open arms and two enclosed arms. Anxiety-associated behavior was determined by counting the number of times mice entered open or closed arms as well as the time spent in each arm.

Analysis

Analysis was performed by comparing each test group to both the host and donor strains (Student's t-test), with the significance threshold set at $p < 0.05$ after Bonferroni correction for multiple testing.

Results

Survey for transgenerational genetic effects

Phenotypic variation among daughters of B6-Y^{A/J} and B6-Y^{PWD} males as well as among CSSs for autosomes and for the X and Y chromosomes was surveyed to characterize transgenerational effects for occurrence (frequency [%] of traits that differed significantly) and strength (average phenotypic effect for multigenic traits). We focused on a total of 120 traits for the B6-Chr^{A/J} CSS panel [101] and 38 traits for the B6-Chr^{PWD} panel [27]. Multigenic traits were then identified by selecting those where at least three CSSs differed from B6, excluding 'B6' from this calculation. A total of 41 traits in females and 23 in males remained for the B6-Chr^{A/J} panel, including 22 behavioral, seven cardiac and 12 hematologic traits in females and nine behavioral, four cardiac and ten hematologic traits in males. A total of 21 traits in females and 26 traits in males remained for the B6-Chr^{PWD} panel, including 17 hematologic in females, and 22 hematologic, three obesity-related traits and one bone mineral density trait in males.

Several traits are highlighted to provide a sense of the nature of the phenotypic variation between 'B6' females and the CSSs (Figure 2; complete results are provided in Online Supplementary Table 2 [see www.futuremedicine.com/doi/suppl/10.2217/epi.10.26]). In particular, we show four representative traits for the B6-Chr^{A/J} panel (mean platelet volume, QT interval, total fatty acid level and startle reflex), and two representative traits for the B6-Chr^{PWD} panel (plasma triglycerides and bone mineral density). The number of CSSs that differed from B6 ranged from three (plasma triglyceride) to 17 for mean platelet volume. In each case, 'B6' females showed phenotypic differences that were generally similar in strength to differences found in other CSSs.

For these multigenic traits, we then compared the occurrence and strength of transgenerational versus conventional effects in these two CSS panels. On average, for the B6-Chr^{A/J} panel, 46.5% of the multigenic traits tested in females for each autosomal CSS (range: 29.3–61.0% for individual autosomal CSS strains) and 34.1% of multigenic traits in B6-Chr^{A/J} females were significantly changed relative to B6 females (Table 1; complete results are provided in Online Supplementary Table 3 [see www.futuremedicine.com/doi/suppl/10.2217/epi.10.26]). The frequency of affected traits was somewhat lower for males (35.9% for autosomal CSSs with range: 17.4–65.6%, 17.4% for B6-X^{A/J} males, and 30% for

B6-Y^{A/J} males; Table 1). Remarkably, for ‘B6’ females, 36.6% of multigenic traits differed from B6 females, a frequency that was similar to other CSSs in the panel.

Results for the multigenic traits for the B6-Chr^{PWD} panel revealed similar patterns of variation (Table 1; complete results are provided in Online Supplementary Table 4 [see www.futuremedicine.com/doi/suppl/10.2217/epi.10.26]). In particular, the average frequency of affected traits was 11.1% (range: 4.8–19.0%) in females for autosomal CSSs and 21.4% (19.0% in X.1 and 23.8% in X.3) for X chromosome CSSs. Again, the frequency of affected traits was lower in males than in females, with 9.7% (range: 0–42.3%) of traits showing significant variation for autosomal CSSs, 13.5% for X chromosome CSSs (7.7 and 19.2% for X.1 and X.3), and 3.8% for B6-Y^{PWD}. For ‘B6’ females, 9.5% of the traits differed significantly from B6 females. Together, these results suggest that transgenerational effects were as common as conventional genetic effects that presumably result from the direct action of substituted chromosomes.

Next, we focused on effect size as a measure of the strength of phenotypic effects. Average effect size was calculated as the percent of the difference between the host and donor strain that was attributable to the substituted chromosome. Interestingly, average effect size was similar between ‘B6’ daughters of B6-Y^{A/J} males and other strains in the B6-Chr^{A/J} panel. Among the 41 multigenic traits tested in females of the B6-Chr^{A/J} panel, 15 were significantly different between ‘B6’ and B6 females. The average effect size of these differences in ‘B6’ was 85.7%, which was remarkably similar to the average effect size of 94.2% (range: 80.2–115.2%) in autosomal CSS females and 74.3% in B6-X^{A/J} females (Table 1). Among ‘B6’ daughters of B6-Y^{PWD} males, the average phenotypic effect was 128.2%, which was remarkably similar to the average effect among B6-X^{PWD} (145.0%) and autosomal CSS females in the panel (113.7%, range: 59.6–242.3%) (Table 1).

