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Cheng-Hsien Chang
Kuei-Hsiang Lin
Min-Muh Sheu
Wen-Loong Huang
Huei-Zu Wang
Chen-Wu Chen

The change of etiological agents and clinical signs of epidemic viral conjunctivitis over an 18-year period in southern Taiwan

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The first two authors contributed equally to this study

C.-H. Chang · M.-M. Sheu · H.-Z. Wang
C.-W. Chen
Department of Ophthalmology,
College of Medicine,
Kaohsiung Medical University,
100 Shih-chuan 1st Road, Kaohsiung,
Taiwan

K.-H. Lin (✉)
Department of Clinical Laboratory,
College of Medicine,
Kaohsiung Medical University,
Kaohsiung, Taiwan
e-mail: Kuhsli@Kmu.edu.tw
Tel.: +886-7-3114449
Fax: +886-7-3114449

W.-L. Huang
Life Eye Clinic,
Kaohsiung, Taiwan

Abstract *Background:* Epidemic viral conjunctivitis is a highly contagious eye disease that occurs worldwide and is caused mainly by adenoviruses and enteroviruses. An 18-year analysis of the changes of pathogens and clinical signs in a subtropical and densely populated island presents certain special features. *Methods:* We retrospectively analyzed the clinical information and laboratory records of the conjunctivitis patients with positive conjunctival swabs from 1980 to 1997. *Results:* The positive rate of laboratory diagnosis of epidemic conjunctivitis was 50.0% (1,233/2,467). From 1980 to 1994, the predominant causative agent of adenoviral keratoconjunctivitis was adenovirus type 8 (Ad8), with six genotypes being evolved. Three of the new Ad8 genotypes each caused a new epidemic. After 1995 the predominant adenoviral pathogens shifted to Ad37 and Ad19, and no more Ad8 was isolated. Enterovirus type 70 (EV70) was isolated from four outbreaks of acute hemorrhagic conjunctivitis (AHC) from 1980 to 1984, but rarely in later years. Coxsackievirus A type 24 variant (CA24v), which first appeared in 1985, appeared later as the causes of four major epidemics of AHC from 1985 to 1994. The overall clinical symptoms of viral conjunctivitis were more severe in the 1990s than in the 1980s. *Conclusion:* In southern Taiwan, outbreaks of adenoviral keratoconjunctivitis caused by new genomic variants could be associated with the long-term endemic co-circulation of Ad8, Ad19, and Ad37, while epidemics of CA24v AHC were caused mainly by introduction of new viral strains from neighboring countries. The aggravation of host symptoms in the 1990s needs further investigation and close follow-up.

Introduction

Epidemic viral keratoconjunctivitis is a common infectious eye disease in the subtropical region where southern Taiwan is located. The viruses are isolated all year round from patients with conjunctivitis [6, 17]. This highly contagious disease is generally attributed to two categories of causative pathogens. The first group is adenoviruses, including adenovirus (Ad) types Ad8, Ad11, Ad19, and Ad37, which cause epidemic keratoconjunctivitis (EKC), and Ad3, Ad4, and Ad7, which cause pharyngoconjunctival fever (PCF). The other group is enteroviruses, including enterovirus type 70 (EV70) and coxsackievirus A type 24 variant (CA24v), which induce acute hemorrhagic conjunctivitis (AHC) characterized by an acute course and subconjunctival hemorrhage (SCH).

Taiwan is an island on the west Pacific Rim, at the junction of the Far East and Southeast Asia. With a high density of population (23 million in the 1990s) and warm climate, any contagious disease can spread quickly. A chronological analysis of the causative agents of epidemic viral conjunctivitis for nearly two decades in this subtropical island would improve our understanding of the evolution and host–virus interaction of the viral pathogens.

Adenoviruses are the viruses most often isolated from the epidemic viral keratoconjunctivitis. The types and genotypes have changed frequently. According to Kemp et al. [20], Ad8 was the predominant pathogen of adenoviral keratoconjunctivitis in North America and Europe before 1973, but then Ad19 and Ad37 gradually emerged as the dominating pathogens. However, the timing of the shift of predominant pathogen varied in different regions of the world.

