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Genetics

Pleiotropy in microdeletion syndromes: Neurologic and spermatogenic abnormalities in mice homozygous for the p^{6H} deletion are likely due to dysfunction of a single gene

EUGENE M. RINCHIK*†, DONALD A. CARPENTER*, AND MARY ANN HANDEL‡

*Biology Division, Oak Ridge National Laboratory, P.O. Box 2009, Oak Ridge, TN 37831-8077; and ‡Department of Zoology, University of Tennessee, Knoxville, TN 37996-0810

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ABSTRACT **Variability and complexity of phenotypes observed in microdeletion syndromes can be due to deletion of a single gene whose product participates in several aspects of development or can be due to the deletion of a number of tightly linked genes, each adding its own effect to the syndrome. The p^{6H} deletion in mouse chromosome 7 presents a good model with which to address this question of multigene vs. single-gene pleiotropy. Mice homozygous for the p^{6H} deletion are diluted in pigmentation, are smaller than their littermates, and manifest a nervous jerky-gait phenotype. Male homozygotes are sterile and exhibit profound abnormalities in spermiogenesis. By using *N*-ethyl-*N*-nitrosourea (EtNU) mutagenesis and a breeding protocol designed to recover recessive mutations expressed hemizygously opposite a large *p*-locus deletion, we have generated three noncomplementing mutations that map to the p^{6H} deletion. Each of these EtNU-induced mutations has adverse effects on the size, nervous behavior, and progression of spermiogenesis that characterize p^{6H} deletion homozygotes. Because EtNU is thought to induce primarily intragenic (point) mutations in mouse stem-cell spermatogonia, we propose that the trio of phenotypes (runtiness, nervous jerky gait, and male sterility) expressed in p^{6H} deletion homozygotes is the result of deletion of a single highly pleiotropic gene. We also predict that a homologous single locus, quite possibly tightly linked and distal to the *D15S12 (P)* locus in human chromosome 15q11–q13, may be associated with similar developmental abnormalities in humans.**

Heritable chromosomal deletions are frequently associated with variably expressed pleiotropic syndromes, and it has often been difficult to determine whether a variable phenotype is due to the deletion of one pleiotropic gene or is due to the additive effects of phenotypes caused by the deletion of a number of genes. Nonetheless, such deletions have been useful in the development of genetic and physical maps of large stretches of the genome in humans and experimental organisms. Moreover, association of specific clinical phenotypes with cytogenetically detectable deletions in humans has been of major importance in placing loci responsible for genetic disease within defined regions of the genome. For example, the Prader–Willi (PWS) and Angelman (AS) syndromes, which are distinct neurobehavioral disorders with complex, and often variable, phenotypes, have been associated with deletions of human 15q11–q13 (for review, see ref. 1). In many of these so-called "microdeletion" or "contiguous-gene" syndromes (2), it is often not clear whether the phenotype is due to deletion of several tightly linked genes or to the effect of deletion of one gene only. Mouse models, where point mutations can be induced with appropriate protocols, provide one approach to resolving this issue.

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The region of mouse chromosome 7 (Mmu7) surrounding the pink-eyed dilution (*p*) locus is being characterized by genetic and molecular approaches (3–7). This region shares homology with at least two regions of the human genome: 15q11–q13 and 11p15 (3, 8, 9). The homology with 15q11–q13 has provided an impetus for exploring whether mutations mapping to the mouse *p* region could have potential use as models for features of human 15q11–q13 syndromes, including imprinted and nonimprinted components of PWS and AS (3, 9). Thus, the phenotypes specified by any mutations (deletions or otherwise) mapping to the region surrounding *p* could be useful for dissection of microdeletion phenotypes within the corresponding human region.

