DHPLC is a more efficient method than denaturing gradient gel electrophoresis (DGGE) [19-21] for detecting gene alterations. DHPLC has been used successfully to screen a single gene, *ABCA4*, for macular degeneration [22] and in 43 ADRP families [23]. In the present study, we used this method to screen all the exons containing reported mutations in the genes linked to RP. The utilization of the Wave system was amenable to the mass screening by validation test, and the introduction of this screening system significantly reduced the cost and labor for genetic diagnosis as compared to direct sequencing of all the corresponding exons, regardless of initial investments. By using the Wave system to prescreen likely candidates, we reduced the number of fragments required for sequencing analysis to one-fifth of the total number of screening exons. This ratio can be further reduced considering the fact that we used stringent selection criteria for fragments for sequencing in order to prevent the risk of missing any mutations. Thus far, we have reduced the total cost of gene diagnosis per patient to less than one-third of that required for complete direct sequencing. In cases wherein a genetic mutation was found in a patient, we determined whether this same mutation was also present in his/her family members, when available, and whether it co-segregated with the disease.

It should be noted that our large-scale screening revealed still less than 20% of the total patients investigated. We propose several possibilities: (1) we screened only those exons that harbored reported mutations, and the mutation spectrum may not accurately reflect the mutations found in Japanese population; (2) a large number of genes responsible for RP are yet to be cloned or identified; (3) mutations in the noncoding regions such as promoter regions were not included in the present study; and (4) the disease may reflect DNA copy number abnormalities, some of which are known to cause genetic disorders. For a better performance of mutational screening, the targets may be extended to all other genes/exons in the next stage; furthermore, the screening of chromosomal defects is a potentially complementary approach.

Using linkage analysis and location cloning or pathway candidate gene approaches, geneticists have discovered causing genes of inherited disorders. Still, genetic background has not been well elucidated in many complex diseases such as hereditary deafness and mental retardation that have a number of disease-causing genes. Our screening methods will be potentially useful for identifying genetic causes in other disorders with a wide range of responsible genes.

Identified mutations and spectrum

It is always problematic to predict whether the newly detected gene alterations are pathogenic, particularly when they are missense substitutions and when the segregation pattern among the family members cannot be tested. In such cases, the following aspects

are generally considered for evaluation: (1) if the same gene alteration is found in normal subjects, (2) if gene alterations are observed in conserved sequences among different species, (3) if the gene alteration is suggestive of protein malfunction, and (4) if the gene alteration is located within a "mutation hot spot" of pathogenic gene. However, even with all these considerations, we are far from conclusive prediction. In the present study, among 4 independent computational programs, we obtained fairly consistent results of 7 pathogenic and 1 neutral judgments among the 8 reported mutations. These results suggest that the clinical use of combined computational methods is a helpful tool to interpret missense variants in genes associated with RP.

An unexpected finding was the high frequency of mutations in the dominant genes in sporadic patients. As discussed above, autosomal recessive inheritance has been assumed to be the major cause of sporadic RP. The *RHO* gene mainly causes ADRP, except several mutations in exons 2 and 4 that have been reported in ARRP family [14, 24]. The mutation G174S has been reported to cause ARRP [14], and 4 other *RHO* mutations identified in this study are considered as dominant causes [25-27]. Among the 11 mutations identified in simplex RP patients, 7 are found in dominant genes, which are assumed to be *de novo* mutations, 3 in recessive gene, and 1 in X-linked gene. These findings lead to the argument that the genetic bases of sporadic RP also comprise a number of dominant mutations, which may be helpful for developing future screening strategy for simplex RP. A homozygous mutation in the dominant gene *CRX* was identified in a sporadic patient (No. 0003). The patient had severe RP symptoms; however, no other family member was affected with night blindness or visual impairment. Since we did not examine the patient's parents, a possible explanation is that the parents carried the same heterozygous mutation but did not exhibit apparent symptoms. Four other variants of the *RHO* gene (G89D, G174S, R135W, and N15S) identified in this study have been previously reported as causative mutations [14, 25, 27].

Additionally, we excluded a frameshift variant, c.72delG, in the *FSCN2* gene from the mutation list. This variant has been reported to cause ADRP in 3.3% of Japanese population [28]. Furthermore, a transgenic mouse line carrying the c.72delG mutation demonstrated haploinsufficiency of the *FSCN2* gene, which may hamper the maintenance and/or elongation of the OS disks and result in photoreceptor degeneration [29]. Nevertheless, other investigators stated that it is not a causative mutation because of the presence in normal controls [15].

Among the 52 simplex RP patients, 11 were found to have mutations (21.15%). Furthermore, among 141 probands from multiplex RP patients, mutations were identified in 14 probands (9.93%). A total of 28 probands including 1 patient with areolar atrophy

(14.07%) in 199 patients were identified to have mutations. These results represent the first survey of RP with unknown inheritance pattern. Six and 5 mutations in the *RDS* and *RHO* genes were identified in 9 and 6 unrelated patients, respectively, indicating the relatively high frequency of these mutations in the Japanese RP population.

