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our assay, both Gli3C′ΔClaI, which approximates the PAP-A form, and Gli3C′ΔBal#8, which approximates the PHS form, bind Smads.

Our findings suggest a physiologically relevant interaction between C-terminally truncated Gli3 proteins and Smads. C-terminally truncated Gli proteins are nuclear repressors that may resemble endogenous processed forms (A.R.A., submitted). Thus, production of C-terminally truncated Gli3 mutant forms, as in PHS, may inhibit activating Gli function and may also affect the outcome of TGFβ-family signalling in tissues that express *Gli3*. How Gli-Smad complexes act, however, is not clear. Full-length or truncated forms of Gli1 or Gli3 were unable to differentially affect transcriptional activity from the BMP-inducible *Vent2* enhancer[13], the TGFβ/activin-inducible *Mix2* promoter[14], or by Gal4-Smad2 from a *Gal4* reporter gene[10] (data not shown). It remains possible that the Gli3-Smad complex has novel binding or transcriptional specificities. One role Smad-Gli3 (and possibly Smad-Gli2) complexes could have is to partially coordinate the actions of the two regulatory systems. For example, in instances where Shh and BMP signalling act antagonistically, BMP-induced dissociation of truncated Gli3-Smad complexes could induce a two-tier antagonism of the Shh pathway. On one hand, Smads would be free to bind specific partners to induce BMP-responsive gene expression. On the other hand, C-terminally truncated Gli3 proteins would antagonize the activating function of Gli1 and Gli2, and thus Shh signalling. In addition, complex formation may occur in the cytoplasm where inactive Smads normally reside[9], suggesting that depending on the relative abundance of each protein, Smads could render Gli3 repressors inactive by cytoplasmic sequestration until signalling occurs. The regulation of the production of C-terminally deleted forms would thus appear to be critical for determining the signalling outcome.

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MSH2 genomic deletions are a frequent cause of HNPCC

Hereditary non-polyposis colorectal cancer (HNPCC) is a common, autosomal dominant, cancer susceptibility condition characterized by early onset colorectal cancer[1]. HNPCC is caused by germline mutations in one of five DNA mismatch repair genes (MMR): *MSH2* (ref. 2), *MLH1* (refs 3,4), *PMS1* (ref. 5), *PMS2* (ref. 5) and *MSH6* (refs 6,7). To date, more than 200 different predisposing mutations in these genes have been characterized in HNPCC patients, the majority of which occur in *MSH2* and *MLH1* (ref. 8; see also http://www.nfdht.nl/index.htm). Here, we report that genomic deletions at *MSH2* also represent a frequent cause of HNPCC. In fact, these deletions comprise more than one-third of all pathogenic *MSH2* mutations among Dutch HNPCC families and account for 6.5% of HNPCC defined by the Amsterdam criteria.

In previous studies[9,10], we determined the prevalence of mutations at *MSH2* and *MLH1* among 184 kindreds, 92 of which comply with the Amsterdam criteria (AMS+) and 92 of which have a familial clustering of colorectal cancer reminiscent of HNPCC (AMS–). Approximately one-half (41) of AMS+ families revealed a pathogenic germline mutation in *MSH2* or *MLH1*, whereas only 6 of 92 AMS– families had a mutation in either gene. In the present study, the remaining 137 families, 51 AMS+ and 86 AMS–, were investigated by Southern-blot analysis of genomic DNA. We analysed *Nsi*I, *Eco*RI and *Hin*dIII genomic digests with two different *MSH2* cDNA probes encompassing exons 1–7 (5′ probe) and exons 4–16 (3′ probe), respectively. Eight patients, six of which are from AMS+ families, revealed aberrant restriction fragments with these enzymes when hybridized with a 5′ *MSH2* probe, thus indicating the presence of genomic rearrangements. Of the four distinct restriction patterns indicative of a genomic deletion in *MSH2*, three were identified in more than one kindred (Fig. 1). Hybridization with exon-specific probes revealed the presence of a deletion of approximately 2.1 kb encompassing exon 1 (NLB50162), a 5.4-kb deletion of exon 2 (NA17 and NA86), an exon 3 deletion of approximately 2.2 kb (NA64, NLB50696 and NLB51971) and a large (approximately 13 kb) deletion of exon 6 (NA33 and NLB50490; Fig. 1*a*–*c*). In four kindreds (NA17, NA33, NA64 and NLB51971) from which more family members were available, the genomic deletion co-segregated with the disease phenotype (data not shown). The deletions of exon 3 and 6 were confirmed by nucleotide sequencing of the shorter RT-PCR product from affected individuals (Fig. 2). No RNA was available from the carriers of the other deletions. To date, only two germline genomic deletions in *MLH1* have been described, both resulting from recombination between two *Alu*-repeats located in introns 12, 15 and 16 (refs 11,12). Moreover, a deletion of *MLH1* exons 4–19 has also been reported in a mismatch repair-deficient cell line[13]. All the filters we employed for the South-