In mice, homozygosity for the p^{6H} deletion results in dilution of eumelanin (due to loss of function at the *p* locus) and in three other seemingly unrelated phenotypes: runtiness, a nervous jerky gait, and male sterility with abnormalities in sperm acrosome differentiation and sperm head shape (10–12). Thus far, analyses of p^{6H} and other phenotypically similar mutations of the same region have not yet resolved whether this pleiotropic syndrome results from alterations of a single gene or of several genes. The chromosomal subregion in mouse responsible for these three phenotypes has been mapped close to *p* (4), and more finely (3) to a region proximal to *p* close to *D7H15F37S1* (formerly *D15F37S1h*), a locus defined by a human microclone that detects a family of sequences in human chromosomes 15q11–q13 and 16p (13).

We report here the isolation of three noncomplementing *N*-ethyl-*N*-nitrosourea (EtNU)-induced mutations that map to the region implicated in the p^{6H} deletion syndrome. We also demonstrate that the phenotype specified by mice expressing any of these mutant alleles is identical to that exhibited by p^{6H}/p^{6H} deletion homozygotes in terms of reduced fitness, neurological phenotype, and defects in spermiogenesis and male sterility. Because EtNU is known to induce primarily point mutations in spermatogonial stem cells (14–17), the trio of phenotypes observed in p^{6H} deletion homozygotes is probably due to deletion of a single gene, mapping proximal to *p*, and is not a result of the deletion of several individual genes, each adding a subcomponent to the overall syndrome. Furthermore, the definition and mapping of this locus in mouse suggests the existence of a homologous single locus in human 15q11–q13 that may be involved in similar developmental systems in humans.

MATERIALS AND METHODS

Mice. The p^{6H} mutation, originally imported in a segregating p^{d}/p^{6H} stock from The Jackson Laboratory, was maintained in p^{7R75M}/p^{6H} heterozygotes. All other animals originated and

Abbreviations: EtNU, *N*-ethyl-*N*-nitrosourea; PWS, Prader–Willi syndrome; AS, Angelman syndrome; Mmu7, mouse chromosome 7.

†To whom reprint requests should be addressed at present address: Sarah Lawrence College, 1 Mead Way, Bronxville, NY 10708.

were bred at the Oak Ridge National Laboratory. The p^{7R75M} mutation was detected in the progeny of a neutron-treated male, and p^{7R75M}/p^{7R75M} animals are darker in color than p/p^{7R75M} animals. The $p^{46DFiOD}$ deletion is prenatally lethal (18) and includes a number of mapped loci, including *Myod1, ru2, D7H15F37S1, p(D7H15S12)*, and *Gabrg3* (3, 5, 7, 19). The $p^{46DFiOD}$ deletion stock is maintained by alternate crosses of $+ p/p^{46DFiOD}$ and $ru2 + /p^{46DFiOD}$ heterozygotes to $ru2 + /ru2 +$ and $+ p/+ p$ mice, respectively. *ru2* is another coat-color marker that maps ≈3 centimorgans proximal to *p* (20). $p^{3RD300H}$ (E.M.R., unpublished data) and p^{25DVT} (5) are deletions of *p* that extend proximally to include at least one prenatal lethal factor mapping proximal to *D7H15F37S1* (3, 4). $p^{4THO-II}$ is a deletion whose proximal breakpoint lies within the *p (D7H15S12)* transcription unit and whose distal breakpoint maps between *Gabrb3* and *Znf127* (formerly *D15S9h1*) (3, 6).

Mutagenesis. EtNU was obtained from Sigma. Two groups of BJR-*a/a*; *ru2 p/ru2 p* males (35 and 65 males, respectively) that were >8 weeks old were given a total fractionated dose of 400 mg/kg i.p. as described (21).