Digenic mutations

Unexpectedly, we identified 3 digenic mutations in this study; this again raised the important issue of digenic inheritance, which was first reported in RP. Understanding the genetic basis of phenotypic variations in human population is currently one of the major but extremely challenging goals in human genetics. Conversely, a previous study on genetic subtyping based on clinical examinations of RP patients indicated that the 3 types of RP demonstrate some detectable phenotype distinctions [30]. This study improved the understanding of clinical features in different types of RP and thus provided considerably useful clues for genetic counseling. However, marked phenotype variations have been well known in either inter-individual RP or inter- and intra-familial RP. A good example is that several single amino acid substitutions in the *RDS* gene cause diverse disease severity, which has been proven in both affected patients [2] and animal models [31]. These findings prompted us to postulate that digenic mutation is a reasonable cause of phenotype variation in RP. In the present study, our screening strategy allowed to search for possible multigenic mutation in 30 known genes. Consequently, in 2 sporadic patients, we found 2 patterns of digenic mutation; one was the combined mutation in the $RPGR^{ORF15}$ (ORF15+568_571delAGAG) and the *CRX* (S152Y) genes in a male patient (No. 0107), while the other was in the *RHO* (G174S) and *RDS* (W316G) genes. Another digenic pattern with a heterozygous mutation in the dominant *RDS* gene and a heterozygous mutation in the recessive *PDE6B* gene was found in a multiplex family with RP and was co-segregated with the disease. All these mutations were not identified in 115 normal controls. The *RPGR* gene is responsible for X-linked RP or X-linked cone-rod dystrophy [32]. The g. ORF15+568_571delAGAG mutation is a frameshift mutation in exon ORF15, a mutation hotspot that harbors 60% of XLRP mutations. This mutation is predicted to result in an early termination protein. This mutation in patient no. 0107, a male sporadic patient, is postulated as a gonadal mosaic mutation. Other 5 single base substitution mutations were predicted as pathogenic mutations by computational methods (Table 2). The *CRX* and *RDS* genes are the dominant causes of RP, while the *RHO* G174S mutation and the *PDE6B* mutation may cause ARRP. Thus, it is tempting to speculate that a direct or indirect relationship exists between these mated genes or that one mutant is a modifier allele for the other one. It should be noted that we were unaware of the presence of anticipant "digenic pattern" distribution in the patient's family and we could not

provide direct evidence that the "digenic" patients who had distinct phenotypes compared to the "monogenic" patients carried one of the paired mutants. An in vitro or in vivo test is required to elucidate the functional importance of these combined mutations.

In conclusion, we established a new screening system based on dHPLC sequencing for comprehensive screening of RP-causing genes in RP patients, independent of the determination of specific inheritance trait. This efficient strategy that enables to screen more genes with lower costs may help to address the desire of RP patients to know the genetic cause of their affliction and to provide a more comprehensive understanding of the molecular factors that contribute to RP. Our study also demonstrated that a proportion of sporadic and multiplex RP patients had a broad spectrum of mutations and identified 3 possible novel patterns of digenic mutation. Further studies are required to improve the success rate of mutation identification and to identify complex genetic factors such as modifier gene(s), DNA copy numbers, or epigenetic factors.

ACKNOWLEDGMENTS

We thank all the patients their families for their participation in this study. We are thankful to Kaori Higuchi, Aiko Kido, Tsukiko Shoji, Katsuyuki Ohmori (Department of Clinical Laboratory, Kyoto University Hospital) and Kuniko Ishibashi (Center for Developmental Biology, RIKEN) for there excellent technical assistance. We also thank all the members in the retinitis pigmentosa clinic of Kyoto University Hospital for their assistance. This work was supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology (Japan), the Leading Project to M.T. and partly supported by Grants-in-Aid for JSPS Fellows (ZB. J.).

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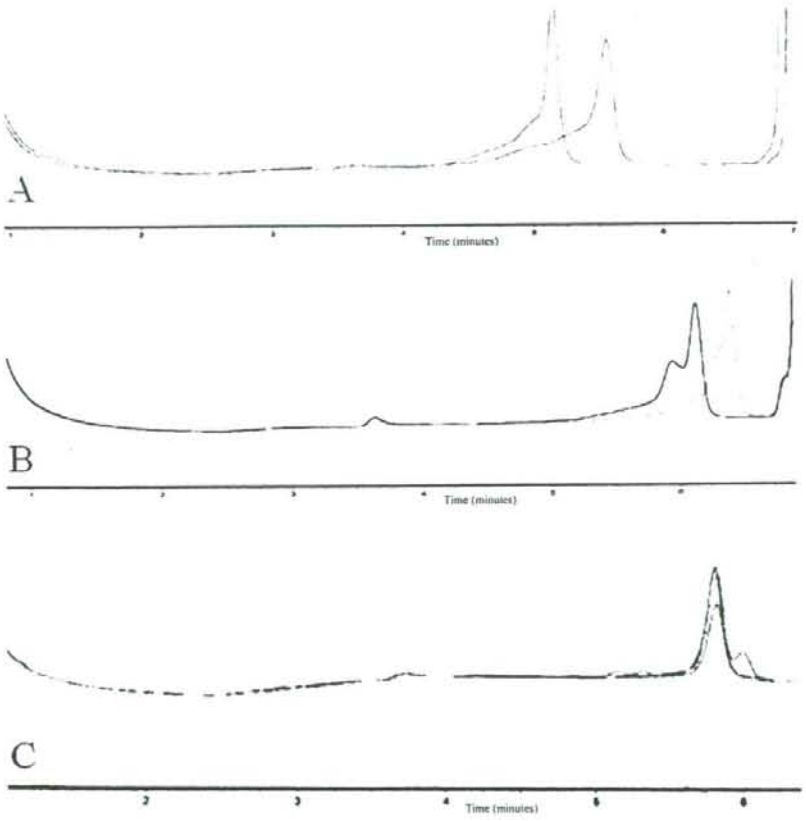
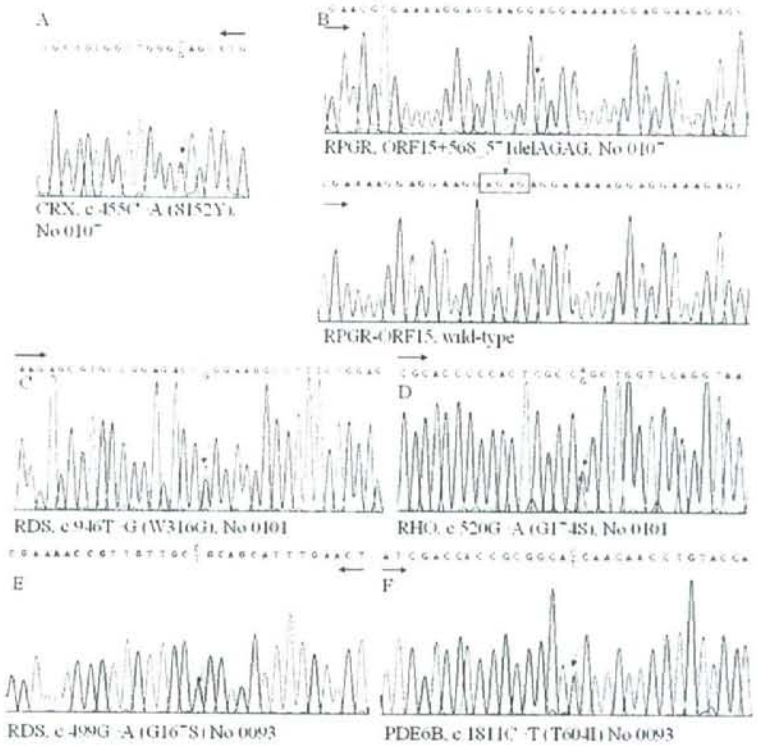
Legends

