From 1949 to 1995, the annual frequency of days exceeding the thresholds increased at most stations. These increases are largest for daily-minimum *A*, and the number of high heat-stress nights increased by 25% or more at some locations. The largest and most statistically significant trends occurred in some of the most populated areas, in the eastern and western thirds of the United States (Fig. 1).

The spatial distribution of the trends allows us to present results for three regions of the country (Fig. 1) bounded approximately by the Mississippi River and the continental divide (Rocky Mountains). Regional trends in the frequency of extreme daily-maximum *A* are substantially smaller than those for extreme daily minima, and are statistically significant only in the western region (Table 1). In the eastern and central United States, trends in extreme *A* are larger than trends in extreme *T*. In the western region, where summertime humidity increases are less marked[4], the *A* and *T* trends are similar.

These increases in single-day heat stress events are associated with increases in heatwaves, defined as runs of three or four consecutive days with daily-average *A* exceeding the 85th percentile value. On average, each weather station experiences 1.7 three-day and one four-day heatwaves per year. Upward trends in the frequency of heatwaves are highly significant ($P < 0.01$) in the eastern and western regions (Table 1) and indicate an increase of about 20% in the number of heatwaves over the period from 1949 to 1995.

These trends may be partly associated with increased urbanization. If the spatial extent of urban heat islands has been growing, weather stations at airports near large cities might experience high temperatures more frequently, especially at night[12]. However, the regional consistency of the trends suggests that their origins are not strictly local.

If these climate trends continue they may pose a public health problem[13], particularly as there are increasing numbers of elderly people, who are most vulnerable to heat-related sickness and mortality[10].

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p 3 pol mo phi m nd i ko i l n

Storey and co-workers[1] recently presented results indicating that the allele encoding arginine in the codon-72 polymorphism of the *p53* gene represents a significant risk factor in the development of cancers associated with human papilloma virus (HPV). The form of the p53 protein carrying an arginine residue at this position was found to be significantly more susceptible to degradation by the HPV E6 protein than by the proline form. Genotype analysis of 30 cervical tumours and 12 skin carcinomas revealed that the homozygous Arg/Arg genotype was overrepresented compared with 41 controls. We have now analysed this polymorphism in leukocyte DNA from a larger sample of cancer patients and controls but have found no significant overrepresentation of this genotype.

We analysed leukocyte DNA from 77 cervical-cancer patients[2] who were positive for 'high-risk' HPVs and 92 patients with cervical intraepithelial neoplasia (CIN) grades II–III, of which 72 were positive for 'high-risk' HPVs. For controls, we used 225 females who were also tested for the presence of HPV in DNA from cervical smears, and 109 patients with breast cancer. The CIN patients and controls were from a population-based case-control study[3].

To analyse the codon-72 polymorphism, we digested a 199-base-pair (bp) product from polymerase chain reaction (PCR) with the restriction enzyme *Bst*UI and separated the fragments by polyacrylamide gel electrophoresis[4]. Only the arginine allele is cleaved, giving two fragments of 113 and 86 bp, whereas the proline allele is not cut (the fragment remains 199 bp long). More than 100 samples have been analysed both by this method and by constant denaturant gel electrophoresis, giving the same results. Cytological specimens from the CIN patients and from the controls were analysed for HPV using nested PCR and type-specific primers[3]. The patients with breast cancer were included as additional controls.

Our results on genotype distribution are presented in Table 1. We did not find any significant overrepresentation of homozygotes for the arginine allele either among the cervical-cancer patients or the CIN II–III patients compared with controls. The frequency in patients with breast cancer was similar to the other controls. Comparison of the total patient group with cervical malignancy with the 334 controls, regardless of HPV status, revealed no significantly increased risk for women carrying the Arg/Arg genotype (odds ratio, 1.09; 95% confidence interval, 0.73–1.61; $P = 0.74$). The power of this study to detect a twofold increase in the susceptibility to HPV-associated malignancy for Arg/Arg homozygotes was 92%.

HPV-16- or HPV-18-positive cervical-cancer patients revealed an odds ratio of 1.24 (95% confidence interval, 0.51–2.97; $P = 0.74$) for the Arg/Arg homozygotes, compared to HPV-positive controls. The probability of detecting a sixfold-increased risk among HPV-positive Arg/Arg homozygous individuals, as found by Storey *et al.*, is 96% in our study. We were therefore not able to confirm that HPV-positive women carrying the Arg/Arg genotype have an increased risk of developing cervical cancer.