The first outbreak of AHC caused by EV70 was in Ghana, West Africa, in 1969 [5]. In the following 3 years, there was a pandemic of EV70 in Africa, Europe, and Asia. It was not until the early 1980s, however, that America and Australia were affected in the second worldwide pandemic. EV70 was first isolated in Taiwan in 1971 [43]. After 1985, EV70 was hardly isolated as an etiologic pathogen of AHC anywhere in the world. The other causative agent of AHC, CA24v, was first isolated in Singapore in 1970 [21]. In contrast to EV70, CA24v was long restricted to Southeast Asia. Not until the mid-1980s was the Far East affected [30, 41, 46] To date CA24v outbreaks have been mainly restricted to the eastern hemisphere with only occasional reports in the western hemisphere.

In this study, we retrospectively reviewed the viral pathogens of epidemic viral conjunctivitis in southern Taiwan from 1980 to 1997. The clinical features of keratoconjunctivitis were also analyzed.

Materials and methods

From January 1980 to December 1997, a total of 2,467 consecutive specimens of conjunctival swabs were sent for laboratory diagnosis of clinically suspected epidemic viral keratoconjunctivitis in the Viral Laboratory of Kaohsiung Medical University Hospital. The clinical records and laboratory test results of these specimens were retrospectively reviewed. In the clinical record, the patient's age, sex, address, transmission patterns, dates of visit and disease onset, clinical symptoms and signs, and drug use before and after the visit were all recorded. Clinical symptoms include discharge, tearing, pain, foreign body sensation, and extraocular symptoms, and clinical signs include lid swelling, chemosis, conjunctival follicles, hemorrhagic patterns, keratitis patterns, and preauricular adenopathy. All the examinations were performed by senior ophthalmologists (C.-W.C., M.-M.S., C.-H.C., and W.-L.H.).

Laboratory diagnosis was made by culturing the transport medium of conjunctival swabs in HeLa, MRC5, and A549 cell lines. The viral culture consisted of two passages, 7 days for each passage. Types of the samples with positive cytopathic effect (CPE) were further determined by means of the neutralization test (NT) using specific antisera. For genotyping of adenoviruses, the nucleic acids were extracted according to Lin's method [23] and subjected to restriction fragment length polymorphism analysis using six restriction endonucleases, *Pst*I, *Bam*HI, *Hin*dIII, *Sal*I, *Sst*I and *Sma*I [2, 39, 40]. To determine the phylogenic relationship of CA24v viral strains isolated from each outbreak in Taiwan and those from different countries, the nucleotide sequence of the 3C proteinase ($3C^{pro}$) region was amplified by reverse-transcription polymerase chain reaction (RT-PCR) and analyzed using primers flanking the $3C^{pro}$ region, followed by autosequencing, GCG pileup program, and SINCA package (Fujitsu, Tokyo, Japan) [28].

From 1990 onward, RT-PCR diagnosis for CA24v and EV70 was used to screen all culture-negative samples. After 1995, all samples were subjected to PCR–restriction fragment length polymorphism (PCR-RFLP) for the identification and typing of adenoviruses [3, 35] and RT-PCR for the detection of both EV70 and CA24v in addition to culture isolation.

The proportions of patients presenting clinical signs of epidemic conjunctivitis caused by different etiologic agents or from different outbreaks were compared using Chi-square test or Fisher's exact test.

Results

Chronological analysis of viral agents causing epidemic conjunctivitis

Among 2,467 collected samples of conjunctival swabs from patients with conjunctivitis, 1,233 samples (50.0%) were positive for virus identification. The positive results were diagnosed either by culture isolation or by molecular diagnosis, e.g., PCR and RT-PCR. The numbers of each viruses identified in each year is shown in Table 1.