Detection of Mutations. The genetic crosses used to identify mutations mapping to the region of Mmu7 that is missing in the $p^{46DFiOD}$ deletion are shown in Fig. 1. Male BJR mice (in the G_0 generation) that had been exposed to EtNU were mated with (C57BL/10R1 × C3Hf/R1)F_1 females. All G_1 offspring from this cross carried a mutagenized paternal genome, including a Mmu7 marked with the *ru2* and *p* mutations. G_1 females were then crossed to $a/a; ru2 + /p^{46DFiOD}$ males. Three coat-color phenotypes were observed in the G_2 progeny: wild type ($ru2 +/+ +$ or $p^{46DFiOD}/+ +$), ruby ($ru2\ p/ru2 +$), and pink-eyed dilute, ruby ($ru2\ p/p^{46DFiOD}$). [Because agouti (*A*) and nonagouti (*a*) alleles were also segregating, 50% of each phenotypic class was agouti and 50% was nonagouti, but this posed no problem in the accurate genotyping of the G_2 segregants.] Any EtNU-induced recessive mutations closely linked to *ru2* and *p* and included within the segment deleted

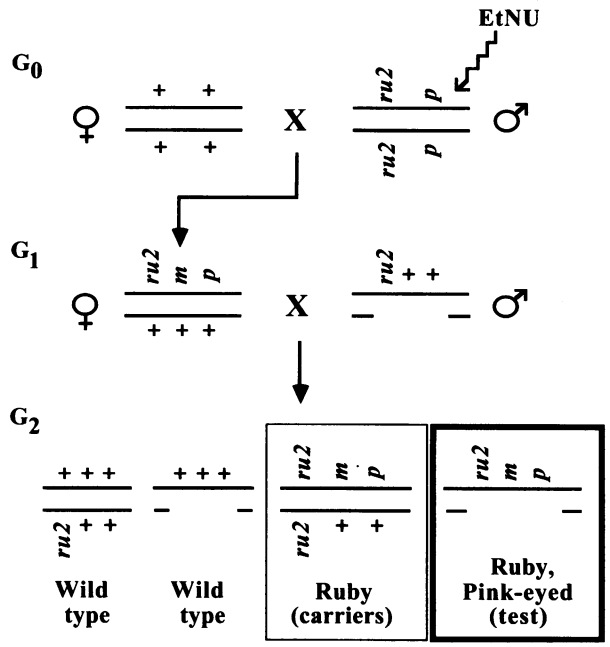

FIG. 1. Breeding protocol used to recover EtNU-induced recessive mutations within the region corresponding to the ≥4-centimorgan $p^{46DFiOD}$ deletion. The heavily outlined box highlights the G_2 ruby pink-eyed dilute class, which is the test class for mutations in this screening protocol. The lightly outlined box highlights the G_2 ruby class, from which the mutations can be recovered and propagated. *m* is a mutation induced by EtNU; *ru2*, ruby-eye 2; *p*, pink-eyed dilution. The G_1 male is a ruby-colored mouse that is heterozygous for the $p^{46DFiOD}$ deletion [$ru2 + +/Df(ru2\ p)^{46DFiOD}$].

in the $p^{46DFiOD}$ deletion should be hemizygously expressed in the pink-eyed dilute, ruby ($ru2\ p/p^{46DFiOD}$) G_2 progeny. The pink-eyed dilute, ruby G_2 animals were observed at weaning for visible differences in body size/weight, hair quality, obvious skeletal abnormalities, ability to swim, other balance problems, or abnormal nervous activity. Equally important was the finding of an absence of pink-eyed dilute, ruby animals in a particular pedigree, which indicated that that particular $ru2\ p/p^{46DFiOD}$ genotype was lethal. Whenever possible, at least 30 G_2 offspring from each $ru2\ p/+ +$ G_1 female were produced to score for the presence of such a lethal mutation. This was not always possible, however, because the G_1 female often became sterile or died before 30 progeny could be reared.