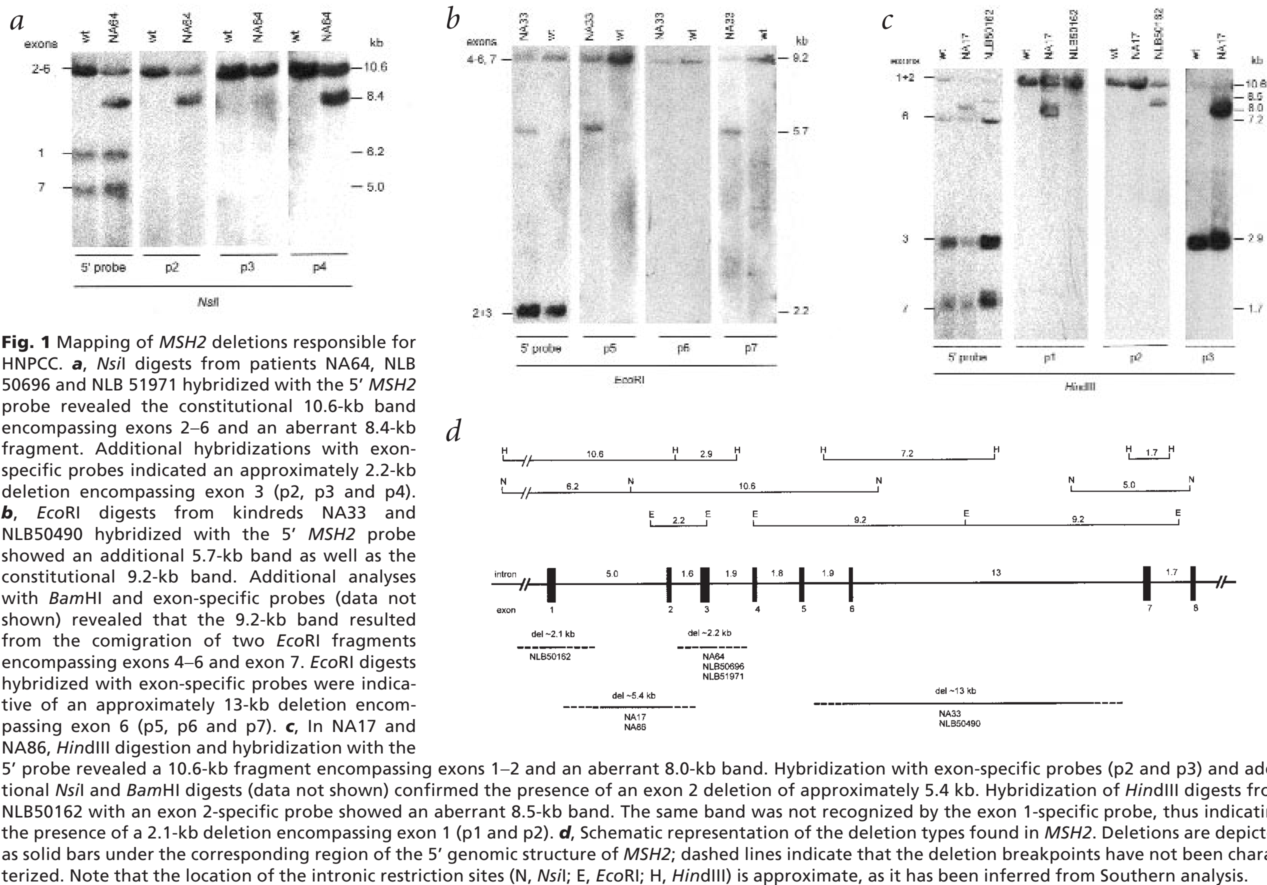

Fig. 1 Mapping of *MSH2* deletions responsible for HNPCC. ***a***, *Nsi*I digests from patients NA64, NLB 50696 and NLB 51971 hybridized with the 5′ *MSH2* probe revealed the constitutional 10.6-kb band encompassing exons 2–6 and an aberrant 8.4-kb fragment. Additional hybridizations with exon-specific probes indicated an approximately 2.2-kb deletion encompassing exon 3 (p2, p3 and p4). ***b***, *Eco*RI digests from kindreds NA33 and NLB50490 hybridized with the 5′ *MSH2* probe showed an additional 5.7-kb band as well as the constitutional 9.2-kb band. Additional analyses with *Bam*HI and exon-specific probes (data not shown) revealed that the 9.2-kb band resulted from the comigration of two *Eco*RI fragments encompassing exons 4–6 and exon 7. *Eco*RI digests hybridized with exon-specific probes were indicative of an approximately 13-kb deletion encompassing exon 6 (p5, p6 and p7). ***c***, In NA17 and NA86, *Hin*dIII digestion and hybridization with the 5′ probe revealed a 10.6-kb fragment encompassing exons 1–2 and an aberrant 8.0-kb band. Hybridization with exon-specific probes (p2 and p3) and additional *Nsi*I and *Bam*HI digests (data not shown) confirmed the presence of an exon 2 deletion of approximately 5.4 kb. Hybridization of *Hin*dIII digests from NLB50162 with an exon 2-specific probe showed an aberrant 8.5-kb band. The same band was not recognized by the exon 1-specific probe, thus indicating the presence of a 2.1-kb deletion encompassing exon 1 (p1 and p2). ***d***, Schematic representation of the deletion types found in *MSH2*. Deletions are depicted as solid bars under the corresponding region of the 5′ genomic structure of *MSH2*; dashed lines indicate that the deletion breakpoints have not been characterized. Note that the location of the intronic restriction sites (N, *Nsi*I; E, *Eco*RI; H, *Hin*dIII) is approximate, as it has been inferred from Southern analysis.

ern analysis of *MSH2* were also hybridized with *MLH1* cDNA probes to assay the presence of deletions in the chromosome 3p locus; no aberrant restriction pattern indicative of a genomic rearrangement at the *MLH1* locus was found with the restriction endonucleases used here.