The frequencies of the *p53* codon-72 genotypes vary according to ethnic group. The frequency in our control group is similar to that found in a Swedish study[5]. Storey *et al.*[1] report frequencies similar to those found in a Japanese population[6]. As infection with cancer-associated HPV types is relatively common among cytologically normal women as well[7], other environmental or genetic cofactors are required for cervical carcinogenesis. It may be that the virus load or the status of HPV integration influences the susceptibility to HPV-associated cancers. An association with HLA specificity has been found among both cervical-cancer and CIN patients[8,9], and a strong interaction between tobacco smoke and HPV-16 is indicated[10]. Further investigation is needed in different ethnic populations to

l 1 Genotype distribution in *p53* codon-72 polymorphism

	o/ o	o/ g	g/ g
on ol (*n*=22)	13 (6%)	90 (40%)	122 (4%)
po i i on ol (*n*=29)	0 (0%)	14 (4 %)	1 (2%)
i l inom (*n*=77)	10 (13%)	23 (30%)	44 (7%)
po i i (*n*=72)	2 (3%)	29 (40%)	41 (7%)
n g i (*n*=19)‖	1 (%)	7 (37%)	11 (%)
inom (*n*=109)	6 (%)	40 (37%)	63 (%)

on ol po i i o 'high i k (12 on ol no p d).
i l n p i n po i i o 16 o 1 .
dd io 1.24 o g/ g homoz gou i l n p i n omp d ih on ol ; =0.74.
– p i n po i i o 'high i k .
‖ – p i n n g i o 'high i k (on p i n no p d).

determine the influence of this polymorphism on HPV-associated carcinogenesis.

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Storey and co-workers[1] claim that there is an association between a common variant of the *p53* tumour-suppressor gene and the development of invasive cervical carcinoma. Here we present evidence to refute this, based on a reassessment of the importance of the polymorphism at codon 72 in the *p53* gene for the development of cervical cancer.

We genotyped a large set of both *in situ* and invasive squamous-cell cervical cancer cases and controls. Our material was derived from a strictly population-based epidemiological study of women with a diagnosis of cervical cancer *in situ* ($n=488$), as well as age-matched controls ($n=626$). We also analysed samples from 63 cases of invasive cancer from the same population. All *in situ* cases were collected in the county of Uppsala, Sweden, and only women born in Sweden were included[2].

We found no statistically significant differences in the distribution of *p53* genotypes between the control women and patients with either *in situ* or invasive cervical cancer (Table 1). Homozygosity for arginine at residue 72 was not associated with an increased risk for *in situ* (odds ratio, 1.06; $P=0.648$) or invasive cancer (odds ratio, 1.12; $P=0.684$). We also compared the frequencies of *p53* codon-72 genotypes, using only cases and controls with a positive human papilloma virus (HPV)-16 history, as determined by amplification of DNA from archival smears using the polymerase chain reaction (PCR), and found no significant difference between them (Table 1). Thus, there is no indication that the *p53* genotype increases the risk of developing cervical cancer in HPV-16-exposed women

There are at least four possible explanations for the discrepancy between our results and those of Storey *et al.*[1]. First, their results could be due to chance. Our study was based on a sample size more than ten times larger than theirs and so gives a more accurate estimate of the association between the p53 polymorphism and cervical carcinoma. This allows us to detect a true odds ratio of two for association with the arginine-homozygote genotype with more than 95% certainty.

Second, selection bias may be introduced when a convenience sample of blood donors is used as a reference group. By contrast, our study was population based, so the controls were representative of the population in which the cases arose.

Third, the p53 polymorphism might be relevant only for women from certain populations. This would require that variants of the E6 protein differ in their ability to degrade p53, and that geographical differences exist in their distribution. But, given the similarity of British and Swedish populations, ethnic differences are unlikely to explain the discrepancy.