Table 1 Viral isolations of epidemic conjunctivitis from 1980 to 1997 in southern Taiwan

Years	Ad3	Ad4	Ad7	Ad8	Ad11	Ad19	Ad37	Adu[a]	EV70	CA24v	Total no. positive/ tested
1980–1981	16	1	1	149	14	12	17	1	22		233/482
1983–1984	32	48		74	23	32	6	9	59		283/692
1985	1									68	69/90
1986	1					1		1		139	142/181
1987				21	1	8	4	34	1	3	72/142
1988	5			19	2	17	2	7		74	126/233
1989	2			16		2	3			43	66/195
1990–1991				5	2		1	22		74	104/229
1994	2			16			1			72	91/110
1995–1996	0					1	14	3			18/43
1997	2					8	18	1			29/70
Total	61	49	1	300	42	81	66	78	82	473	1,233/2,467

[a] untyped adenovirus

Table 2 Adenoviral genotypes of Ad8, Ad19 and Ad37 from 1980 to 1997 in southern Taiwan

Years	Ad8						Ad19					Ad37		
	C	D	E	F	G	H	P	A	B	C	D	P	A	B
1980–1981	22	2	11	3				12				16	1	
1983–1984	1		16		1			24	1			4		1
1985														
1986														
1987			15					8				3		
1988			15			1		15		1	1	2		
1989			16					2				3		
1990–1991						5								
1994						16								
1995–1996								1				14		
1997							2	6				18		

Enteroviral conjunctivitis: Enterovirus type 70 (EV70) was detected in four outbreaks of AHC in 1980–1981 and 1983–1984. Thereafter EV70 was not isolated, except once in 1987. CA24v first appeared in 1985 and subsequently caused four major epidemics (including seven outbreaks) in 1985–1986, 1988–1989, 1990–1991, and 1994. Phylogenetic analysis revealed that the four major epidemics were attributable to three different clusters of genomic CA24v strains.

Adenoviral keratoconjunctivitis: Adenoviruses were detected almost every year during the 18-year period, the exceptions being 1985 and 1986, when CA24v was first prevalent. Ad3, Ad4, Ad7, Ad8, Ad11, Ad19, and Ad37 were detected, but Ad4, Ad7 and Ad11 were hardly isolated after 1984. The more commonly isolated types were Ad3, Ad8, Ad19, and Ad37, of which the genotypes from 1980 to 1997 are displayed in Table 2. From 1980 to 1994, the predominant type of adenoviral infection was Ad8, with either Ad19 or Ad37 in second place. In this period, the genotypes of Ad8 evolved from genotype C (Ad8C) to genotype H (Ad8H). Ad8C, Ad8E and Ad8H were the predominant agents of adenoviral EKC during the periods of 1980–1984, 1987–1989, and 1990–1994 respectively. In contrast, the predominant genotypes of Ad19 and Ad37 were consistently Ad19A and Ad37 prototype (Ad37P) despite the discovery of several new genotypes, namely Ad19B in 1983, Ad19C and Ad19D in 1988, Ad37A in 1981, and Ad37B in 1983. The RFLP pattern of genotypes of Ad19 is displayed in Fig. 1. From 1995 to 1997, no Ad8 was isolated and the predominant adenoviral pathogens shifted to Ad37 (68.1%) and Ad19 (17.0%), of which Ad37P and Ad19A were the predominant genotypes.

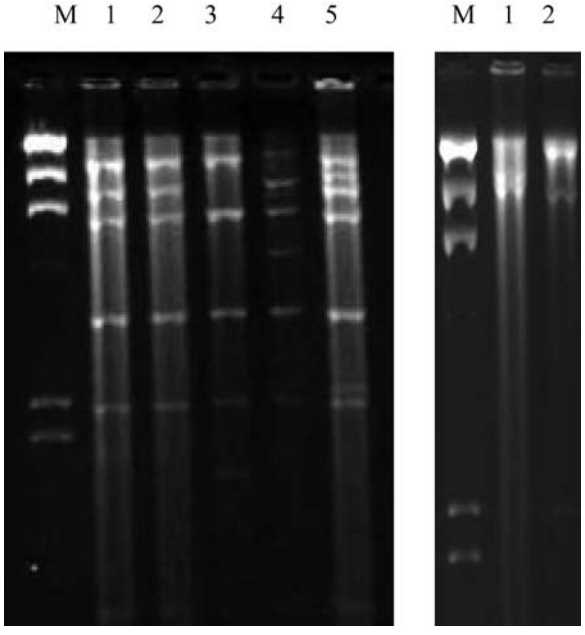

Fig. 1 RFLP pattern of Ad19 genotypes with *Hin*dIII (*left*), *Bam*HI (*right*), including adenovirus types Ad19P, Ad19A, Ad19B, Ad19C, and Ad19D. *Left: M* marker λ *Hin*dIII, *lane 1* Ad19P (AV-587), *lane 2* Ad 19A (023/88), *lane 3* Ad 19B (382/88), *lane 4* Ad19C (001/88), lane 5: Ad19D (152/88), *Right: M* marker λ *Hin*dIII, *lane 1* Ad19P, *lane 2* Ad 19A, B, C, D