If the phenotypes of the G_2 progeny suggested that a mutation (*m*?) had been induced on the *ru2 p* chromosome, the ruby siblings [*ru2* (*m*?) *p/ru2* + +] were crossed to + + $p/p^{46DFiOD}$ mice to verify transmission. Sometimes, this test for transmissibility was done even before the G_1 female had produced 30 offspring; indeed, for a number of pedigrees (including 322SJ and 850SJ, reported here), ruby G_2 siblings [*ru2* (*m*?) *p/ru2* + +] were tested for the transmission of a mutation based on the lack of ruby pink-eyed offspring among 21 and 17 G_2 progeny, respectively. This genetic testing of "suspicious" pedigrees resulted in the recovery of a number of mutant pedigrees that would normally have been missed. Once transmissibility of the mutation was demonstrated, the mutant stock was maintained by alternate crosses of ruby [*ru2* (*m*) *p/ru2* + +] carriers to + + $p/p^{46DFiOD}$ mice with selection for the pink-eyed offspring [*ru2* (*m*) *p*/+ + *p*], followed in the next generation by crossing these *ru2* (*m*) *p*/+ + *p* carriers to *ru2* + + $/p^{46DFiOD}$ mice with selection for the ruby offspring [*ru2* (*m*) *p/ru2* + +] to begin the cycle anew.

Histology. Mice were anesthetized and the testes were removed. For electron microscopy, testes were minced in 3% (vol/vol) glutaraldehyde/2% (wt/vol) paraformaldehyde in 0.1 M sodium cacodylate buffer, rinsed in buffer, postfixed in 1% osmium tetroxide in the same buffer, dehydrated through an ethanol series, infiltrated with propylene oxide, and embedded in Epon. Thin sections were stained with lead citrate and uranyl acetate.

RESULTS

Recovery of EtNU-Induced Mutations Mimicking the Neurological Phenotype Exhibited by p^{6H}/p^{6H} Deletion Homozygotes. A total of 2513 G_1 daughters of EtNU-treated BJR-*ru2 p/ru2 p* males were crossed to $ru2 + /p^{46DFiOD}$ males by the protocol outlined in Fig. 1. Of these 2513 females, 1240 produced large enough G_2 progenies to permit detection of mutations induced by EtNU in the *ru2 p* chromosome in the region corresponding to the $p^{46DFiOD}$ deletion. Cogent to this paper was the discovery of two pedigrees (322SJ and 850SJ) in which the ruby pink-eyed dilute ($ru2\ p/p^{46DFiOD}$) G_2 progeny were absent and one pedigree (932SJ) in which the ruby pink-eyed dilute G_2 progeny were runted and exhibited a nervous phenotype. When ruby [*ru2* (*m*?) *p/ru2* + +] G_2 presumed carriers from the 322SJ and 850SJ pedigrees were crossed to + + $p/p^{46DFiOD}$ mice to test for transmissibility of an EtNU-induced mutation, a few ruby pink-eyed dilute [*ru2* (*m*) $p/p^{46DFiOD}$] G_3 progeny were produced from each carrier, but each of these mice was runted and nervous compared to its littermates. The runted and nervous mice from the 322SJ and 850SJ pedigrees (and from the 932SJ pedigree) had variable lifespans, but most died before 4 weeks of age. Consequently, the 322SJ and 850SJ pedigrees were each segregating a variably expressed EtNU-induced postnatally lethal mutation, similar in phenotype to that segregating in the 932SJ pedigree, and were not carrying a prenatally lethal mutation as the screening experiment had first suggested. The

322SJ, 850SJ, and 932SJ mutations were independent as each was derived from a different EtNU-treated G_0 BJR male.

Mapping and Complementation Analysis of the Neurological Component of the p^{6H} Syndrome. The phenotype of the affected mice in the 322SJ, 850SJ, and 932SJ pedigrees grossly resembled that exhibited by p^{6H}/p^{6H} deletion homozygotes. Therefore, we crossed carriers (*ru2 m p*/*ru2* + +) of each of the three EtNU-induced mutations to p^{7R75M}/p^{6H} mice to determine whether the p^{6H} deletion could complement the neurological and/or runting defects specified by each mutation. The data in Table 1 indicate that the 322SJ, 850SJ, and 932SJ mutations are not complemented by p^{6H} since compound heterozygotes (*ru2 m p*/+ p^{6H}) are small and nervous. The three SJ mutations are, however, complemented by $p^{4THO\text{-}II}$, which breaks within the *p(D7H15S12)* transcription unit (3) and extends distally past the *Gabrb3* locus (Fig. 2). Consequently, these crosses map the 322SJ, 850SJ, and 932SJ mutations proximal to *p*, near the *D7H15F37S1* locus, in a region previously associated with a phenotype of runting, nervousness, and male sterility (3, 4).