Behavioral testing

The similar frequency and size of phenotypic effects in ‘B6’ test and B6 control females suggest that transgenerational genetic effects play a large role in the inheritance of complex traits. However, the possibility remains that the effects do not result from transgenerational effects attributable to the substituted paternal Y chromosome, but instead from other social or environmental factors. We therefore designed a study utilizing ‘B6’ and ‘129’ as test females, and B6 and 129 as control females to test directly for transgenerational effects of the paternal Y chromosome under conditions that rigorously control for social and environmental factors. Social effects that may result from the presence of males were minimized by removing male parents prior to birth and removing male siblings shortly after birth. To control social influences after weaning, females were housed in cohorts containing one age-matched female from each of four test groups (B6, ‘B6’, 129 and ‘129’ females), ensuring that each test mouse in the cohort was exposed to the same environmental and social conditions affecting other mice in the cage (Figure 1C).

An initial behavioral phenotyping survey suggested changes in anxiety-related phenotypes (Online Supplementary Table 1 [see www.futuremedicine.com/doi/suppl/10.2217/epi.10.26]). We therefore performed open-field and elevated plus maze (EPM) testing to examine these phenotypes in detail.

Open-field and EPM assays were used to test directly for anxiety-related phenotypes. 'B6' test and B6 control females showed similar measures of total activity, total distance traveled and average velocity (calculated as the distance travelled per second of testing) during the open-field test, as well as total number of entrances into arms of the maze during EPM testing (Figure 3; complete results are provided in Online Supplementary Tables 5 & 6 [see www.futuremedicine.com/doi/suppl/10.2217/epi.10.26]). Anxiety was measured as a decrease in the amount of time spent in the center region of the open-field, and in parallel, a decrease in the proportion of entrances into and percent of time spent in open arms of the EPM. Anxiety-related measures in '129' test females were not significantly different from the 129 control females, suggesting that the paternal ChrY^{B6} chromosome did not affect anxiety in '129' daughters (Figure 3A).

By contrast, a significant increase in the time spent in the center region of the open-field was observed in 'B6' test females compared with the genetically-identical B6 control females (Figure 3B). In addition, in 'B6' females, we found a significant increase in the percent of time spent in open arms of the EPM and a corresponding, nonsignificant increase in the percent of entrances into open arms, suggesting that 'B6' females have a decreased level of anxiety compared with the genetically-identical B6 control females. Thus at least one factor associated with the paternal 129-derived Y chromosome led to a heritable epigenetic change that reduced anxiety in 'B6' females.

Discussion

We found that transgenerational genetic effects are both common and strong among daughters of males with different Y chromosomes. With conventional modes of inheritance, daughters of CSS-Y males and genetically identical host strain females should be phenotypically indistinguishable. However, the frequency of affected traits and the strength of their effects in daughters of CSS-Y males were remarkably similar to those observed in females with a substituted autosome or X chromosome, and to males with a substituted autosome or with a substituted X or Y chromosome (Table 1). In addition, results based on the careful design of behavioral tests in 'B6' and '129' females strongly argue that transgenerational rather than social and environmental factors led to reduced anxiety in 'B6' daughters of B6-Y¹²⁹ males. These results are especially surprising given the relatively small number of genes on the Y chromosome. At least three important questions emerge:

- Do other chromosomes lead to transgenerational effects?
- Do transgenerational effects occur in humans?
- What is the molecular basis for these effects?

These transgenerational effects appear to depend on interactions between the host strain background and epigenetic factors related to specific Y chromosomes. In particular, the paternal 129-derived Y chromosome affected anxiety-related behaviors during open-field and EPM testing on the C57BL/6J but not the 129S1/SvImJ genetic background, suggesting that combinations of factors, one in fathers and the other in daughters, jointly determine phenotypic outcome. Similar interactions across generations have been reported previously [19,20].

Heritable epigenetic changes could contribute to ‘missing heritability’ in humans and model organisms. The association between genotype and phenotype is central to many studies of heritable traits. However, ongoing genome-wide association studies to discover these associations have yielded a surprising and unexpected result, namely that most genetic variants elude discovery [3]. Among the explanations that are being actively investigated are overestimates of heritability, unexplored regions of the genome, untested classes of genetic variants, and action of many rare variants. We propose that transgenerational genetic effects contribute to ‘missing heritability’. The present study together with related reports suggest that heritable epigenetic effects are as common and as strong as genetic variants whose phenotypic effects are inherited in the conventional manner [13,19,20]. In addition, two recent studies show that these effects can persist for multiple generations [32].