Clinical signs of viral conjunctivitis

The main etiologic agents of the epidemic conjunctivitis in the 18-year period were Ad8C, Ad8E, Ad8H, Ad19A, Ad37P, EV70, and CA24v. Among patients infected with these viruses, conjunctival follicles were present in nearly 100% of cases, while subconjunctival hemorrhage (SCH), keratitis, and preauricular adenopathy (PAL) varied (Fig. 2). CA24v induced the highest incidence of SCH, overall 54.9% (211 of 384 patients). EV70 caused SCH in 44% of infected patients (26/59). SCH occurred in from 8.7% (Ad8C) to 33.3% (Ad8H) of cases of adenoviral conjunctivitis. Keratitis, defined as diffuse superficial epithelial keratitis, focal epithelial lesions, and subepithelial opacities, was observed in from 13.0% of cases (3/23 patients) in Ad8C- to 33.3% of cases (7/21 pa-

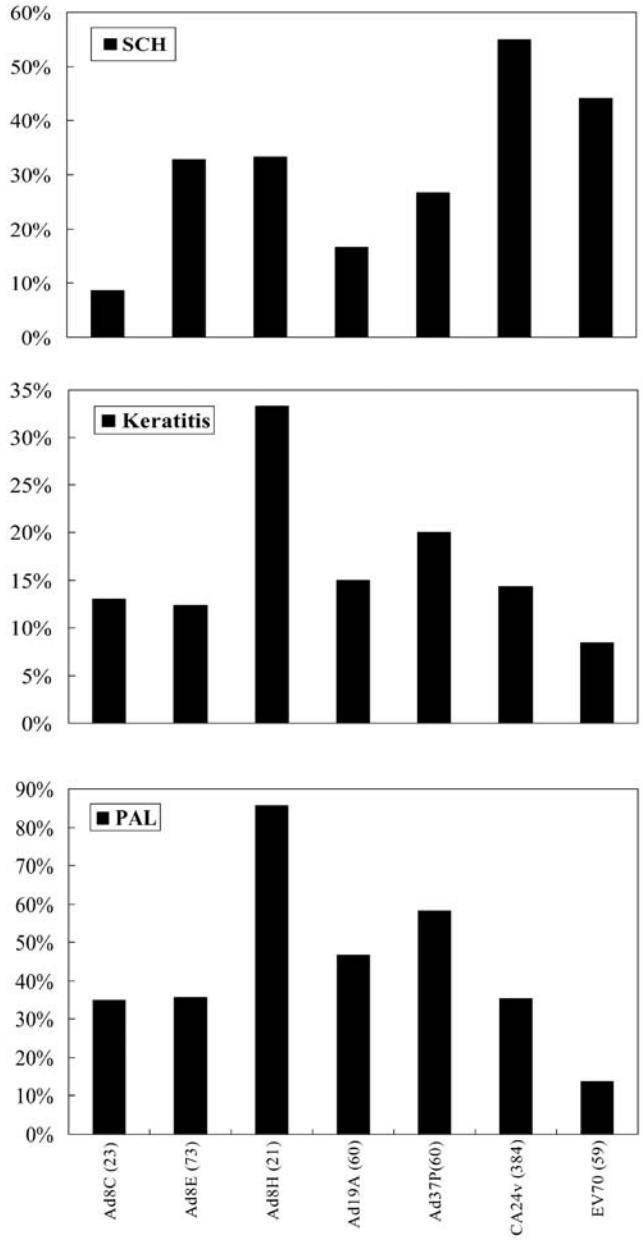

Fig. 2 The crude incidences (an accumulation of 18 years) of clinical signs of conjunctivitis caused by predominant viral pathogens. The incidences of follicles of all pathogens were 100% and are not shown in the figure. Chi-square test or Fisher's exact test. Follicles $P>0.1$, subconjunctival hemorrhage (*SCH*) $P<0.001$, keratitis $P>0.1$, preauricular lymphadenopathy (*PAL*) $P<0.001$. In parentheses, numbers of patients analyzed