Figure 1. Representative examples of wild-type and mutant amplicons analyzed by Wave system.

A: PCR products from an exon of wild type sequence and analyzed by d-HPLC at 2 different melting temperatures.
B; PCR products from the same exon with artificially introduced 1 base pair mutation were mixed with the above wild type PCR products and were run on Wave system after hetero-duplex reaction.
C: detection of SNPs by mass analysis. DNA samples from 19 volunteer controls with no backgroud disease were amplified and the mixture of all the amplicons of selected exons were run on Wave System and compared with a couple of equivalent individual amplicons. *Some individual amplicons show a single peak pattern. **When PCR products of the same exon from the DNAs of 19 volunteers were mixed, 2-peak-pattern was presented, indicating the presence of SNP in the exon.

Figure 2. Digenic mutation patterns in retinitis pigmentosa patients
A and B: two mutations in the *CRX* and the *RPGR* genes in the patient 0107;
C and D: the patient 0101 had two mutations in the *RHO* and the *RDS* genes.
E and F: digenic mutation identified in a multiplex family (0093, 0116, 0117 and 0118) with disease co-segregation.


A
Time (minutes)
B
Time (minutes)
C
Time (minutes)


A
CRX, c.455C>A (S152Y), No.010
B
RPGR, ORF15+568_57delAGAG, No.010
RPGR-ORF15, wild-type
C
RDS, c.946T>G (W316G), No.0101
D
RHO, c.520G>A (G174S), No.0101
E
RDS, c.499G>A (G167S), No.0093
F
PDE6B, c.1811C>T (T604I), No.0093

Supplemental table 1. PCR primers for listed genes

Gene	Exon	Forward primer	Reverse primer
RP1	4	GAATTTGCTCAGTGTGGTTTAAC	CCTAAGCTTATTTTATTTAGTGATCTAG
RHO	1	CACGGGTCAGCCACAAGG	GGCTCATACCGCCCAGGGTG
	2	TAGGCAGTGGGGTCTGTGCTG	CTTCTGCTCAGTGCCATTACCTG
	3	CCCGGCCCGTACCTCCTTGAC	TCCAGGGAGGGAATGTGAAGC
	4	AGGAGCCATGGTCTGGACCCG	CGCAGTAGGCACCTGCTTG
	5	CAGTTCCAAGCACACTGTGG	GAAGGTGTAGGGGATGGGAGAC
FSCN2	1	TGAAGATGCCGACGAACGGCCTGCAC	CCTTGTCCTGGGTCGGGTTCC
RDS	1a	GAAGCAACCCGGACTACACTTG	CGTCGTAGCAGATCTTCCCAG
	1b	GGCTATCTGTGTTCTCTTCAACATC	TCTGACCCCAGGACTGGAAG
	2	AGCCCATCTCCAGCTGTCTG	TGGGACCGGAGGCTCTCCTT
	3	ATTGCCTCTAAATCTCCTCTCCCA	CAGGGCCCTCAGCCAGCCT
CRX	2	GTGGATGACCTGAGGGTCCTG	CTGAGATCACAGGAAGTGTCG
	3	ACCCCCATCTCCGCTCTTATCC	AGCCCAGCCCGCTGCGCCTCAG
HPRP3	11	GCAAATGCTGAAGTGATTGGATGGG	CCCAGAAGAGTTTGGGAAAGGTAC
IMPDH1	7	GTGGTCCACTTTCATCTTCACCC	CTCATAAACCTCCACTCTGCTGAAC
NRL	1	CCCTGGCCATGGAATATGTCAATG	CTCAGGCCAGCTTGCTGAC
PRPF8	42	AGGAGCCCTGTTAACATTGGCTG	TCAGGCATACAGGTCCTCCC
ROM1	1	CACCTCCTGGTCCAGCTAAG	CCAATCCTTGTACCCGTGGC
	2	GCCTCTATCTCCAGACATCCTAAC	GTGGGAGGAGGTGTCAGATG
RP9	5	CTTGTGATCCATGAAGCCTAACC	TGTTTACTGCACCATTCCTCT
	6	CATCCTATACTGCTTTTGAATGACA	GACTTGGAAGATTTGTGCTTCCG
RP11	5	AACTAATTCCCCTGGTCAC	AGCCCCGCCTGCCCCTTCA
	6	AGCAAGAGAGGTTCTCGAGCC	ATCCCATTAGGGCCCGGCGCCT
	7	CAGGCAGGCGGGAGATCCAGGAG	CATGGGACCCAGCCCGGGGA
	8	CTGCTTTCTTCTGACCGCCCCCCC	TCTCACGTCCCATGCCACCCTGTTCC
	11	AGCCGACTCCCTGGCGCCGCCCAC	TGGCGGCTGGCTGGCTGTG
RLBP-1	3	ACGTTCCGCATGGTACCTGAAG	GAAGGACCTTAGAACAGGCC
	6	CTGAGTCCCACTAGGAGGGATG	CCACAGGGTGGGAGCCAG
	7	TTGTTGCGGGTTAGATCTTACAGCC	CCGTGCGACAGAACTCTAAG
	8	AGCAGGGAATGAGTGGGAGCCT	CTCAGCCCTCTTGTCTCATTGTCT
LART	1	GACAACCGTGTTGCCCACATG	ATCTCCGCGGAGCCCACTTCC
	2	AGCCACCTTTCCTAATTTTCC	TTAGCCAGCCATCCATAGGAAG
MERTK	10	GCCAAGTAAGTCTAAAATAAATCAAAGG	TGGCAATGTCTGGCTATCTACTT