Finally, the DNA source and the techniques used for genotyping might have affected the results. Poor-quality DNA, such as that derived from formalin-fixed tissue, can inhibit PCR amplification, with failure being correlated with the length of the fragment[3]. Storey *et al.*[1] used a PCR product for the proline allele that is 25% longer than the product for the arginine allele. This length difference may cause a bias in the PCR in favour of the arginine allele and an overestimate of the number of homozygotes for arginine 72. Such a bias would have less influence on large pieces of DNA, such as that isolated from the blood of the controls. It is dangerous to use an allele-specific assay in a study when the quality of the DNA differs between patients and controls.

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Storey and co-workers[1] have reported data suggesting that individuals homozygous for arginine at residue 72 of p53 (p53Arg) are about seven times more susceptible to invasive cervical cancer than individuals who carry at least one proline at that position (p53Pro)[1]. These preliminary data were supported by *in vitro* evidence demonstrating that the E6 oncoprotein of human papilloma virus (HPV) degrades p53Arg more efficiently than p53Pro. We have now tested specimens from a total of 1,309 women in three studies for p53 polymorphisms. We find that p53Arg is not associated with an increased risk of preinvasive or invasive cervical neoplasia; indeed, there is a tendency for p53Arg to be associated with a decreased risk of neoplasia.

Participants were selected from three projects sponsored by the National Cancer Institute: (1) a 10,000-woman population-based cohort in Guanacaste, Costa Rica[2]; (2) a 24,000-woman cohort in Portland, Oregon, USA[3]; and (3) a 529-woman multicentre study of histological subtypes of cervical neoplasia in the eastern United States. We used a test based on the polymerase chain reaction (PCR) for p53 codon-72 polymorphisms (PCR primers were: p53.5, 5′-gaagacccaggtccagatga-3′; and p53.3, 5′-ggtaggttttctgggaaggg-3′) and single-strand-conformation polymorphism (SSCP) analysis to differentiate between alleles encoding p53Arg and p53Pro (ref. 4). Direct sequencing of 60 randomly selected specimens confirmed our SSCP findings.

In Costa Rica, women diagnosed with preinvasive cervical lesions (known as LSIL and HSIL, for low- and high-grade squamous intraepithelial lesions, respectively) or invasive cervical cancer had an approximately 2–4-fold smaller risk of disease if they were homozygous for p53Arg com-

Table 1 Distribution of codon-72 *p53* genotypes in patients and controls

	Controls		*In situ* cases		Invasive cases	
	n (%)	Expected (%)	*n* (%)	Odds ratio (95% CI)	*n* (%)	Odds ratio (95% CI)
Pro/Pro	69 (11)	(9)	41 ( )	0.77 (0.50–1.1)	( )	0.74 (0.27–2.02)
Pro/Arg	246 (39)	(42)	191 (39)	1.0 (n)	24 (3)	1.0 (n)
Arg/Arg	311 (0)	(49)	2 6 (2)	1.06 (0.83–1.36)	34 (4)	1.12 (0.6 –1.94)
Pro/Pro	12 (11)	( )	27 (9)	0.77 (0.36–1.66)	n.d.	n.d.
Pro/Arg	41 (36)	(41)	120 (39)	1.0 (n)	n.d.	n.d.
Arg/Arg	61 (4)	(1)	1 9 (2)	0. 9 (0. 6–1.41)	n.d.	n.d.

om on ol nd *in i u* x d om i l m ; h om ihin i i l n om o m lin ix d iop i . no p d min d mpli i ion o 164 p i gm n ollo d l g o h p odu h i ion nz m *i*. n.d. no d min d. il d m hod il l om h u ho . xp d inomi l(d – in g) p opo ion . dd io ( ) i h 9 % on id n in l ( ) l ul d.