Table 1 also highlights a high degree of variability in viability of mice hemizygous for these mutations. When the mutations are opposite the large $p^{46DFiOD}$ deletion, viability is substantially reduced when compared to mice carrying the mutations opposite other *p* deletions. It is not clear at this point whether this difference in viability is due to undefined background effects introduced by the $p^{46DFiOD}$ stock or, rather, to a combinatorial effect of hemizygosity for a relatively long stretch of Mmu7 (associated with the $p^{46DFiOD}$ deletion) added to the effects of the loss-of-function EtNU-induced mutation.

Mice heterozygous for one of the mutations (*ru2 m1 p*/*ru2* + + or *ru2 m1 p*/+ + *p*) were also crossed to mice heterozygous for another (*ru2 m2 p*/*ru2* + + or *ru2 m2 p*/+ + *p*) to test whether the EtNU-induced mutations could complement each other. Lack of complementation would be recognized by the runting/nervousness phenotype appearing in the ruby pink-eyed-dilute (*ru2 m1 p*/*ru2 m2 p*) offspring. We found that mice homozygous for 322SJ, 850SJ, or 932SJ express the runting/nervousness phenotype, as do all possible compound heterozygotes (i.e., *m1*/*m2*). A control cross of female carriers of each of these mutations with males carrying 22SJ, another EtNU-induced mutation that specifies a mild runting syndrome that is complemented by the p^{6H} deletion (E.M.R. and D.A.C., unpublished data), showed complementation in each case. Consequently, the three EtNU-induced mutations 322SJ, 850SJ, and 932SJ affect the same genetic function, belong to