	14	ACCCACTCCCCTTAATTGAG	ATGTTGGGGTTATACGAAGTG
	15	TCTGGTCACAGTAACAAGGAC	AGCACTGAACAAAAGTCAACGTC
RPE65	1	GCCTCCCAAAGCCATAACTCC	GCACATTTATCATGAATCCATGAAGGTG
	3	GGATAAGAAGCAATGTTCTGTC	CCTCGTATCCATGCAGACAC
	4	CTTGATGAGGACACATAGAATGGCCA	ATGTCTTGAGTAACATTCAGTTTGGG
	5	ATCCGCACTGATGCTTACGTACG	TCTAAATTCCTGAACATCACCTAGC
	6	GTGCACTTAGGATGAGAGTTC	CTCACAATACAGTAACTTTCTC
	7	CCAAAGCCTTTTAAAACCACTTTATTTC	GTGGTATGCTCAGTTACAAG
	10	TGTCATTGCCTGTGCTCATG	TGAAACATTCTGGTTAAATCTG
	11	ATTCTTTCCTGCTCACTGAGG	GCATATGTGTAAGGTTTCCC
	13	GTATATGCTAGTCAAGTAAAGCAT	GAACTAACATACAGAACTGCAG
CRB1	2	CTGGGTACAGTGGGACAATCTGTG	TCCTATTTCATTGAGGCATGTAG
	3	GGAAATTGACGAATGTTGGTCCC	GAGAGCTCTAAATTCAACAGAGTGG
	6	AACAGGAATCCACTGCGAAG	GACCCACAGGAAGCCATCGC
	7	GAAGAGTATGTGGCAGGCAG	TTGCTAGTCTGCCGCGCTCTAG
	8	GACTTCTCCTGTTCCTGTCC	CCATTTCCTATCCAGACAGC
	9	ACGGTAATTAAGCAAACTATAGA	GCTCTGTCTCCCACATAAATATCTGT
PDE6B	1	TGGGAAGAAACTGAGCCCTGAG	TCCCTGAGGCTCCGCACAGAC
	3	ACAGCTTCCTGGCGTGTCTG	TCTGTGCAGGCAGCGAGATGG
	4	CAGGTGGTCAGATCAGGGAG	AATTCCTGGGCAGAGAAGAGGTGA
	5	AGGTGAGGCTTCCGTGGCTC	GGAAACAGAGGCGTGTGGACAG
	12	AGGTGTCTGAGGCTTGGCAG	ACCTTCTGGGGCACCTGAGG
	13	ACTGTGAAGTCAGCCACAGGTG	TGCACGGTGTCAGAGGGCGTTG
	14	AACCTCCAACCCGACGCCTAG	CATAGCCTGCTATGCGAAGCC
	15	ATCACTTGAGCCCAGGAGGTC	ATACCAACCTCCTCCGAGAGCA
	17	GTCTCCACACTTGCTCCCAC	TCTTCCTGCTCCAGAGCGTCT
	18	GGCAACAGAGCAAGACTCCATCTCA	ATGGTCCGTTGCCATCCGAG
	22	GTAGAGGTCACACCAGGCAG	AGGGTCCCCACGGGACCAGTG
PDE6A	7	CCTGCACTGAGAGTCACCGT	GCTTTTAGAGATCCACACTTGCCAT
	13	GTGTGTCCTGCCTCACTGATGT	GATTGTCTAGTGTGATGCAAGCCCA
	14	CCTGCTTCCAGCCCCTACACA	TGTGGGGAGGGAGGCTGACTC
ABCA4	13	AGCTATCCAAGCCCGTTCCC	AACTGGAGCCTCCGCCTGCA
	23	ATCCACGGGGAGGGAGGCAC	GGCTGCCAGACGGAACCC
	30	GGAGTACCCCTGTGGCAACTC	CCTGGGGCCCCGTTGTTTGG
	40	GGAAGAACCTGTTTGCCATGGTGG	CTAGTACTTTGGATACCCTGTAACC
CNGA1	7	GATGGTGACTACTGTGATTTCACTGC	CACATTGGCTCAAACTGGAAAGGAG