Little is known about the number or location of *Alu*-repeats in *MSH2*, however, *MSH2* deletions encompassing exon 1, exons 1–6 and exons 4–8 have been detected[13–15]. The presence of recurring deletions in several HNPCC families could be indicative of a founder mutation or of the independent occurrence of the same rearrangement due to the presence of recombinogenic sequences such as *Alu* repeats. Haplotype analysis of the kindreds sharing the same deletions failed to show evidence of a founder effect (data not shown). These observations indicate that the deletions reported here arose independently through a common recombination event.

In the present study, the HNPCC series comprises 86 Dutch and 51 Norwegian families. The eight genomic deletions were found exclusively in kindreds of Dutch origin; thus, genomic deletions may show inter-ethnic differences and could be prevalent in the Dutch population, as was previously reported for *BRCA1* (ref. 16).

We had already reported 19 *MSH2* (16 in AMS+) and 28 *MLH1* (25 in AMS+) pathogenic mutations by DGGE (refs 9,11) in our set of 92 HNPCC and 92 HNPCC-like families. Therefore, *MSH2* accounts for 24% of HNPCC defined by the Amsterdam criteria, 6.5% (6/92) due to genomic deletions and 17% (16/92) to point mutations. Moreover, the eight genomic deletions comprise 30% (8/27) of all mutations detected in *MSH2*, and 36% (8/22) of those found among Dutch HNPCC families.

Our results indicate that genomic deletions at *MSH2* are a frequent cause of disease among HNPCC patients. These findings have implications for the improvement of HNPCC mutation screening protocols, as deletions will often escape detection by currently employed methods such as SSCP, DGGE, heteroduplex-analysis and direct sequencing of genomic DNA. RNA-based mutation detection technologies such as RT-PCR and PTT may also fail to detect such rearrangements due to complete deletion of the gene, or because of the instability of