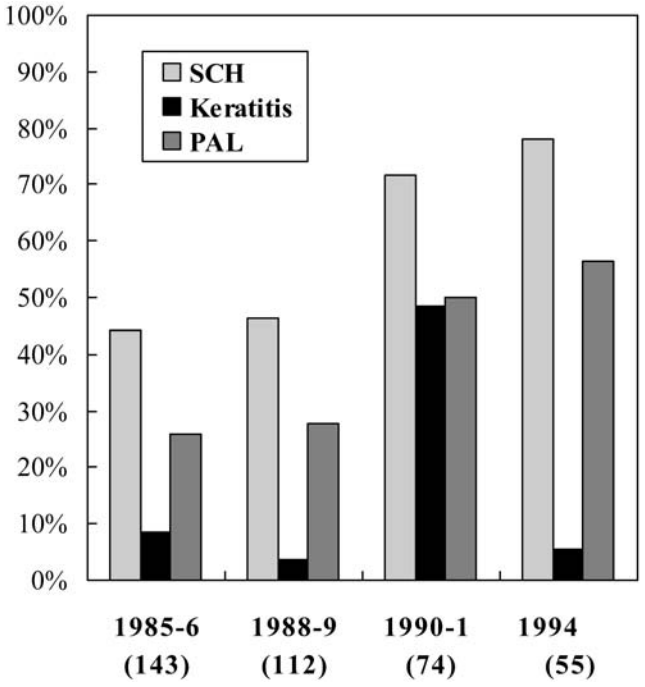

Fig. 3 The incidences of clinical signs of CA24v AHC stratified in each main epidemic. SCH and PAL were more evident in the outbreaks of 1990 and 1994. The incidences of follicles of all different main epidemics were 100% and are not shown in the figure. Chi-square test or Fisher's exact test. Follicles $P>0.1$, subconjunctival hemorrhage (*SCH*) $P<0.001$, keratitis $P<0.001$, preauricular lymphadenopathy (*PAL*) $P<0.001$. In parentheses, numbers of patients analyzed

tients) in Ad8H-associated keratoconjunctivitis. CA24v and EV70 induced keratitis in 14.3% (55/384) and 9.3% (5/59) of infected patients respectively. PAL was observed tin 85.7% of Ad8H conjunctivitis patients (18/21). Ad37P and Ad19A caused PAL in 58.3% (35/60) and 46.7% (28/60) of infected patients respectively. The incidence of PAL in conjunctivitis caused by Ad8C, Ad8E, and CA24v was around 35%. EV70 induced a lower incidence of PAL of 13.6% (8/59) in infected patients.

The data on clinical signs given above are a crude and accumulative incidence over 18 years. However, if the data on each form of viral conjunctivitis are further stratified and compared among different outbreaks, the clinical signs changed from one outbreak to another. SCH occurred in CA24v conjunctivitis with an incidence of 44.1% (63/143 patients) in the 1985–1986 outbreaks, and 46.4% (52/112 patients) in the 1988–1989 outbreaks, whereas the incidence increased to 71.6% (53/74 patients) in the 1990–1991 outbreaks and 78.2% (45/55 patients) in the 1994 outbreaks (Fig. 3). Similarly, the incidences of SCH, keratitis and PAL in adenoviral conjunctivitis increased from Ad8C (1980–1981) to Ad8H (1990–1994) infection (Fig. 2). Ad19 and Ad37 are generally considered to bear antigenic resemblance to one another and cause similar symptoms of conjunctivitis [11]. Therefore all the conjunctivitis cases caused by these two types were analyzed. Comparison of the conjunctivitis signs caused by Ad19/Ad37 in 1983–1984 and in 1995–1997 revealed significantly higher inci-