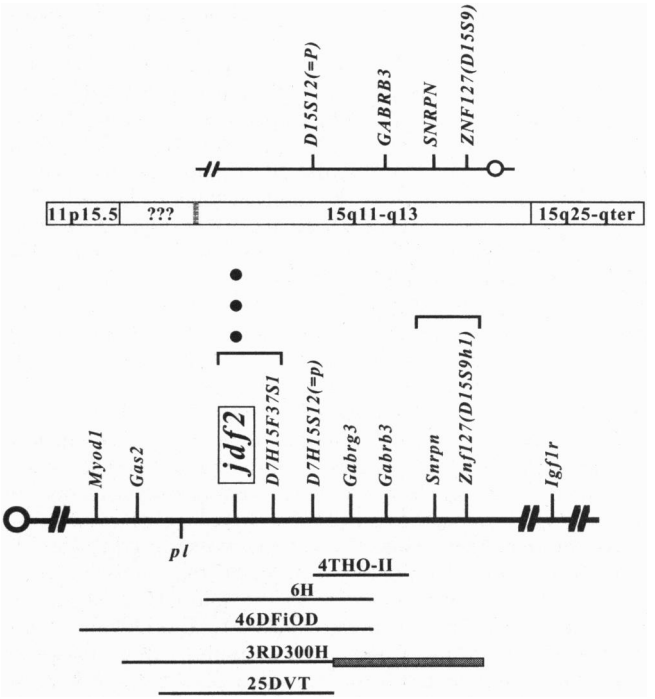

FIG. 2. Map of the *Myod1—Igf1r* region of Mmu7 showing the position of the *jdf2* locus. This map is modified from that presented in refs. 3 and 7 and is based on the deletion-mapping data presented in Table 1. Mmu7 is shown as the thickest line, and its centromere is represented at the left by a circle. The *pl* (prenatal lethal) marker below the chromosome map represents a minimum of one gene defined by lethal *p* mutations that is (are) required for normal prenatal development. Extent of deletions with respect to markers on the genetic map is also indicated below the map, and the name of each deletion is indicated. The bracket on the left indicates that the order of *jdf2* and *D7H15F37S1* (3, 13) cannot be determined from available data. The solid circles above this bracket represent 322SJ, 850SJ, and 932SJ, which are three EtNU-induced alleles of *jdf2*. The bracket on the right designates the unknown order of *Snrpn* and *Znf127* in the mouse. The placement of the *Gas2* locus (22) on this particular panel of deletions, as well as the extent of the $p^{3RD300H}$ deletion, are based on unpublished data from E.M.R. The shaded box represents current uncertainty about the distal extent of the $p^{3RD300H}$ deletion. Known human homology regions are shown at the top of the map. For the 15q11–q13 homology region, an abbreviated map of human chromosome 15 just distal to its centromere shows loci mapped in both mouse and human.

Table 1. Deletion mapping of EtNU-induced mutations specifying a runting/nervousness syndrome

Deletion (p^l)	EtNU mutations (*m*), no.								
	322SJ			850SJ			932SJ		
	Pink eyed			Pink eyed			Pink eyed		
	WT	SN	Total	WT	SN	Total	WT	SN	Total
$p^{4THO\text{-}II}$	12	0	27	8	0	24	4	0	11
$p^{46DFiOD}$	0	11	423	0	7	263	1*	9	267
p^{6H}	0	6	33	0	11	88	0	4	24
p^{25DVT}	0	7	25	0	8	25	ND	ND	ND
$p^{3RD300H}$	0	7	43	0	5	-27	ND	ND	ND

Crosses used in these analyses were *ru2 m p*/*ru-2* ++ × p^{7R75M}/p^l or *ru2 m p*/*ru2* ++ × *ru2* + *p*/+ p^l (where p^l indicates *p*-locus deletions and *m* denotes an EtNU-induced SJ mutation). Progeny were classified at 3 weeks of age. In crosses involving the $p^{46DFiOD}$ deletion, both male and female carriers of *m* were used; in all other crosses, data were obtained only from male carriers. Pink-eyed progeny in either of the two types of crosses are expected to be ≈25% of the progeny. Viability was often highly variable, especially when SJ mutations were heterozygous with the large $p^{46DFiOD}$ deletion. This would often lead to a deficiency of the pink-eyed class. WT, pink-eyed progeny that were normal in size; SN, pink-eyed progeny that were small and had a nervous jerky gait; ND, not done.

*This pink-eyed mouse with normal gait (i.e., WT) was presumed to be a recombinant that had lost *ru2 m* from the original *ru2 m p* chromosome. This presumed *ru2* + *p*/+ p^l recombinant was not progeny tested.

