Limited Genetic Diversity in the HLA Class II DP Region of the Atayal Tribe, with Dominant Alleles *DPA1*02022* and *DPB1*0501* by High Resolution Sequencing Based Typing

SHAOKEE WU[1,2] YU-MING SHIAO[2] CHENG-KUANG SHAW[3] KUAN-YI WU[1] CHEN-ING LEE[1]
ANNE-WIL VAN DER ZWAN[4] MARCEL G. J. TILANUS[4]

Departments of Medical Technology[1], Public Health[3], Institute of Aboriginal Health[2], Tzu Chi College of Medicine and Humanities, Hualien, Taiwan; Department of Pathology[4], University Hospital Utrecht, The Netherlands

*The polygenic HLA (human leukocyte antigen) gene complex is the most polymorphic cluster of functional human genes known. The degree of HLA polymorphism in an ethnic group determines the size of a donor registry for finding an HLA-matched unrelated donor for solid organ or bone marrow transplantation. In this report, we use sequencing based typing (SBT) to analyze the degree of polymorphism of the class II HLA-DP genes from Atayal tribe members living in the Hualien county (Taiwan) at 121° East and 24° North. DNA was isolated from peripheral blood cells from 60 Atayal individuals. HLA-DPA1 and -DPB1 alleles were identified by sequencing the polymorphic second exon. Three alleles were found for the DPA1 locus, among which 02022 had the highest allele frequency (59%), followed by 0103 (17%) and 02011 (9%). Five alleles were found for the DPB1 locus, with 0501 having the highest allele frequency (75%), followed by 0301 (9%), 1401 (7%), 02012 (6%) and 1301 (2%). Five two-locus haplotypes were inferred by a maximum-likelihood method: DPA1*02022-DPB1*0501 (57.7%), DPA1*0103-DPB1*0301 (9.2%), DPA1*02011-DPB1*1401 (7.5%), DPA1*0103-DPB1*02012 (5.8%) and DPA1*02011-DPB1*1301 (1.7%). These results suggest that there is linkage disequilibrium within the HLA-DP region in the Atayal tribe. Remarkable is the high frequency (55%) of individuals potentially homozygous for both DPA1*02022 and DPB1*0501 alleles. The Atayal tribe seems to show a very limited HLA-DP polymorphism. (**Tzu Chi Med J** 1999; **11**: 15-23)*

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Address reprint requests and correspondence to: Dr. Shaokee Wu, Department of Medical Technology, Tzu Chi College of Medicine and Humanities, 701, Section 3, Chung Yang Road, Hualien, Taiwan

INTRODUCTION

The human HLA gene cluster is located on the short arm of chromosome 6 [1]. Both HLA class I and class II regions contain several immunologically important genes specifying cell surface antigens which function in antigen presentation to educate developing thymocytes inside thymus and to activate mature T lymphocytes [1]. To fulfill these important physiological functions, HLA genes, among the known functional human genes, have evolved to become the most polymorphic gene cluster. As the consequences of these important functions, HLA genes also play important roles in the rejection of tissue grafts, and in the susceptibility of individuals to pathogenic and autoimmune diseases.

In the class I region, the polymorphic heavy chain gene HLA-A has 86 alleles, HLA-B 185 alleles, and HLA-C 45 alleles. In the class II region, the polymorphic DRB1 has 192 alleles, DQA1 19 alleles, DQB1 35 alleles, DPA1 13 alleles, and DPB1 83 alleles [2]. The numbers of alleles increase regularly due to the international effort in using the high resolution SBT to type minority ethnic groups. This high degree of polymorphism is a nightmare for transplantation medical biologists. In an era when bone marrow transplantation becomes feasible as a treatment regimen for hematological diseases, the decreasing family size makes it imperative to use unrelated bone marrow transplantation. In the United States where the first large-scale unrelated bone marrow registry was established, it has been estimated that the high degree of HLA polymorphism dictates the establishment of a registry containing at least one million volunteer donors in order to find a six-locus match (HLA-A, -B, and -DRB) at a probability of 84% for the white population [3]. On the other hand, the relatively homogeneous Japanese need a registry of only 50,000 volunteers to find a match at a probability of 82% [3]. For Chinese living in Taiwan, we estimated that it also needs a registry of one million volunteers to have an 80% matching probability [3]. Although the Tzu Chi Taiwan Marrow Donor Registry (TCTMDR) has an impressive record of recruiting more than 160,000 volunteers during a short period of four years, the probability of a six-locus matching is only 59% at present [3]. Furthermore, the TCTMDR recruits mainly the Taiwan (Han) Chinese, and registry for the minority aboriginal tribes remains to be established. To do so, information regarding the HLA allele and haplotype frequencies will be extremely helpful in order to determine the degree of genetic heterogeneity of the aboriginal tribes, and to calculate the practical size of the aboriginal registry. The indigenous people of Taiwan are broadly divided into two groups, the plains people and the mountain people. The mountain people are composed of nine ethnic groups with individually distinct languages, social organizations, and material cultures. These nine tribes are Atayal, Saisiat, Ami, Puyuma, Yami, Bunun, Paiwan, Rukai, and Tsou.