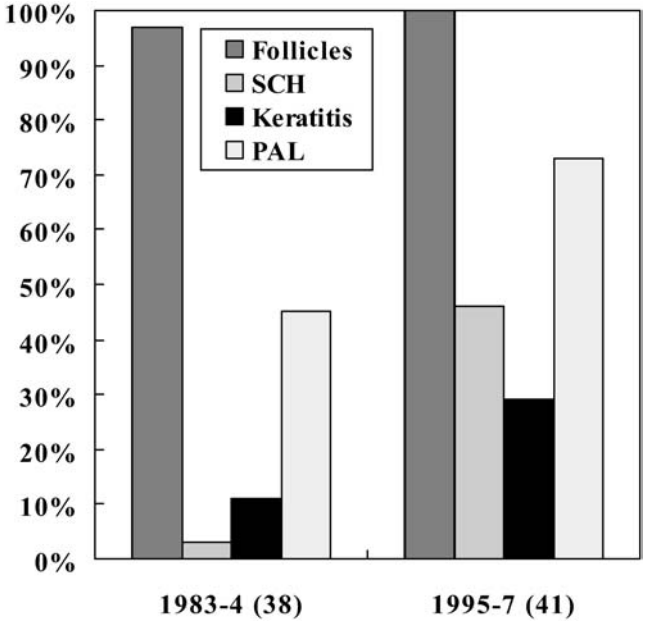

Fig. 4 Clinical signs of Ad19 and Ad37 EKC in the 1983–1984 and 1995–1997 outbreaks. Chi-square test or Fisher's exact test. Follicles $P>0.1$, subconjunctival hemorrhage (*SCH*) $P<0.001$, keratitis $P<0.05$, preauricular lymphadenopathy (*PAL*) $P<0.05$. In parentheses, numbers of patients analyzed

dences of SCH (3% vs 46.3%), keratitis (10.5% vs 29.3%), and PAL (44.7% vs 73.2%) in the latter period (Fig. 4).

Discussion

In this 18-year survey of epidemic viral keratoconjunctivitis in southern Taiwan, adenoviruses were the main etiological agents. Subgenus D of adenovirus, including Ad8, Ad19 and Ad37, comprised 66% of all cases of adenoviral keratoconjunctivitis, while Ad3, Ad7, and Ad11 of subgenus C and Ad4 of subgenus E were hardly isolated after 1984. However, subgenus D is not always the predominance of adenoviral keratoconjunctivitis in every geographical region. In recent reports from Japan (1982–1994) [36, 47] and Glasgow, Scotland (1981–1991) [32], Ad3 (subgenus B) and Ad4 (subgenus E) were the predominant pathogens of adenoviral keratoconjunctivitis, while Ad8, Ad19, and Ad37 accounted together for around 30–40% of cases or even fewer.

In southern Taiwan, the shift of the predominant type of adenoviral conjunctivitis from Ad8 to Ad19 or Ad37 occurred almost 20 years later than that in the western hemisphere reported by Kemp et al. [20]. However, it would be more appropriate to regard it as a multiple and alternative infection with Ad8, Ad19, and Ad37. Similarly, many outbreaks of Ad8 EKC were reported in the Far East, Australia and Europe from the 1970s to the 1990s [2, 8, 9, 12, 13, 14, 15, 16, 18, 29, 31, 34, 37, 39, 42].

During its 15-year period of predominance in southern Taiwan, Ad8 evolved into six genotypes, namely Ad8C through Ad8H. Ad8A (1975–1978) and Ad8B (1976–1981) were recovered in Sapporo, Japan [13]. Similar variability of the Ad8 genome was also found in France from 1983 to 1988, with several genotypes appearing in four consecutive epidemics [12]. However, prototype Ad8 (Ad8P, Trim type) was stable in geographically different areas of the world during this period [19, 33]. In contrast, Ad19 and Ad37, though having evolved into several genotypes, did not change their predominant genotypes, which were Ad19A and Ad37P. It would be of interest to investigate why Ad8 evolved quickly to several consecutive predominant genotypes and caused new outbreaks while the predominant genotypes of Ad19A and Ad37P remained the same.