Several molecular mechanisms could mediate transgenerational effects. Considerable evidence supports heritable changes in DNA methylation and histone modifications [12,33]. However, growing evidence from plants, flies, worms and mice implicate aspects of RNA biology such as small RNAs [13–15,34,35], RNA binding proteins that edit RNA [36] and control access of miRNA to target mRNAs [37], and RNA editing enzymes that also methylate DNA [38,39]. Several of these processes control translation in RNA granules that are abundant in gametes in both males and females [40–42]. Delineating the sequence of molecular events that initiate epigenetic changes in one generation and lead to phenotypic changes in subsequent generations is perhaps the major question in studies of transgenerational effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Executive summary

- Several published studies report evidence for genetic variants in one generation affecting phenotypes in subsequent generations.
- It is unclear, however, whether these effects involve many traits or are merely interesting exceptions, and it is also unclear whether they compare with phenotypic consequences to genetic variants that are inherited in a conventional manner.
- The work reported in this study sought to determine whether transgenerational genetic effects are common or rare, and strong or weak.
- We took advantage of inbred strains of mice whose Y chromosome has been replaced with the Y chromosome from another inbred strain. Females from these substitution strains are genetically identical to females from the host strain, and should be phenotypically indistinguishable in the absence of heritable epigenetic changes resulting from the paternal Y chromosome.
- In several strain combinations, physiological and behavioral tests for hundreds of traits showed unequivocally that transgenerational and conventional genetic effects are remarkably similar in frequency and strength.
- These results demonstrate that transgenerational effects are both common and strong, and rival conventional genetics effects in phenotypic consequences.

Figure 1. Study design testing for the effects of the paternal Y chromosome on daughters' phenotypes

(A) Generation of substitution strains for the Y chromosome. Males were backcrossed to the C57BL/6J host strain, with selection for males from at least 10 backcross generations to reconstitute the genetic background of the host strain [23,24]. (B) Genetic identity for daughters of CSS and host strain males. (C) Controlling for social effects. Male parents and siblings were removed at birth from home cages and then one from each of the two tests and two control females (total of four females) were housed together after weaning. CSS: Chromosome substitution strain.

Figure 2. ‘B6’ test versus B6 control females in the B6-Chr^{A/J} and B6-Chr^{PWD} chromosome substitution strain surveys

Results highlighted in light blue represent significant differences from C57BL/6J. ‘B6’ test and B6 control groups are boxed to highlight the primary contrast in this test. $p < 0.05$ after Bonferroni correction for multiple hypothesis testing [22,30]. (A) MPV (fL) for B6-Chr^{A/J} chromosome substitution strains (CSSs); (B) QT interval (ms) for B6-Chr^{A/J} CSSs; (C) total fatty acids (mg/dl) for B6-Chr^{A/J} CSSs; (D) startle reflex (response to airpuff) for B6-Chr^{A/J} CSSs; (E) plasma triglyceride (mg/dl) for B6-Chr^{PWD} CSSs; (F) BMD (g/cm²) for B6-Chr^{PWD} CSSs.

BMD: Bone mineral density; MPV: Mean platelet volume.

Figure 3. Elevated plus maze and open field tests for anxiety-related behavior

(A) '129' test and 129 control females, and (B) 'B6' test and B6 control females. In total, 16 independent tests were made, with four mice in each test (Figure 1C) and a total of 64 mice. For the elevated plus maze test, the percent of time spent in and entrances into the open rather than closed arms of the maze was measured, and for the open field test, the time spent in the center of the open field was measured. The y-axis shows the level (percentage) of particular traits that are provided for each data set along the x-axis. Data shown as mean $\pm$ SEM.

* $p < 0.05$ after Bonferroni correction for multiple testing.

Frequency and strength of transgenerational effects in daughters of CSS-Y males versus conventional genetic effects in females and males from the B6-ChrA/J and B6-ChrPWD CSS panels.

Table 1

Chromosome	Traits with significant phenotypic variation (%)		Average phenotypic effect (%)	
	Females	Males	Females	Males
B6-Chr^{A/J}				
Transgenerational effects	'B6' (Y [father])	36.6	85.7	
Conventional effects	Y (brother)	30.0		73.6
	X	34.1	74.3	92.6
	Autosomes	46.5	94.2	82.0
B6-Chr^{PWD}				
Transgenerational effects	'B6' (Y [father])	9.5	128.2	
Conventional effects	Y (brother)	3.8	80.9	
	X	21.4	13.5	145.0
	Autosomes	11.1	9.7	179.0