	9	CGAAAATAAAAACGACCCAGAGAAG	CCTAGAAACCTAATCCTCCTTGGAACC
	12a	CACAATGACATAAAAAGGAGAAACACTG	CAGGATCATTAATATCAGGGTAGACC
	12b	GGACAAACTATCCAAACATCTTCAGG	GTGTCTAAGTGAACGTTGATGGC
CNGB1	31	CTGGTGACTTTGGGGGATGAACA	TGCTGCATCTACCAAGCCGTAG
NR2E3	9	GCCATAACAGGCACCCCT	TTGGGCAGAGACTCAGTTAC
RGR	1	CAGCTCTCTCCGGTCTCA	AGGGGTCAGGCCCCAGGC
	6	GAGACCAGAGAGAGGATCAGTGGC	TCACTTGGTTCGGTCCTTCTCC
SAG	11	CTCCTCTGTTCTTCTTCCTCTAGAG	CCTGAGCCTCGAGGACTCAC
TULP1	2	AGGGAGACAGGCTGATCCCT	CTGTCCCACCCCTAGACCCC
	5	GAAGGGCAGGCTCCCCTT	AGCTCTCTCAGCACCCTC
	9	CTGCCCCCACCCAGAAGGAGGA	CAGGGAGAAATCAGGCCCGT
	11	CACAGCGTGGGTACAGCACT	CGGTCATGCGCCGGGGGCCA
	12	GGCTGCTCCCCTTACAGGGG	CTCCTGTTTCTCACATAGGGAGCC
	13	ACTCCCCTGGGGCACCCCAG	CTGGGCCCTCAGGTACTCAC
USH2A	2a	GCAGTAGCATTGTTTGTGTCTCGTCTAT	CTCCAAACATTTATTGCACTGTCTCC
	2b	GTGACACAGCTGGATCCCTCCCTG	GGAATGTCATTGTGCACTGAAAATGT
	3	GGGAATTAGTGCCTTGGTAGAG	GTGTGAGCCACCAAGCCGGG
	9	CTGCCAAGGTTATATTTTCACACTG	CAGAGTTAGTGAGGGAGGAGAAGAC
RP2	1	TGAGCTGGCCAACGAGCTC	CTGAGACGGCGGCTACACGACT
	2	CACTCTGCTACAGTTACCATTGATGAC	GTTGAGTTCTCCTGACACAGGTG
	4	CAATACATGGAGTAACATTCATGAC	TGGCATTTGCAATAGTGTAATCACC
RPGR	1	GTGTTAAGAAAATGTTTCTTGGG	CCAAGCAACATTAGGGACAAAAG
	2	GGCTGCCCGTACTGCCCGTG	CTACAGGGCCGATCCGGAGG
	3	CTCAAAAGTTATTTTAATAACAGGC	GTATTACTGTCCTTATTCAGG
	4	AGCTTTTTATTGCTTTGTGGT	GACATTAAAGAACTACACAGTC
	5	GTTACCATTTGTCCTGGACTAC	CAAAGCCACGTTACTGGAATG
	6	GTCTCATAAAAAGGGGGACTCTA	CAGGAAAGGAATGTGTCC
	7	TAGCTTCAGAGCCTGGCTACC	TAACAACATAGAAGTGGGAGATA
	8	TTTGACGGTAAGACCAGCT	TAGCCACCACAGAACGCAG
	9	AACGTGACAGTTTTTCCAGAT	TCTCAGCCATTAATTCCTTTAC
	10	CAAATAGATCCATACAAGTAACAC	TAAACAGGGAAATGTGATGCC
	11	TTGAAGCCAATGTTGATGAG	TTCTTCTAGTTTTCTTTGCAG
	12	TGTTGGCATACTTTGAACTTTC	AGGCACATTTATCCTGAGAG
	13	TTTCTGTTTTCTGTCCAGTTGC	ACACATTCACATACACAATGAAC
	14	CGGATTCTATTACTATCTGAAC	AACTTAAACTGCTCTCACCAAC
	15	GCTATTTTGATTCTCCTAAACT	TCAGTGTCAGCCTGAGGTCC

ORF15-1	CATACGGTATGGCAGGAAATTG	CCATCCTCTGCTTCTCCCACTG
ORF15-2	ATGGAATAGAGGAGCAAGAGGT	CCTCTTCTTCCATTCTTCC
ORF15-4	GGGGAGAAAGACAAGGGTAG	TTTTCCTCCCCTCTCCCCTCTG

BIOCHEMISTRY AND MOLECULAR BIOLOGY EDUCATION
Vol. 36, No. 6, pp. 395–401, 2008

Articles

Multimedia Presentations on the Human Genome

IMPLEMENTATION AND ASSESSMENT OF A TEACHING PROGRAM FOR THE INTRODUCTION TO GENOME SCIENCE USING A POSTER AND ANIMATIONS*

Received for publication, May 29, 2008, and in revised form, July 17, 2008

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Genome science, including topics such as gene recombination, cloning, genetic tests, and gene therapy, is now an established part of our daily lives; thus we need to learn genome science to better equip ourselves for the present day. Learning from topics directly related to the human has been suggested to be more effective than learning from Mendel's peas not only because many students do not understand that plants are organisms, but also because human biology contains important social and health issues. Therefore, we have developed a teaching program for the introduction to genome science, whose subjects are focused on the human genome. This program comprises mixed multimedia presentations: a large poster with illustrations and text on the human genome (a human genome map for every home), and animations on the basics of genome science. We implemented and assessed this program at four high schools. Our results indicate that students felt that they learned about the human genome from the program and some increases in students' understanding were observed with longer exposure to the mixed multimedia presentations.