The rapid shift from Ad8 to Ad19 and Ad37 has been speculated to have been caused by a special mechanism of recombination during replication in which two single-stranded DNAs from different parental adenoviruses may have hybridized efficiently in co-infected cells [20]. Systemic organ infection simultaneously with conjunctivitis is possible with Ad8, Ad19, and Ad37 [3, 16, 29, 31, 38, 44]. Adenoviruses can be isolated from throat, urogenital tract, and feces [16, 38]. Ad19 has been cultured from symptom-free eyes [16] and 12 months after onset of conjunctivitis [10]. Therefore, different types of adenovirus can exist in the same human body with an increased chance of genetic recombination. In southern Taiwan, the co-existence of Ad8, Ad19 and Ad37 over a long period might thus have predisposed to the creation of new genotypes.

The severity of conjunctivitis increased from Ad8C to Ad8H infection. Without any shift of genotypes, Ad19/37 keratoconjunctivitis presented more severe signs in the 1990s outbreaks than in those during the 1980s. Therefore it is difficult to tell whether the genomic alteration of viruses or a change in host immune responses caused the shift in patients' clinical signs.

In contrast to the year-round prevalence of adenoviral conjunctivitis, enteroviral conjunctivitis in Taiwan displayed several distinct outbreaks over the 18-year period. There were four epidemics of EV70 AHC in 1980–1981 and 1983–1984 and none thereafter, though we used both RT-PCR and the cell culture method to increase the sensitivity of detection owing to the difficulty of culturing EV70 [48]. Despite two epidemics in neighboring areas, i.e., Guangzhou, China in 1988 [41] and Okinawa, Japan in 1994 [45], EV70 was not re-introduced to Taiwan despite the high number of people traveling to and fro. According to a Japanese report [1], anti-EV70 antibodies in EV70-infected patients gradually declined over a period of 7 years. Serologic studies in southern Taiwan in the 1980s revealed that inhabitants' anti-EV70 antibodies

were positive in 34–46% of cases [22, 26]. It has been around 15 years since the last outbreak of EV70 in southern Taiwan. The herd immunity against EV70 may have decreased to a level such that an outbreak could occur at any time if EV70 were reintroduced to the island.

In contrast to the rapid spread of EV70 into Taiwan in 1971 shortly after the first outbreak in Africa, CA24v was first recovered in Taiwan from an AHC outbreak in 1985 [7, 24], almost 15 years after the initial outbreak in South Asia. Although two outbreaks (1971 and 1974) of CA24v AHC occurred in Hong Kong [4], which is geographically close to Taiwan, CA24v did not reach Taiwan. The absence of CA24v in Taiwan before 1985 was supported by another survey demonstrating generally low serum antibody titers against CA24v in this area prior to that time [26]. Phylogenetic analysis of the $3C^{pro}$ sequence of CA24v [24, 25, 28] revealed that CA24v was introduced into Taiwan twice in the 1985–1986 and 1988–1989 epidemics; the isolates were genetically close to isolates from Japan. The isolates from 1990s epidemics were genetically distinct from those of the 1980s and close to those from Thailand [28], indicating another introduction of CA24v into Taiwan.

Compared to the AHC associated with CA24v in 1985, the severity of the AHC in 1989 mildly abated [27]. However, the symptoms of CA24v-associated AHC of 1990–1991, especially SCH, aggravated so strikingly that they were mistaken for those of EV70 AHC. A similar fluctuation in severity of conjunctivitis has also been observed among different outbreaks of AHC caused by EV70. For example, in a recent (1994) outbreak of EV70-associated AHC in Okinawa, Japan, the conjunctivitis was found to be milder than before [45]. Study of the $3C^{pro}$ sequence may help delineate the phylogenetic relationships of viral strains from different areas or epidemics; however, it may not explain the fluctuations of their clinical signs. Further genetic investigation of these two hemorrhagic viruses would be of more clinical significance if the concurrent pathogenic change could be included as well.

In conclusion, it was noted in this long-term survey of epidemic viral conjunctivitis that the Ad8, Ad19, and Ad37 forms of adenoviral conjunctivitis co-circulated in southern Taiwan, each evolving several genomic variants. Outbreaks of adenoviral keratoconjunctivitis could be caused by the emerging inbred genotypes, while epidemics of enteroviral conjunctivitis were mainly caused by the introduction of new strains to Taiwan island from other areas. The general aggravation of conjunctivitis symptoms warrants close surveillance of the genetic alteration of the viruses.

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