The HLA-D region contains several class II genes and has three main subregions: HLA-DR, -DQ, and -DP. The DP heterodimers are, among these three class II genes, expressed at a lower level on the cell surface and elicit much weaker responses in both antibody production and primary mixed lymphocyte reaction. DP was originally identified using the primed lymphocyte typing (PLT) method [4]. However, this allows for the definition of only six DP subtypes (DPw1 to DPw6). The application of polymerase chain reaction (PCR) and DNA-based typing technology has greatly facilitated DP typing. Currently, there are 13 DPA1 and 83 DPB1 alleles [2]. In terms of DNA-based typing techniques, three are now widely used. They may be used either individually or in combination. They are SSP (sequence-specific primers), SSOP (sequence-specific oligonucleotide probes) and SBT. Among them, SBT allows the unambiguous identification of new HLA alleles, which are more frequently found in the minority ethnic groups.

We have initiated a long-term project within the Tzu Chi organization to (1) find out the HLA allele and haplotype frequencies of the various aboriginal minorities in Taiwan in order to estimate the practical size of an aboriginal bone marrow registry, (2) study the genetic affinity among these aboriginal groups by using

HLA genes, among others, as the first group of genetic markers, and (3) establish SBT as a routine and reliable HLA typing technique. We report the initial successful application of using the high resolution SBT to type the HLA-DPA1 and -DPB1 alleles of the aboriginal Atayal tribe from members living close to the Tzu Chi College of Medicine and Humanities. We found that there is only very limited genetic diversity with characteristic dominant alleles *DPA1*02022* and *DPB1*0501*.

MATERIALS AND METHODS

Peripheral blood cells were drawn from 60 donors of the Atayal tribe living in the Showlynn hsiang, Hualien county at 121^0 East and 24^0 North. Genomic DNA was isolated using the Qiagen (Valenica, CA, USA) blood kit and quantitated by a Hoefer's (Hoefer Scientific Instrument, San Francisco, CA, USA) DyNA Quant 200 fluorometer.

The PCR technique was used to amplify the second exon of the DPA1 and DPB1 genes. Primers were synthesized on a 392 ABI oligo-synthesizer (Applied Biosystems, Foster City, CA, USA). Amplification primers are located within the first and second introns: DPA5 with the M13 universal primer (as indicated with lower case) at the 5' end (5' tgt aaa acg acg gcc agt ACA TTT TGT CGT GTT TTT CTC T 3'; the first A in ACA is 37-base upstream from the first base of the DPA1 second exon; see [5] for the DPA1 genomic sequence), DPA3 with the M13 reverse primer (as indicated with lower case) at the 5' end (5' cag gaa aca gct atg acc CTC TCA TCC CTT CCA GTT G 3'; the first C in CTC is 52-base downstream from the last base of the DPA1 second exon [5]), DPB5 primer (5' AGG ACC ACA GAA CTC GGT ACT AGG A 3'; A in AGG is 153-base upstream from the first base of the DPB1 second exon; see [6] for the DPB1 genomic sequence), and DPB3 primer (5' TGA ATC CCC AAC CCA AAG TCC CC 3'; T in TGA is 129-base downstream from the last base of the DPB1 second exon [6]). For DPA1 PCR, the reaction volume was 50 μL with 50 ng genomic DNA in 1X PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 8.4, and 1.5 mM $MgCl_2$) containing 1 U of AmpliTaq, 5 pmol of each primer, and 200 μM dNTPs. PCR was performed using a Perkin Elmer 9600 or 9700 Thermal Cycler with the following programs: 95°C for 2 min and then 30 PCR cycles (20 s at 96°C, 30 s at 54°C, and 45 s at 72°C). For DPB1 PCR, the reaction volume was 25 μL with 50 ng genomic DNA in 1X PCR buffer [18.7 mM $(NH_4)_2SO_4$, 75 mM Tris-HCl pH 9.0, and 1.75 mM $MgCl_2$] containing 0.75 U of AmpliTaq, 5 pmol of each primer, and 200 μM dNTPs. PCR was performed with the following programs: 98°C for 2 min and then a two cycling procedure. The first eight PCR cycles are 5 s at 98°C, 30 s at 64°C, and 60 s at 72°C, and the second 32 PCR cycles are 10 s at 96°C, 30 s at 64°C, and 60 s at 72°C. Positive PCR products were detected as a 335 bp band for DPA1 and as a 546 bp band for DPB1 by running a 1% agarose gel in 0.5X TBE buffer.