Keywords: Human genome, genome science, multimedia presentation, poster, illustration, animation, teaching program, high school.

With the rapid development of genome science, the number of species whose genome has been decoded has increased exponentially, with genomes of over 700 species decoded to date [1]. The outcomes of these genome-sequencing projects are used extensively, for example, in the fields of life science, medicine, and industry. Biotechnology products such as genetically modified organisms, cloned animals, genetic tests, and gene therapy are now part of our daily lives. To better equip us to live with such developments, we need to understand and learn the fundamental knowledge and technology of genome science.

However, studies from different countries have shown that most students do not fully understand the basics of genome science (*i.e.* what a gene is, where it might be found, and how it relates to other structures) even if they started to learn from Mendelian genetics [2–6]. Banet and Ayuso suggested that the reason was simply the fact that many students do not understand that plants are made up of cells, contain chromosomes and genes, reproduce sexually, and also they had little interest in exercises involving Mendel's peas [3].

In Japan, students do not learn genetics until they undertake a standard course of biology (Biology 1) at high schools, where 97.7% (2007) [7] of graduates of junior high school (compulsory education) enter. It is only at this stage that they start to learn the basics of Mendelian genetics. To make things worse, they do not learn the relationships between chromosomes and DNA, and DNA and genes, although they learn the relationships between chromosomes and genes (Boveri-Sutton chromosome theory of inheritance and Morgan's genetic map). In this situation, it is expected that many students do not fully comprehend the basics of genome science, as other studies have reported [2–6].

To improve these situations, Banet and Ayuso [3] and Marbach-Ad and Stavy [4] suggested that it is important to use as many examples as possible that are related to human genetics. In particular, the human genome is a hot topic that can provide an interesting and coherent framework for the discovery of multiple layers of biological information [8]. Therefore, we plan to start teaching topics based on the human genome as the basics of genome science. To do this, we have chosen to use multimedia presentations.

Multimedia presentations are methods of presenting instructional messages in two formats—as words (spoken or printed text) and pictures (animations or

* This work is supported by KAKENHI (Grant-in-Aid for Scientific Research) on Priority Areas "Applied Genomics" from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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 DOI 10.1002/bmb.20231

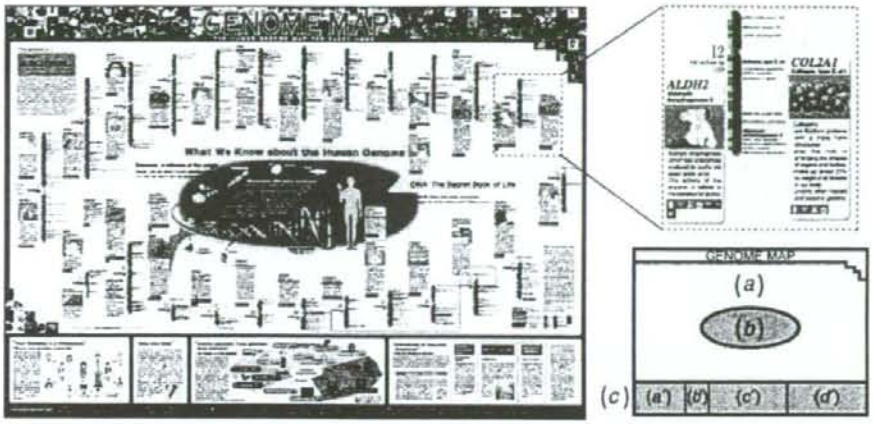

FIG. 1. **The genome science poster A human genome map for every home: what we know about the human genome contains three sections.** (*a*) Whole image of human genome. (*b*) Relationships between the terms "chromosomes," "DNA," "genes," and "proteins." (*c*) Four topics of genome science: (*a'*) Transferring the genome from generation to generation, (*b'*) single nucleotide polymorphisms (SNPs), (*c'*) diversity of the genome in various organisms, and (*d'*) application of genome science including ELSI. The poster is available for downloads from the web site http://stw.mext.go.jp/20080714 (in English) or http://www.lif.kyoto-u.ac.jp/genomemap/html/img/pdf/genomemap_A2.pdf (in Japanese). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

illustrations). It has been shown that students learn more deeply from words and pictures than from words alone, in both book-based and computer-based environments ("multimedia principle") [9, 10].

In particular, animations are new educational tools in biology teaching that allow students to learn effectively by calling attention to objects' simulated motion (*e.g.* representing biological processes) [11–14]. O'Day showed that animations with narration can lead to greater long-term memory retention than illustrations with printed text [14]. However, his work also suggested that the study of the value of animations in life sciences is fundamentally in its infancy [14]. In fact, he also showed that some students prefer to use a combination of both figures with text and animations with narration [13]. Stith also reported that learning issues based on definition (rote memory) are not enhanced by animations with narration but that principles based on the biological processes are [11]. Considering these findings, we believe that both animations with narration and illustrations with printed text have instructional value. Therefore, we decided to juxtapose the illustrations with animations.

In this study, we developed several kinds of multimedia presentations: One is a large poster with illustrations and printed text that allows students to view a whole image of the human genome, and others are animations with narration explaining the basics of genetics. These multimedia presentations were carefully designed in line with the "coherence principle," in which learning is increased when irrelevant information is reduced, and the "contiguity principle," in which learning is enhanced when words are placed in close proximity to pictures [9, 10], following Guideline 1 (take cognizance of current theories on how individuals learn from, and visualize, external representations) proposed by Schönborn and Anderson [15]. We then included them in a teaching program we have developed for the introduction to genome science and assessed this program.