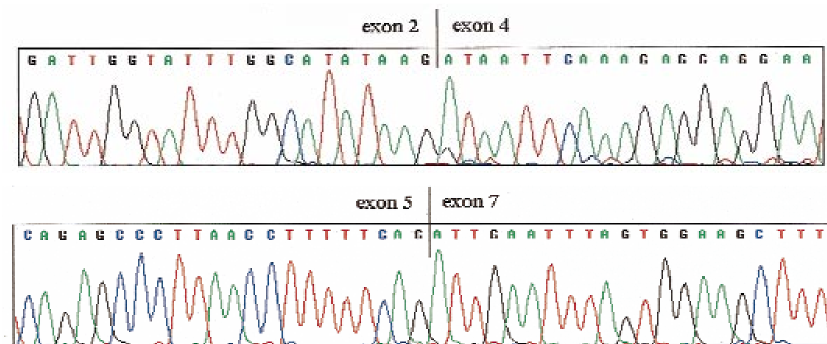

Fig. 2 Nucleotide sequence analysis of deleted *MSH2* transcripts. RT-PCR of exons 1–7 from patients NA64 and NLB50490 yielded the expected 1.3-kb fragment in addition to two shorter products of approximately 1.0 and 1.15 kb, respectively. Sequence analysis of the latter fragments revealed the loss of exon 3 (top) and 6 (bottom) sequences.

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the altered mRNA. Therefore, a thorough mutation analysis of *MSH2* should include the examination of its genomic structure by Southern analysis.

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Allelic variation in *ABCR* associated with Stargardt disease but not age-related macular degeneration

Age-related macular degeneration (AMD) is a potentially blinding disease that has been estimated to affect as many as 30% of people over age 65 (refs 1,2). Clinical manifestations of AMD include deposition of debris within and beneath the retinal pigment epithelium (RPE), atrophy of the RPE and haemorrhage and exudation beneath the retina from aberrant choroidal blood vessels. The latter complication (sometimes referred to as 'wet' AMD) occurs in approximately 10% of eyes with AMD overall, but is present in approximately 90% of eyes that have become legally blind from this condition[3]. There is no evidence at this time that patients with this complication have a pathophysiologically distinct form of macular degeneration.

Recently, mutations in a gene (*ABCR*) encoding an ATP-binding transmembrane transporter protein have been associated with Stargardt disease[4–6], an autosomal recessive retinal disease that, similar to AMD, affects the central retina (macula). Mutations in *ABCR* were later reported to be associated with up to 16% of AMD (ref. 7), although the methodology used in this study was controversial (http://www.sciencemag.org/cgi/content/full/279/5354/1107a). To further investigate the role of *ABCR* in Stargardt disease and AMD, we studied three populations: (i) 215 individuals with a clinical diagnosis of Stargardt disease; (ii) 182 patients with AMD diagnosed at the University of Iowa; and (iii) 96 unrelated subjects also from Iowa. The latter group was chosen to represent an ethnically matched sample of the population which would be expected to develop the population rate of AMD. Despite the loss of power, we decided not to choose an elderly, 'AMD free' control group, as this would require the subjective differentiation between the retinal changes involved in normal ageing and early AMD, a distinction which remains poorly understood. Sixty percent of the AMD group had, by the time of the study, developed a choroidal neovascular membrane in at least one eye. This rate of choroidal neovascularization is typical for a retina specialty clinic of a tertiary care hospital, and reflects the fact that the more severely affected AMD patients in a population are more likely to be cared for in such a venue than their less affected family members. Our study was designed to compare the three groups with respect to the proportion of non-conservative nucleotide

Table 1 • Distribution of *ABCR* sequence variants among three study groups

	Controls (n=96)	AMD (n=182)	Stargardt (n=215)	Total
non-conservative changes	26	59	204	289
conservative missense changes	12	20	92	124
synonymous codon changes	96	177	316	589
intronic changes	49	82	188	319
total	183	338	800	1321

51 Primer pairs[4] were used for SSCP analysis of the entire coding sequence as well as all exon-intron boundaries of the 50 exons of *ABCR* in 493 individuals. Amplimers showing a band shift were reamplified and sequenced using an ABI 373 automated sequencer. Non-conservative variants were defined as those that would be expected to cause a change in the charge, polarity, or number of amino acids of ABCR.

Table 2 • Individuals harbouring non-conservative *ABCR* variants

	Controls (n=96)	AMD (n=182)	Stargardt (n=215)
all non-conservative changes	26	57 (P=0.49)	137 (P<0.0001)
rare non-conservative changes	2	3 (P=1.0)	82 (P<0.0001)

The number of AMD and Stargardt patients harbouring one or more non-conservative *ABCR* variants was compared with the number of control subjects with such changes. *P*-values were calculated with Fisher's exact test. The 52 'rare variants' were all present in less than 1% of controls. The 3 common variants (Asn1868Ile, Arg943Gln and Ser2255Ile) were all present in more than 4% of all 3 groups.