Cycle sequencing and allele assignment

The DPA1 PCR products were used directly for cycle sequencing. The DPB1 PCR products were diluted 1 to 10 in dd H_2O before performing cycle sequencing. For DPA1, the forward and reverse sequencing primers were the M13 forward and reverse sequence primers purchased from Perkin Elmer (the -21M13 TaqFS PE kit and the M13 reverse TaqFS PE kit; Perkin Elmer, Foster City, CA, USA). The cycle sequencing protocols in the kits were followed. For DPB1, the fluorescent forward sequencing dye primer is: 5' GAG AGT GGC GCC TCC GCT C 3' (the first G in GAG is 40-base upstream from the first base of the DPB1 second exon [6]). The DPB1 reverse sequencing dye primer is: 5' CCG GCC CAA AGC CCT CAC TC 3' (the first C in CCG is 22-base downstream from the last base of the DPB1 second exon [6]). The standard cycle sequencing protocol for dye primers from Perkin Elmer was followed using a Perkin Elmer 9600 or 9700 Thermal Cycler with the following programs: 96°C for 20 s and then a two cycling procedure. The first 15 PCR cycles were 10 s at 96°C, 5 s at 55°C, and 60 s at 70°C, and the sec-

ond 15 PCR cycles are 10 s at 96°C and 60 s at 70°C. After ethanol precipitation of the cycle sequencing products, the precipitate was resuspended in 4 μL of gel loading buffer (5:1 mixture of formamide and 50 mM EDTA, pH 8.0), denatured at 90°C for two minutes, quenched on ice and then loaded into wells on a standard 6% denaturing acrylamide gel of an ABI 373 or ABI 377 Automated DNA Sequencer. Data was collected and analyzed using the ABI sequencing data collection and analysis software.

Allele frequency, haplotype frequency and statistical analysis

Allele (i.e., gene) and two-locus haplotype frequencies were estimated using a maximum-likelihood method with the Imanishi's program [7]. Calculation of linkage disequilibrium (LD) and chi-square value follows the methods outlined in [7]. The p-values were corrected for the number of comparisons made (i.e., 15 potential haplotypes in this study) [7] and values less than 0.05 are considered as significant.

RESULTS

Allele frequencies of HLA-DPA1 and HLA-DPB1

Table 1 lists the DPA1 and DPB1 typing results of the 60 Atayal samples identified using the high resolution SBT method. Thirty-three individuals (55% of the samples) were typed for only one allele of these two genes (*DPA1*02022* and *DPB1*0501*). Similarly, there was one individual each typed for only *DPA1*0103/-* with *DPB1*0301/ -* and *DPA1*02011/ -* with *DPB1*1401/-*. Nine individuals were typed for only the *DPA1*0103* allele with heterozygosity at the DPB1 locus (0501/02012 or 0501/0301 DPB1 heterozygotes).

The allele frequencies (Table 2) were calculated [7] from the typing results shown in Table 1. Out of a total of 13 defined DPA1 alleles [2], only three alleles were found: 02022 at 59%, 0103 at 17% and 02011 at 9%. Out of a total of 83 defined DPB1 alleles [2], only five alleles were found: 0501 at 75%, 0301 at 9%, 1401 at 7%, 02012 at 6% and 1301 at 2%. These results show the potentially limited degree of allelic diversity in the DP genes, especially the DPB1 gene, in the Atayal members. Furthermore, the representation of both *DPA1*02022* (59%) and *DPB1*0501* (75%) is quite high.