MATERIALS AND METHODS

Producing and Distributing the Poster for the Introduction to Genome Science—A Human Genome Map for Every Home

As one of the multimedia presentations for the introduction to genome science, on April 14th of 2006, which was exactly 3 years after the date of declaration of completion of deciphering the human genome, we produced a large poster showing a map of the human genome "A human genome map for every home: What we know about the human genome" ("Ikka ni ichi mai hito genomu mappu: Koko made wakatta!! Hito genomu"). This was produced under the general editorship of the Japanese Educational Administration Agency, Ministry of Education, Culture, Sports, Science and Technology (MEXT)[1] of Japan. So far, over 140,000 posters have been distributed. Of them, 40,000 copies were sent to all elementary, junior, and senior high schools throughout Japan. In addition, PDF files of various sizes have been freely downloadable from the website version of a human genome map for every home (http://www.lif.kyoto-u.ac.jp/genomemap/).

This poster included three topics summarizing the basic knowledge of genome science (Fig. 1). On the basis of the origin of the word "genome," "gene + -ome (whole)" or "gene + chromosome," genome has a broader meaning. Therefore, the bird's-eye view of the whole image of the genome is essential for understanding the human genome. In the content (*a*), all 24 human chromosomes are illustrated and the locations of genes on each chromosome are indicated. However, as it is impossible to depict all genes (~28,900 genes including RNA genes and pseudo genes [16]) in this limited space, just as a world

[1] The abbreviations used are: MEXT, Ministry of Education, Culture, Sports, Science and Technology; ELSI, ethical, legal, and social implications; CDB, Center for Developmental Biology.

map cannot show all of the cities on earth, we selected 266 genes and positioned them on the map-like metropolises in a world map. As the names of genes are difficult for the public to understand, we gave them aliases. We also selected 35 genes and added detailed descriptions and attractive illustrations to help describe their biological function. The aliases and descriptions were checked by consulting a number of researchers.

Hence, the human genome map poster is an epitome of a genome-wide map at a scale of 1:100 and it provided the viewer with an overview of the essence of the human genome. The illustration of content (*b*) allowed readers to visually understand the relationships between chromosomes, DNA, genes, and the products of genes. In addition, one can also understand them in words after reading the printed text. The content (*c*) was prepared for the purpose of generating interest in how the human genome is inherited (*a'*), how genomes differ between people (*b'*) and other species (*c'*), and application of genome science including ethical, legal, and social implications (ELSI) (*d'*).

The original poster had limited use because the text was written in Japanese. Therefore, we produced an English version of "a human genome map for every home" and released it through the MEXT in July 2008 so that other instructors around the world could use it (http://stw.mext.go.jp/20080714/).

Producing Seven FLASH Animations on the Basics of Genome Science

Besides the human genome map poster, we also produced seven animations on the basics of genome science using FLASH (Adobe, San Jose, CA) as a multimedia presentation for the introduction to genome science. In addition, we produced an English version of animations without narration for other instructors around the world. The FLASH animations are freely downloadable from the Web (Table I).

Developing a Teaching Program for the Introduction to Genome Science

We developed a teaching program for the introduction to genome science using mixed multimedia presentation: The poster, a video associated with the fourth edition of Molecular Biology of the Cell (Alberts *et al.*, 2002; 18.2 Animal Cell Division), and 10 animations. Of the 10 animations, we produced seven, and three animations on the structure and function of cadherins were produced by the RIKEN Center for Developmental Biology (CDB;

TABLE I
List of animations used

No.	Title	Animation length (s)	Content of the animation	Web address
Basics of genome science (animations that we produced)				
1	The genome is enclosed in the cell	37	The location of genome DNA. Relationship between double-helical DNA and nucleosome	http://www.lif.kyoto-u.ac.jp/labs/biosoc/7animations/animation1.html (in English) http://www.zinbun.kyoto-u.ac.jp/~kato/atgenome/cont02/p03.html (in Japanese)
2	Chromosomes are folded genome DNA	33	The relationship between chromosome and genome DNA	http://www.lif.kyoto-u.ac.jp/labs/biosoc/7animations/animation2.html (in English) http://www.zinbun.kyoto-u.ac.jp/~kato/atgenome/cont02/p05.html (in Japanese)
3	The genetic information is coded as A, T, G, C	38	DNA is composed of four kinds of nucleotides and their sequence codes the genetic information	http://www.lif.kyoto-u.ac.jp/labs/biosoc/7animations/animation3.html (in English) http://www.zinbun.kyoto-u.ac.jp/~kato/atgenome/cont02/p04.html (in Japanese)
4	Nongene DNA?	36	DNA is composed of both gene and nongene regions	http://www.lif.kyoto-u.ac.jp/labs/biosoc/7animations/animation4.html (in English) http://www.zinbun.kyoto-u.ac.jp/~kato/atgenome/cont02/p06.html (in Japanese)
5	Are genes secret codes?	35	Genetic information codes the sequence of amino acids (protein)	http://www.lif.kyoto-u.ac.jp/labs/biosoc/7animations/animation5.html (in English) http://www.zinbun.kyoto-u.ac.jp/~kato/atgenome/cont02/p07.html (in Japanese)
6	Individual variation of the genome	50	99.9% of our genome is the same. The rest of the genome cause individual differences	http://www.lif.kyoto-u.ac.jp/labs/biosoc/7animations/animation6.html (in English) http://www.zinbun.kyoto-u.ac.jp/~kato/atgenome/cont05/index.html (in Japanese)
7	The influence of SNPs—an example	205	Single Nucleotide Polymorphism (SNP) of ALDH2 gene determines how much you can tolerate alcohol.	http://www.lif.kyoto-u.ac.jp/labs/biosoc/7animations/animation7.html (in English) http://www.zinbun.kyoto-u.ac.jp/~kato/atgenome/cont05/p03.html (in Japanese)
Structure and function of cadherins (produced by RIKEN CDB)				
8	Molecular structure and function of cadherin	102	Molecular structure and function of cadherin	http://www.cdb.riken.jp/en/cadherins/wmv/scene2e.wmv (in English) http://www.cdb.riken.jp/jp/cadherins/wmt/Scene2J.wmv (in Japanese)
9	Adherens junctions and tissue morphogenesis	87	Adherens junctions and tissue morphogenesis	http://www.cdb.riken.jp/en/cadherins/wmv/scene3e.wmv (in English) http://www.cdb.riken.jp/jp/cadherins/wmt/Scene3J.wmv (in Japanese)
10	Conclusion	42	Cell adherence is essential for embryogenesis and the maintenance of the adult body; improperly regulated cell adherence is related to some cancers	http://www.cdb.riken.jp/en/cadherins/wmv/scene4e.wmv (in English) http://www.cdb.riken.jp/jp/cadherins/wmt/Scene4J.wmv (in Japanese)