l 1 **Distribution of and risk associated with *p53* codon-72 polymorphisms**

ud g oup	*n*	p 3 o/ o (%)	p 3 g/ o (%)	g/ o (9 %)	p 3 g/ g (%)	g/ g (9 %)
u						
opul ion	123	6.	4 .		44.7	
	1	16.1	.6	0.46 (0.1 1.2)	2 .4	0.26 (0.09 0.7)
	117	12.	4 .3	0.47 (0.19 1.2)	41.9	0.4 (0.09 1.2)
n	49	14.3	44.9	0.42 (0.14 1.3)	40.	0.42 (0.13 1.3)
u						
(−) no m l	109	3.7	29.4		67.0	
(+) no m l	10	.3	3 .0	0. 7 (0.16 2.0)	3.7	0.3 (0.10 1.2)
	93	.4	31.2	0.73 (0.1 3.0)	63.4	0.6 (0.17 2.)
HSIL	11	9.6	3 .7	0.47 (0.14 1.6)	4.	0.31 (0.10 1.0)
		u u u				
on ol	24	.2	40.0		1.	
	47	6.4	40.4	1.30 (0.3 4.)	3.2	1.30 (0.36 4.)
n		10.2	40.9	0. 2 (0.34 2.0)	4 .9	0.7 (0.32 1.)
u						
on ol	24	.2	40.0		1.	
	3	.7	2 .6	1.0 (0.21 .0)	6 .7	1. 0 (0.40 .3)
n	99	9.1	4 .	1.1 (0.46 2.6)	42.4	0.74 (0.31 1.7)

p 3 o/ o on id d h lin h n l ul ing im o l i i k ( ); 9 % on id n in l ( ) l ul d.
ndom mpl o h o i n (u n) popul ion.
n lud 93 pu d no inom 22 d no qu mou inom nd 19 o h hi ologi l p .
d no inom *in i u.*

pared with women homozygous for p53Pro (Table 1). The risk for heterozygotes was also roughly halved. These findings were reproduced in our Portland study, in which women who were heterozygous or homozygous for p53Arg had a 1.5–3-fold decreased risk of disease (Table 1).

Cervical cancers with a glandular component are known to be more strongly associated with infection by HPV-18 than are the more common squamous-cell carcinomas of the cervix[5], and Storey *et al.*[1] focused on the HPV-18 E6 protein. We therefore assessed the effect of p53 polymorphisms on disease risk by looking at tumour histology in our third case-control study of squamous cell tumours and adenocarcinomas of the cervix. We found no evidence that women with p53Arg were at increased risk of disease (Table 1).

There was no indication either of a positive association between p53Arg and cervical neoplasia in analyses restricted to participants who were positive for HPV-16. Analysis of pooled data from the three studies restricted to HPV-18 suggested only a weak, non-significant association between p53 status and cervical neoplasia, despite the 79% power of our study to detect a risk factor of seven, the estimate of Storey *et al*[1]. Among the 67 women positive for HPV-18, the prevalence of p53Arg was 40% among cytologically normal individuals ($n=10$), 59% among LSIL patients ($n=17$), 60% among HSIL patients ($n=20$), and 60% among cancer patients ($n=20$; $P=0.72$). The comparable proportion of HPV-18-negative controls carrying p53Arg was 54%.

Longitudinal data from our Costa Rican cohort were also analysed ($n=75$ women; median follow-up time was 44 months). We tested whether carriers of p53Arg had an altered risk of incident HSIL or of persistent LSIL during follow-up, but found no evidence for a detrimental effect caused by p53Arg (data not shown).

Given the consistent results from these three studies, it is unlikely that p53Arg is associated with any increase in susceptibility to cervical neoplasia.

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Storey et al. *reply* — These reports assess the frequency of the p53Arg allele in different populations and conclude that homozygous p53Arg is not a risk factor for cancer associated with human papilloma virus (HPV). The functional differences between the p53 isoforms that we have described[1,2] provoked our initial epidemiological study. As we concluded then, it is crucial that investigations should be extended to different populations, and we are encouraged that such studies are underway.

There are several potential reasons for the apparent discrepancies between our original study and those reported here. Our study comprised a smaller number of samples from a different population. In addition, rather than leukocyte DNA, we screened microdissected tumour material, in which we demonstrated that it was always the proline allele that was lost in cases of loss of heterozygosity. Furthermore, it is important to compare the different screening methodologies that have been used as this may also be a source of incorrect allelic assignment, which would lead to a bias towards a null hypothesis.

In such studies, the composition of the control population is a major factor affecting the outcome. We are now concentrating our efforts on populations with a higher frequency of the proline allele. Preliminary results from a Brazilian population strongly support our original observations. In addition, when the control population consists of cytologically normal but HPV-positive individuals, the association of the p53Arg allele with the risk of tumour development is greatly increased; a similar trend is reported here by Hildesheim *et al.* All of these factors need to be taken into consideration in any future studies of this polymorphism.

Continuing exploration of the differential effects of this polymorphism should further unravel the complexities of p53 function.

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