Table 1. SBT Typing Results of 60 Atayal Members

n	DPA1	DPA1	DPB1	DPB1
33	02022	-	0501	-
1	0103	-	0301	-
1	02011	-	1401	-
6	0103	-	0501	0301
3	0103	-	0501	02012
7	02022	02011	0501	1401
4	02022	0103	0501	02012
3	02022	0103	0501	0301
2	02022	02011	0501	1301

n: number of individuals who were typed by SBT to have the indicated specific HLA-DPA1 and HLA-DPB1 alleles. The "-" sign in the table indicates a "blank" allele which may be a homozygous allele or an untyped and/or potentially new allele due to PCR failure in the annealing of the PCR primers.

Table 2. Allele Frequencies of HLA-DPA1 and HLA-DPB1†

Allele	n	N	AgF	AF
DPA1*0103	17	60	0.28	0.17
DPA1*02011	10	60	0.17	0.09
DPA1*02022	49	60	0.82	0.59
DPB1*02012	7	60	0.12	0.06
DPB1*0301	10	60	0.17	0.09
DPB1*0501	58	60	0.97	0.75
DPB1*1301	2	60	0.03	0.02
DPB1*1401	8	60	0.13	0.07

† n: total number with the indicated allelic phenotype from Table 1; N: total number of individuals in the sample; AgF: (phenotypic) antigen frequency; AF: allele (or gene) frequency as calculated by the Imanishi's program using a maximum-likelihood method [7].

Table 3. Two-locus Haplotype Frequencies of and Linkage Disequilibrium between HLA-DPA1 and HLA-DPB1 Alleles†

Haplotype	HF(%)	a_ib_j(%)	LD(%)	χ^2	Pc
DPA1*02022-DPB1*0501	57.7	43.9	13.8	66.5	5.2×10^{-15}
DPA1*0103 -DPB1*0301	9.2	1.5	7.6	80.6	4.1×10^{-18}
DPA1*02011-DPB1*1401	7.5	0.6	6.9	178.1	1.9×10^{-39}
DPA1*0103 -DPB1*02012	5.8	1.0	4.8	47.9	6.8×10^{-11}
DPA1*02011-DPB1*1301	1.7	0.1	1.5	29.1	1.0×10^{-6}

† Haplotype frequency (HF) is estimated by a maximum-likelihood method [7]; a_i is the allele frequency of DPA1 allele and b_j the allele frequency of DPB1 allele; a_ib_j is the expected haplotype frequency; linkage disequilibrium LD= HF - a_ib_j; $\chi^2 = (2N \times LD^2) \div [a_i (1-a_i) b_j (1- b_j)]$; N is the sample size and equal to 60.

Two-locus haplotype frequency and linkage disequilibrium

Table 3 lists the two-locus haplotype frequency (HF) as estimated using a maximum likelihood method [7], the expected haplotype frequency a_ib_j as calculated from the allele frequencies, the linkage disequilibrium value (LD), the chi-square value and the corrected p-value (Pc). Out of the 15 potential combinatorial haplotypes between the three DPA1 alleles and the five DPB1 alleles, only five two-locus combinations were obtained. They are *DPA1*02022-DPB1*0501* (57.7%), *DPA1*0103-DPB1*0301* (9.2%), *DPA1*02011-DPB1*1401* (7.5%), *DPA1*0103-DPB1*02012* (5.8%), and *DPA1*02011-DPB1*1301* (1.7%). All these five two-locus haplotypes show significant positive linkage disequilibria.