TABLE II
Content of the two different teaching programs that we implemented

(1) Explanation of the basics of genome science										
	Introduction	Animation 1	Animation 2	Movie	Animation 3	Quiz 1[a]	Quiz 2[b]	Animation 4	Animation 5	Summary[c]
Short program	+	−	−	−	+	+	−	−	+	−
Long program	+	+	+	+	+	+	+	+	+	+

(2) Characterization of selected genes in the human genome map poster					
	Evolutionarily conserved genes (*SOD1, POLA*)	Pseudo-genes (*GULOP, CMAH*)	Familiar genes (*AMY1A, ABO*)	Sex-related genes (*SRY, DAZ*)	Animation 8–10
Short program	+	+	+	−	+
Long program	+	+	+	+	+

(3) Explanation of the application of genome science and ethical, legal, and social implications (ELSI)				
	Animation 6	Animation 7	Ethics guidelines for human genome in Japan	Explanation of the Nagahama project
Short program	+	−	+	+
Long program	+	+	+	+

[a] How many phone books do 3 billion letters correspond to?
[b] How long is the human genome DNA?
[c] Summarizing the part (1) using the content (B) of the human genome map for every home.

Table I). In this program, all narration attached to animations were set on mute; instead, the lecturer explained them in a conversational tone in line with the "personalized principle" in which students learn more deeply when words are presented in a conversational rather than a formal style [9, 10]. The length of the program was 50 min (short program) or 100 min (long program).

During this program, students were allowed to put the poster (A1 size) on their desk and watch the PowerPoint presentations including 10 animations and the video. This program included three parts: 1) explanations of the basics of genome science, 2) characterization of selected genes in the human genome map poster, and 3) explanations of the application of genome science and ELSI. Part (1) explained the content (*b*) of the poster with some animations and simple quizzes for a little rest (Quiz 1: "How many phone books do 3 billion letters correspond to?" Quiz 2: "How long is the human genome DNA?"). Part (2) explained the content (*a*) of the poster by selecting some attractive genes and using some animations. Part (3) explained the content (*c*) (*b'*) and (*d'*) with some animations, picking up the ELSI in Japan and the Nagahama project, a large genome cohort study that the Graduate School of Medicine Kyoto University and the Nagahama City Government are working together to promote. The purposes of the project were to promote the early detection of diseases and health of citizens, using epidemiological studies as the scientific foundation. The project also tried to realize the concept of personalized medicine and personalized prevention by analyzing individual's genomes.

In this way, the juxtaposition of the poster with animations is a key feature of this teaching program. The difference between the short and long programs was the depth of content, which is mostly reflected in the number of animations (Table II).

Implementation of the Teaching Program at High Schools

We implemented the teaching program at four high schools in Nagahama city or nearby cities as part of the Nagahama Project. High school A included 39 second-year students (aged 16–17), high school B included 33 third-year students (aged 17–18), high school C included 25 second-year students, and high school D included 37 first-year (aged 15–16) and three third-year students. The short program was implemented in high schools A and B, and the long program was implemented in high schools C and D. All students learned Mendelian genetics but had not learnt the relationships between chromosomes, DNA, genes, and proteins before this program was implemented.

Questionnaires

To investigate the students' background knowledge of the term genome and the Nagahama project, a questionnaire (preprogram questionnaire) was completed before students took part in the program. The questionnaire asked: "Have you heard about the term genome?," and "Have you heard about the Nagahama project?"

Immediately after they had finished the program, to assess students' subjective perceptions from the standpoint of the introduction to genome science, another questionnaire (postprogram questionnaire) was completed. The postprogram questionnaire included the following three questions: "How well can you explain the term genome to your parents or grandparents?," "How well can you explain the Nagahama project to your parents or grandparents?," and "How did your view of the term genome change after attending our program?"

Focus-Group Interviews

We conducted focus-group interviews with two groups of students about 10 months after the program. Group 1 consisted of 10 students from high school D and Group 2 consisted of 14 students from high school C. All interviewees had participated in the long program. These interviews, using a semistructured interview protocol, were conducted by the lecturer who had implemented the teaching program.

Using the KJ method [17], transcripts from the two interviews were coded and these codes were intuitively grouped and structured by mutual agreement between the interviewer and the assistant.

RESULTS AND DISCUSSION

Subjective Perceptions on the Educational Capacity of the Teaching Program as an Introduction to Genome Science

To access the perception of students about the potential of the teaching program as an introduction to