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Effects of compound heterozygosity at the *Xpd* locus on cancer and ageing in mouse models

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ABSTRACT

XPD is a helicase subunit of transcription factor IIH, an eleven-protein complex involved in a wide range of cellular activities including transcription and nucleotide excision DNA repair (NER). Mutations in NER genes including *XPD* can lead to a variety of overlapping syndromes with three general categories of symptoms in addition to sun (UV) sensitivity: severe skin cancer predisposition as in xeroderma pigmentosum (XP), segmental progeria as in trichothiodystrophy (TTD) and Cockayne syndrome (CS), and a combination of both as in XP/CS and XP/TTD. Genetic background and compound heterozygosity are two factors potentially complicating straightforward interpretations of genotype–phenotype relationship at the *XPD* locus. Previously we showed that the presence of two different mutant *Xpd* alleles in compound heterozygous mice could in principle contribute to disease heterogeneity through biallelic effects, including dominance of one mutant allele over another and interallelic complementation between mutant alleles, in a tissue-specific manner. Here we report on the interaction between different mutant alleles in compound heterozygous mice carrying one XP/CS-associated allele (Xpd^{G602D}) and one TTD-associated allele (Xpd^{R722W}) relative to homozygous controls in an isogenic background over a range of metabolic and UV-induced DNA damage-related phenotypes. We found complementation of metabolic phenotypes including body weight and insulin sensitivity, but none for any of the measured responses to UV irradiation. Instead, we found dominance of the partially functional TTD allele over the XPCS allele in most aspects of the response to UV irradiation including sunburn and skin cancer *in vivo* or cellular proliferation and DNA damage foci formation *in vitro*. These data support to a model of genotype–phenotype relationship at the *XPD* locus in which interactions between different recessive diseases alleles are a potent source of disease heterogeneity in compound heterozygous patients.

1. Introduction

Defects in genome maintenance are associated with a number of pathologies ranging from cancer to metabolic syndrome to premature ageing [1]. Nucleotide excision repair (NER) is a DNA repair system that eliminates a wide diversity of helix-distorting DNA lesions including UV-induced cyclobutane pyrimidine dimers and (6–4) photoproducts, intrastrand cross-links and bulky chemical adducts. In addition, NER has been implicated in the repair of endogenously occurring oxidative lesions such as cyclopurines [2]. The NER reaction can be initiated by two different sets of recognition proteins. The first recognizes helix-distorting lesions anywhere in the genome, activating global genome repair (GG-NER). The second recognizes transcription-blocking lesions only on the transcribed strand at the site of the stalled RNA polymerase, initiating the transcription-coupled branch of the pathway (TC-NER) [3–6].

Abbreviations: AUC, area under the curve; CH, compound heterozygote; CS, Cockayne syndrome; GG-NER, global genome NER; IGF-1, insulin-like growth factor 1; IGF-1R, insulin-like growth factor receptor; IR, ionizing radiation; MDF, mouse dermal fibroblast; MEF, mouse embryonic fibroblast; mRNA, messenger RNA; NER, nucleotide excision repair; RRS, recovery of RNA synthesis; qPCR, quantitative real-time RT-PCR; SCC, squamous cell carcinoma; SSP, squamous cell papilloma; TC-NER, transcription-coupled NER; TFIIH, transcription factor IIH; TTD, trichothiodystrophy; UDS, unscheduled DNA synthesis; UV, ultraviolet radiation; WT, wild type; XP, xeroderma pigmentosum; XPA–G, xeroderma pigmentosum complementation group A–G (protein); XP/CS, XP combined with CS.

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XPD is a helicase component of basal transcription/DNA repair factor IIH (TFIIH). TFIIH consists of eleven subunits [7], which together form a multifunctional complex that is essential for multiple processes, of which basal transcription initiation and DNA damage repair *via* the NER pathway are two [8,9]. Mutations in *XPD* affect TFIIH function and are associated with an overlapping set of UV-sensitive disorders including xeroderma pigmentosum (XP), trichothiodystrophy (TTD), XP combined with Cockayne syndrome (XP/CS) or XP combined with TTD (XP/TTD) [10–14]. XP presents with pigmentation abnormalities and greatly elevated rates of skin cancer in sun-exposed skin, an increased frequency of internal tumors, and accelerated neurodegeneration in some but not all patients [12,15]. CS is caused by mutations in the TC-NER specific *CSA* or *CSB* genes and characterized by progressive post-natal growth failure, myelination defects resulting in severe neurodysfunction, and UV sensitivity but without a clear cancer predisposition [16–18]. The combined disease XP/CS is characterized by both the cancer predisposition of XP and the neurodevelopmental complications of CS [12]. Finally, TTD is a condition sharing many symptoms with CS, but with the additional hallmarks of brittle hair, nails and scaly skin [19–21].