DISCUSSION

The results presented here suggest a limited HLA-DP polymorphism with potentially high degree of homozygosity (see next paragraph) in the Atayal tribe members. There were only three DPA1 and five DPB1 alleles identified. Fifty-five percent of the individuals analyzed were typed for only one allele of these two genes (*DPA1*02022* and *DPB1*0501* alleles); 58% potentially homozygous for both DPA1 and DPB1; and 73% potentially homozygous for DPA1. Both *DPA1*02022* and *DPB1*0501* alleles had the highest frequencies in the Atayal tribe members: the allele frequency of *DPA1*02022* is 59% and that of *DPB1*0501* is 75%. Furthermore, statistically significant positive linkage disequilibria were found for the following five two-locus haplotypes: *DPA1*02022-DPB1*0501* (57.7%), *DPA1*0103-DPB1*0301* (9.2%), *DPA1*02011-DPB1*1401* (7.5%), *DPA1*0103-DPB1*02012* (5.8%), and *DPA1*02011-DPB1*1301* (1.7%).

According to the Mendel's laws and Hardy-Weinberg equilibrium, 75% allele frequency of *DPB1*0501* has a theoretically expected *DPB1*0501* homozygous individuals of 56% (i.e., 0.75^2). In other words, the 33 individuals (55%, a value close to the theoretical 56%, of the 60 samples) who are typed as *DPB1*0501/-* are potentially *DPB1*0501/0501* homozygotes and the blank alleles in Table 1 may, in most of cases, be homozygous alleles, rather than untyped and/or potentially new alleles. This conclusion may also be expected by a different way of reasoning: there were already 13 DPA1 and 83 DPB1 alleles identified after typing various ethnic populations [2] and the number of potential new alleles may, thus, be minimal by now.

The potentially high degree of homozygosity in the Atayal tribe is in marked contrast to the well-known fact about the high heterozygosity index of HLA genes among major human populations [8]. This may reflect the facts regarding the potential founders' effect, the small population size of Atayal members (about 76,000),

relative geographical isolation in the central northeast area of Taiwan, and the inter-marriage among the Atayal members. By using SSOP to type the DPB1 locus (DPA1 was not tested), Hu et al [9] previously obtained results which are essentially similar to our findings of the high allele frequency of the *DPB1*0501* (84%) with limited number of DPB1 alleles.

Aldener-Cannava and Olerup recently typed and summarized the DPA1 allele distributions in the three major human populations: Caucasoids (Swedish), Negroids (Gambian) and Mongoloids (Japanese and Chinese) [10]. The Swedish had a high 0103 allele frequency (86.5%), the Gambian had a high combined 02011 (48.5%) and 0103 (32.5%) alleles of 81.0%, the Japanese had a combined 0103 (45.0%) and 02022 (38.8%) alleles of 83.8%, and the Chinese had a combined 0103 (43.8%) and 02022 (41.2%) alleles of 85.0%. The Atayals apparently have a different allele distribution with a combined 02022 (59%) and 0103 (17%) alleles of 76%. Compared with the patterns of both the Swedish and the Gambian, the DPA1 allele distribution of Atayals is more similar to the patterns of Japanese and Chinese as reflected by an increased 02022 frequency. Since Atayals are geographically much closer to both Japanese and Chinese, these results may be expected. However, change in allele frequency has occurred already: while both Japanese and Chinese have near equal allele frequencies of 0103 and 02022 with 0103 being slightly higher, the Atayals show a marked increase in the 02022 allele frequency (59%) and a corresponding decrease in the 0103 allele frequency (17%). Thus, the DPA1 allele frequency may be used to differentiate not only the three major human populations (Caucasoids, Negroids and Mongoloids), but also the subgroups of Mongoloids: the Austronesian Atayals vs. Japanese/Chinese.

A similar pattern was found for the DPB1 alleles. The Caucasoid CEPH (Center d'Etude du Polymorphisme Humain) families have a high percentage of 0401 allele (39.8%) and low percentage of 0501 allele (3.0%) [11]. The Negroid Zulu of central Bantu in Africa have a high percentage of 0101 (30.5%) and low percentage of 0501 allele (1.1%) [12]. Both Japanese [13] and Taiwan (Han) Chinese [9] are high in 0501 allele frequency (39.8% and 44.4%) and low in both 0401 (4.5% and 5.6%) and 0101 (0.2% and 0.0%) allele frequencies. The Atayals, similar to both Japanese and Taiwan (Han) Chinese, are high in 0501 (a much higher 75%) and low in both 0401 (0.0%) and 0101 (0.0%). Similar to the DPA1 allele distribution discussed above, the characteristic allele distribution of DPB1 may also be used to mark different human populations.