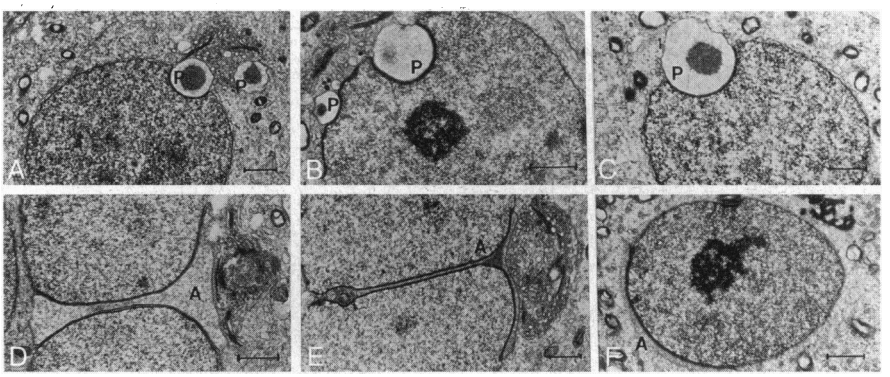

FIG. 3. Micrographs of thin sections of mutant and normal testes demonstrating spermatogenic defects characteristic of p^{6H}/p^{6H} deletion homozygotes. (*A*) Two unfused proacrosomes (P) in an early round spermatid from a mouse that is homozygous for the p^{6H} deletion. (*B*) Two proacrosomic vesicles (P) attached to the nucleus of an early round spermatid of a $jdf2^{322SJ}$ hemizygote [$ru2\ jdf2^{322SJ}\ p/+\ p^{3RD300H}$]. For comparison to *A* and *B*, *C* illustrates the normal single proacrosome (P) of a spermatid from a wild-type CD1 mouse. (*D*) Two round spermatid nuclei conjoined by a common acrosome (A) from testis of a mouse homozygous for the p^{6H} deletion. (*E*) Two spermatid nuclei with a common acrosome (A) from the testis of a mouse that is a $jdf2^{850SJ}$ hemizygote [$ru2\ jdf2^{850SJ}\ p/p^{46DFiOD}$]. For comparison to *D* and *E*, *F* illustrates a spermatid of the same stage from a wild-type CD1 mouse, showing a normal acrosome (A) and single spermatid nucleus. (Bars = 1 μm.)

the same complementation group, and, therefore, presumably represent alleles of a single locus. We define this locus as *jdf2* (juvenile development and fertility 2; identified as "R," "JG," and "S" in ref. 4), which maps proximal to *p* but within the p^{6H} deletion (Fig. 2).

Analysis of Spermiogenesis in Males Carrying EtNU-Induced *jdf2* Mutations. In addition to abnormalities in size and neurological phenotype, male p^{6H}/p^{6H} homozygotes also exhibit characteristic defects in spermiogenesis and sterility (10–12). These defects are primarily in the deposition and elaboration of the acrosome and in spermatid-head morphology. Many spermatids of p^{6H}/p^{6H} homozygotes are characterized by the secretion and attachment to the nuclear envelope of more than one acrosomal vesicle (Fig. 3*A*). This aberration is only occasionally found in other male-sterile mutants. Another frequent and characteristic defect found in p^{6H}/p^{6H} homozygotes is the presence of binucleate spermatids with the nuclei conjoined by a single acrosome (Fig. 3*D*). This defect is also found in male-sterile mice that exhibit meiotic defects (23).

Analysis of spermiogenesis in males carrying each of the three EtNU-induced *jdf2* alleles presented a unique opportunity to determine whether the neurological and spermatogenic defects exhibited by p^{6H}-deletion homozygotes are likely to be due to mutation in a single gene. Accordingly, we used electron microscopy to examine morphological aspects of spermiogenesis in testes from p^{6H}/p^{6H} deletion homozygotes and from males carrying EtNU-induced *jdf2* alleles opposite *p*-locus deletions [$ru2\ jdf2^{322SJ}\ p/+\ p^{3RD300H}$, $ru2\ jdf2^{850SJ}\ p/p^{46DFiOD}$, and $ru2\ jdf2^{932SJ}\ p/p^{46DFiOD}$]. Male hemizygotes bearing any of the three EtNU-induced *jdf2* alleles displayed defects characteristic of p^{6H}/p^{6H} deletion homozygotes—namely, multiple acrosomal vesicles and nuclei conjoined by a single acrosome. Representative micrographs showing each type of defect are presented in Fig. 3 *B* and *E* for the $jdf2^{322SJ}$ and $jdf2^{850SJ}$ mutations, respectively. Males heterozygous for each of these three alleles (i.e., $+/jdf2$) were fertile and were characterized by morphologically normal spermiogenesis (data not shown).

DISCUSSION

Application of a mutagenesis strategy similar to that used for the albino (*c*)-locus region of Mmu7 (21) has identified a number of EtNU-induced mutations mapping within the ≥4-centimorgan $p^{46DFiOD}$ deletion. Among these mutations are three that, when hemizygous or homozygous, cause a phenotype similar to that exhibited by mice homozygous for the p^{6H} deletion. Genetic analyses have demonstrated that the three mutations 322SJ, 850SJ, and 932SJ are likely to be alleles of a single locus, *jdf2*, which maps near the *D7H15F37S1* locus (3) within the proximal portion of the p^{6H} deletion that has been previously associated with the p^{6H} runting and nervousness syndrome (3, 4).