Homozygous mouse models of TTD and XP/CS engineered to mimic known causative point mutations in *XPD* (R722W and G602D, respectively) in the mouse *Xpd* locus (henceforth designated 'TTD' ($Xpd^{R722W/R7222W}$) and 'XPCS' ($Xpd^{G602D/G602D}$) mice) recapitulate many disease characteristics generally in a mild form, notably developmental delay, reduced body size and mild segmental progeroid phenotypes including accelerated bone demineralization and kyphosis [22,23]. TTD mice also display characteristics of a dietary restricted-like phenotype with respect to liver gene expression profiles and reduced spontaneous cancer frequency, particularly lymphomas and pituitary adenomas [24,25]. As in the human disorders, XPCS mice are highly susceptible to UV-induced skin cancer at relatively low UV doses [23]. On the other hand, TTD mice develop UV-induced skin cancers only when subjected to a high dose of UV, and are therefore considered more susceptible to UV carcinogenesis than wildtype (WT) mice [26,27]. However, because existing studies on UV-sensitivity and skin cancer susceptibility were performed on mice and cells in different genetic backgrounds, the isolated effects of these mutations are difficult to disentangle from those of genetic background. This is one of the reasons for performing the studies described herein.

In addition to differences in environment (*e.g.* sun exposure) and genetic background, compound heterozygosity is a major confounding factor in interpretation of genotype–phenotype relationship at the *XPD* locus [28]. Many patients with NER deficiencies, including approximately half of those in the XP-D complementation group, are compound heterozygotes carrying a different mutation in each of the two *XPD* alleles. In the absence of a WT allele, genetic interactions between recessive alleles (referred to here as "biallelic" effects) can result in a variety of phenotypic outcomes ranging from dominance of one mutant allele over another to interallelic complementation between two different mutant alleles [29,30].

Biallelic effects in mouse models of NER disorders were first observed using engineered *Xpd* alleles modeling XP- or XP/CS-associated mutations that were embryonic lethal in a homozygous state likely due to reduced mRNA expression levels [28]. Combining these homozygous lethal XP/CS or XP alleles with a viable TTD allele results in compound heterozygous (CH) mice with a TTD mutation in one *Xpd* allele and an XP/CS or XP mutation in the other. For some phenotypes associated with these alleles in the homozygous state (brittle hair in TTD homozygotes; embryonic lethality in XPCS and XP homozygotes) the compound heterozygous mice are more like wildtype than either of the corresponding homozygous animals, consistent with interallelic complementation between mutant alleles. Other phenotypes (UV sensitivity, developmental delay) show dominance of one mutant allele (TTD) over the other (XPCS, XP). In severe NER progeria, for example in XPCS/TTD compound heterozygous animals in a NER deficient *Xpa* knock-out background, interallelic complementation between the two different *Xpd* allele products was observed for some progeroid phenotypes (perinatal mortality, glucose homeostasis, serum insulin-like growth factor 1 (IGF-1) levels) but not for others (dwarfism) [31–34].

Mouse models of NER deficiency have obvious limitations in their ability to recapitulate disease characteristics, including important differences in tumor spectrum (XP patients develop more basal cell carcinomas than squamous cell carcinomas), neurological complications (XPA mice do not develop neurodegeneration) and disease severity (TTD and XP/CS are much more severe in humans than mouse models). However, mouse models do offer the possibility of controlling for genetic heterogeneity and environmental exposure to a degree that is impossible in patients. Thus, biallelic effects in NER-related disorders are best investigated in isogenic mouse models under controlled environmental conditions. A limitation to previous studies involving compound heterozygosity in *Xpd* mutant animals was the use of alleles that were lethal in the homozygous state. An additional complication was the use of animals that were not fully backcrossed onto an isogenic background [28,32]. Moreover, in previous NER-deficient mouse mutants, different genetic backgrounds were found to be associated with significant differences in the severity of clinical features, with C57BL/6 being generally most severe [35]. Here, in order to overcome such limitations of previous studies, we used homozygous viable *Xpd* alleles of TTD and XPCS in an isogenic C57BL/6 background to isolate the contribution of biallelic effects to phenotypes observed in compound heterozygous XPCS/TTD animals. We examined a number of *Xpd* phenotypes including developmental delay, altered glucose homeostasis, cellular hypersensitivities to acute genotoxic stress and UV-induced carcinogenicity.

2. Materials and methods

2.1. Mice

C57BL/6 mice (ten to sixteen weeks of age with the start of the experiment) were kept under standard laboratory conditions (temperature 20–24 °C, relative humidity 50–60%, 12 h light/12 h dark) with three to four animals per cage before and after the experiment, and allowed free access to water and food (Hope Farms, Woerden, The Netherlands) except where noted. XPA, XPCS, TTD mice were described previously [22,23,36]. CH mice were derived from crosses between heterozygous XPCS and TTD mice. All animal experiments were performed with the approval of the appropriate ethical board.

2.2. Glucose tolerance and insulin sensitivity tests

Mice were fasted overnight prior to testing. Following baseline glucose determination from tail blood of restrained mice, animals were injected intraperitoneally with a bolus of glucose (18 mmol/kg body weight D (+)-glucose monohydrate, Fluka Biochemica, Germany), or insulin (0.75 U/kg body weight NovoRapid, Novo Nordisk, Denmark). Blood glucose determinations were performed as above at the indicated times following injection using a HemoCue glucose 201 RT blood glucose analyzer (HemoCue, Ängelholm, Sweden) according to the manufacturer's instructions.

2.3. Minimal UV dose required to induce edema

The hairless skin of the ear was put in contact with a filtered broadband UVB source (Hanovia Kromayer Lamp Model