The pattern of linkage disequilibrium between DPA1 and DPB1 alleles in the Atayal members (Table 3) has similarly been noted in other ethnic groups [14]. This may be partially accounted for by the short physical distance of 2402 base pairs between DPA1 and DPB1 loci [6]. However, physical distance does not necessarily correlate with linkage disequilibrium. The TAP1 and TAP2 genes within the HLA class II region (and telomeric to the DP region) are separated by a distance similar to that between DPA1 and DPB1, but they do not show linkage disequilibrium [15]. A possible alternative explanation, among others, for linkage disequilibrium may be the constraints in cell surface heterodimer formation, as was reported for certain combinations of DQA1 and DQB1 alleles [16]. This would be interpreted as the primary reason of the strong linkage disequilibrium between alpha and beta chain genes, because individuals carrying new recombinant *cis* combinations of alleles may on the average have a lower fitness, resulting in selection against new haplotypes.

Recent results suggest that the polymorphism at both DPA1 and DPB1 loci may be more important functionally than originally thought [17]. Sequence variability in the DPA1 molecule was found to affect antigen presentation [18]. It was also recently found in the Thais that the presence of *DPB1*0501* allele may correlate with an enhanced vaccine-induced antibody response to an immunodominant repeat region of the *Plasmodium falciparum* circumsporozoite protein [19]. In the Japanese patients with early-onset Graves' disease, the *DPB1*0501* allele frequency increased significantly to 88.9%, while the frequency for normal controls in this

particular study was 55.0% [20]. Thus, while the relative high representation of *DPB1*0501* allele in the Asian populations may offer protective immune responses to intracellular pathogens such as *Plasmodium falciparum*, it may also confer susceptibility to certain autoimmune diseases such as Graves' disease. It deserves further investigation to see whether these observations also hold true for the Atayal members specifically and for the various Taiwan populations in general.

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使用高解析度的DNA定序法探討泰雅族人的HLA-DP型：以*DPA1*02022*及*DPB1*0501*型爲主的頻率分佈

吳紹基[1,2] 蕭育民[2] 蕭正光[3] 巫冠毅[1] 李建瑛[1]
Anne-Wil van der Zwan[4] Marcel G. J. Tilanus[4]

慈濟醫學暨人文社會學院醫技系[1] 原住民健康研究所[2] 公衛系[3]
荷蘭 Utrecht 大學醫院病理系[4]

人類的白血球抗原 (HLA) 基因是已知的功能基因中最具多態性的基因群。在非親屬間的器官與骨髓移植時，供體與受體間HLA配型成功的可能性乃取決於供體資料庫的大小。而所需資料庫的大小又決定於該族群HLA基因多態性的複雜程度。在本文中，我們使用DNA定序法(SBT)分析花蓮縣秀林鄉泰雅族人的HLA-DP基因。先從六十位泰雅族人的血液細胞抽取DNA，然後以DNA定序法定出DPA1與DPB1基因具有變異多態性的第二個表現序列。在DPA1方面，發現有三個等位基因：其中02022型有最高的頻率(59%)，跟著是0103 (17%) 及02011 (9%) 兩型。在DPB1方面，發現有五個等位基因：其中0501型有最高的頻率 (75%)，跟著是0301 (9%)，1401 (7%)，02012 (6%)及1301 (2%)型。 用最大可能性法可推論出五種雙基因位 (two-locus)的單倍體 (haplotype)：*DPA1*02022-DPB1*0501* (57.7%)，*DPA1*0103-DPB1*0301* (9.2%)，*DPA1*02011-DPB1*1401* (7.5%)，*DPA1*0103-DPB1*02012* (5.8%)及*DPA1*02011-DPB1*1301* (1.7%)。這些結果顯示：泰雅族人的DPA1與DPB1基因可能具有連鎖不均衡的特性。最引人注意的乃是高達55%的人可能是*DPA1*02022*與*DPB1*0501*的純合子型. 泰雅族人似乎僅有較簡單性的HLA-DP多態性。(**慈濟醫學** 1999; **11**: 15-23)

關鍵語：HLA-DPA 與 HLA-DPB 區域，連鎖不均衡，DNA 定序法的 HLA 定型，泰雅族，等位基因及單倍體頻率

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抽印本索取及聯絡地址：花蓮市中央路3段701號 慈濟醫學暨人文社會學院醫事技術系 吳紹基教授