Each of the three mutations results in runting, a nervous behavior, and male sterility, the latter being associated with abnormalities in spermatid acrosome and head structures that are similar to those observed in p^{6H}/p^{6H} deletion homozygotes. EtNU is thought to induce primarily intragenic changes (24) and has been found to induce single-base-pair changes in all of the stem-cell spermatogonial mutations analyzed to date (14–17). Consequently, the recovery of three EtNU-induced *jdf2* mutations that express the same trio of phenotypes observed in p^{6H} homozygotes makes it likely that these major aspects of the pleiotropic p^{6H}-deletion syndrome are due, directly or indirectly, to a loss of function of the single *jdf2* gene and that this phenotypic trio does not represent a contiguous gene syndrome whose manifestation depends on the combinatorial effects of deletion of a number of closely linked genes mapping proximal to the *p* locus.

This particular example of single-gene mutational pleiotropy is interesting from a number of viewpoints. Perhaps not surprisingly, a wide range of pleiotropy has been encountered in the study of the effects of mutations in the mouse. For example, it is known that intragenic mutations within the c-*kit* tyrosine kinase receptor affect pigmentation, germ cells, and hematopoiesis (25). Mice homozygous for the c^{14CoS} deletion at the albino (*c*) locus exhibit a perinatal-death syndrome characterized by liver and kidney dysfunction and by abnormalities in cell ultrastructure (26); however, all of these phenotypes result from a lack of the enzyme fumarylacetoacetate hydrolase (FAH) (27–29). It would appear that the fitness/neurological/male sterility subcomponent of the p^{6H} deletion syndrome falls into this group, in which a mutation in one gene affects more than one developmental system. On the other hand, the entire p^{6H} deletion syndrome can be viewed as more complex, since homozygosity for the deletion results in the *jdf2* trio of phenotypes and a defect in pigmentation due to loss of function of the *p* gene. This situation is similar to that observed for the c^{14CoS} deletion, in which affected homozygotes suffer from the FAH deficiency and its clinical ramifications but are also nonpigmented (from loss of the tyrosinase gene). Significantly, it is also similar to cases in which PWS or AS patients exhibit hypopigmentation or complete tyrosinase-positive oculocutaneous albinism, due to alterations in the human *P* (*D15S12*) gene in addition to mutations in the imprinted gene(s) responsible for PWS or AS itself (5).

The single-gene pleiotropy defined for the *jdf2* gene also has implications for predicting whether a similar pleiotropic gene may map to a homologous region in the human genome. The conservation of fine-structure synteny between human 15q11–q13 and Mmu7 is evident with respect to gene composition and gene order (3). However, the proximal extent of the 15q homology in Mmu7 is still not known (the *Gas2* gene is not yet mapped in humans; Fig. 2), and although the *D7H15F37S1* locus in mouse is defined by a human 15q11–q13 microclone (3, 13), the uncertain order of *jdf2* and *D7H15F37S1* still leaves the possibility that *jdf2* may not map to human 15q. Nonetheless, this laboratory had suggested (3) that if any of the phenotypes observed in the p^{6H} (*jdf2*) syndrome could be recognized in humans as nonimprinted components of PWS, AS, or more complex clinical syndromes associated with longer 15q deletions, the locus (loci) responsible would probably map distal to *P* (*D15S12*) (e.g., see Fig. 2). Because our data indicate that a mutation in a single *jdf2* gene results in defects in fitness, nervous/neurological function, and spermiogenesis, we speculate that a single gene mapping distal to *P* (*D15S12*) may be involved in similar developmental processes in humans and should be considered when evaluating patients with genetic disorders associated with cytogenetic abnormalities that extend distally from the PWS- and AS-critical regions in 15q.

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