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Research Article

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Gene Card: A Personalized Guide to Genetic Counselling for Low Resource Countries

Anjali Aryamvally[1], Anwayee Das[1], Kriti Menon[1], Santhanam Rekha[1], Suman Jain[2] and Madasamy Parani[1]*

Abstract

Objectives: Even with today's widespread reach of healthcare technology, a large number of children are affected with genetic diseases that could be prevented with proper healthcare interventions. High statistics show that the awareness and information needed for preventing the next generation from inheriting these diseases is lacking. One reason for this situation is the insufficient number of trained genetic counselors around the globe. This calls for alternative platforms to convey personalized genetic information to the people concerned.

Method: Genetic testing was done for individuals from Sickle Cell Anemia (SCA)-affected families and the data was compiled to make "Gene Cards" which was then used for genetic counseling. The gene cards proposed in the study were designed to contain client-specific genetic test results and genetic counseling information in an easy-to-understand format. Additionally, gene cards were also designed for genetic counseling of all diseases with known Mendelian inheritance pattern.

Results: 24 gene cards were designed to represent autosomal, X-linked and Y-linked diseases. Genetic counseling using gene cards was met with both client and genetic counselor satisfaction.

Conclusion: The ability of the gene card to convey genetic information to non-specialists will be useful for effective genetic counseling and management of genetic diseases.

Keywords

Gene card; Genetic counseling; Consultands; Mendelian inheritance; Genetic disease; Sickle cell anemia

Introduction

The global prevalence of Mendelian diseases is approximately 10 in 1000 and accounts for a significant loss of life [1]. One possible reason for such mortality could be the gap between patients getting the genetic test results and understanding those results in order to take corrective measures. Bridging this gap would help the patient to make informed decisions. To make such a decision is no ordinary task: it involves understanding the disease, the genes involved, and its implications for future generations. Genetic counseling is the means through which a common man can understand and interpret the implications of his disease for current and future generations. However, the number of available genetic counselors does not meet this demand, which especially holds true in case of low- and middle-income countries. The data on the number of genetic counselors available for a particular population or country emphasize the need for more genetic counselors worldwide. For example, India has one genetic counseling center for every 46 million people, Malaysia has one genetic counselor for every 14 million people, and the US and Canada combined have one genetic counselor for every 90,000 people [2-6]. Due to the lack of inadequate number of trained genetic counselors, genetic counseling in resource-poor countries is largely provided by medical health professionals who are not trained in the field [7]. While the solution to this problem includes developing human resources in genetic counseling and setting up more genetic counseling centers and programs, the task is time-consuming and expensive. Therefore, it will be useful if a common platform is set up wherein genetic test data and counseling information are available to genetic counselors and patients in a simple format. The gene card, the platform proposed in this study, contains personalized details on the genetic disease, the results of genetic testing, and counseling information in a simple and easy-to-understand pictorial representation.

The concept of the gene card came into light when a study was carried out on the genetic testing and counseling of families affected by sickle cell anemia (SCA). When the sample group was studied, it was observed that SCA was concentrated in the later generations of the family, possibly due to consanguinity: 46% of the marriages in the study group were consanguineous. The high prevalence of consanguinity calls for immediate ways to prevent the inherited diseases from spreading within families [8]. If every individual is informed about his or her genetic condition and advised to get their partner tested before marriage, a lot of these diseases could be contained to a greater extent.

Methods

Participants

All participants who volunteered for the current study were beneficiaries of the Thalassemia and Sickle Cell Society, a non-governmental organization in Hyderabad, India that is a primary care unit for people suffering from hemoglobinopathies. This study included 55 individuals from 15 SCA-affected families, including 16 patients who had high HbS levels, and were undergoing treatment. The study group included 31 unmarried individuals, of which 21 were minors.

Genetic testing

Genomic DNA was isolated from 0.5 ml of blood following modified Millers method [9]. A pair of primers (forward primer 5'-TGTCATCACTTAGACCTCACC-3', reverse primer 5'-GAAGTTCTCAGGATCCACG-3') flanking the A>T mutation in the sixth codon of HBB gene was designed using Primer 3 software [10,11]. PCR reaction mixture was made up to 30 µl using 21.8 µl of water, 3 µl of 10X PCR buffer, 1 µl of 10 mM dNTPs, 1 µl of 5 pmol/µl forward primer, 1 µl of 5 pmol/µl reverse primer, 0.2 µl of 5 units/µl *Taq* DNA polymerase (NEB, USA) and 2 µl of genomic

***Corresponding authors:** Madasamy Parani, Genomics Laboratory, Department of Genetic Engineering, SRM University, Kattankulathur, Kancheepuram District, Tamil Nadu, India; Tel: +91 (044) 27417817; Fax: +91 (044) 2745 2343, E-mail: parani.m@ktr.srmuniv.ac.in

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DNA (100 ng approx.). The PCR reaction condition included initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 54°C for 30 sec, and extension at 72°C for 45 sec. Final extension was carried out at 72°C for 5 min. PCR-RFLP assay contained 15 µl of PCR products, 0.5 µl of 10 units/µl *Eco*81I restriction enzyme (NEB, USA), 2 µl of 10X PCR buffer, and made up to a 20 µl reaction using nuclease free water. The reaction mix was incubated at 37°C for 3 h. The digested PCR products were separated on 2% agarose gel electrophoresis. All the experiments were conducted in triplicates.

Gene card

The gene card was designed to contain two types of information: the genetic test information and the information required for genetic counseling based on the test results. The card includes a title with a tagline, a logo, disease information, genetic test results, a pictorial representation of the inheritance pattern, and the personal information of the individual who has undergone testing, hereafter referred to as the consultand.

Genetic information: The disease-specific genetic information is given on the front side of the card. This includes test information such as the name of the disease, gene(s) tested, mutation(s) identified, inheritance pattern, and disease status.

Genetic counseling information: The genetic counseling information is given in a pictorial format on the front side of the card. Each card is specific to the consultand's gender, disease status, and inheritance pattern. Instead of using pedigree symbols, like circles and squares, male and female parents, and offspring are pictorially represented to help the lay person understand the inheritance pattern.

Results

General features of gene cards

Separate gene cards were designed for autosomal recessive, autosomal dominant, X-linked recessive, X-linked dominant and Y-linked genetic diseases. The symbols and general layout of the gene card are shown in Figures 1 and 2, respectively. For each pattern of inheritance, a set of gene cards were made, which applied to all the possible genetic statuses of the consultands, such as homozygous mutant, homozygous wild type, hemizygous mutant, hemizygous wild type, heterozygote, etc. Based on the genetic testing results and the pattern of disease inheritance, a specific card can be given to the consultand.

In all the gene cards, the first individual in the inheritance pattern always refers to the consultand. The possible genetic consequences of the consultand marrying a person with different genotypes are given in the gene card.

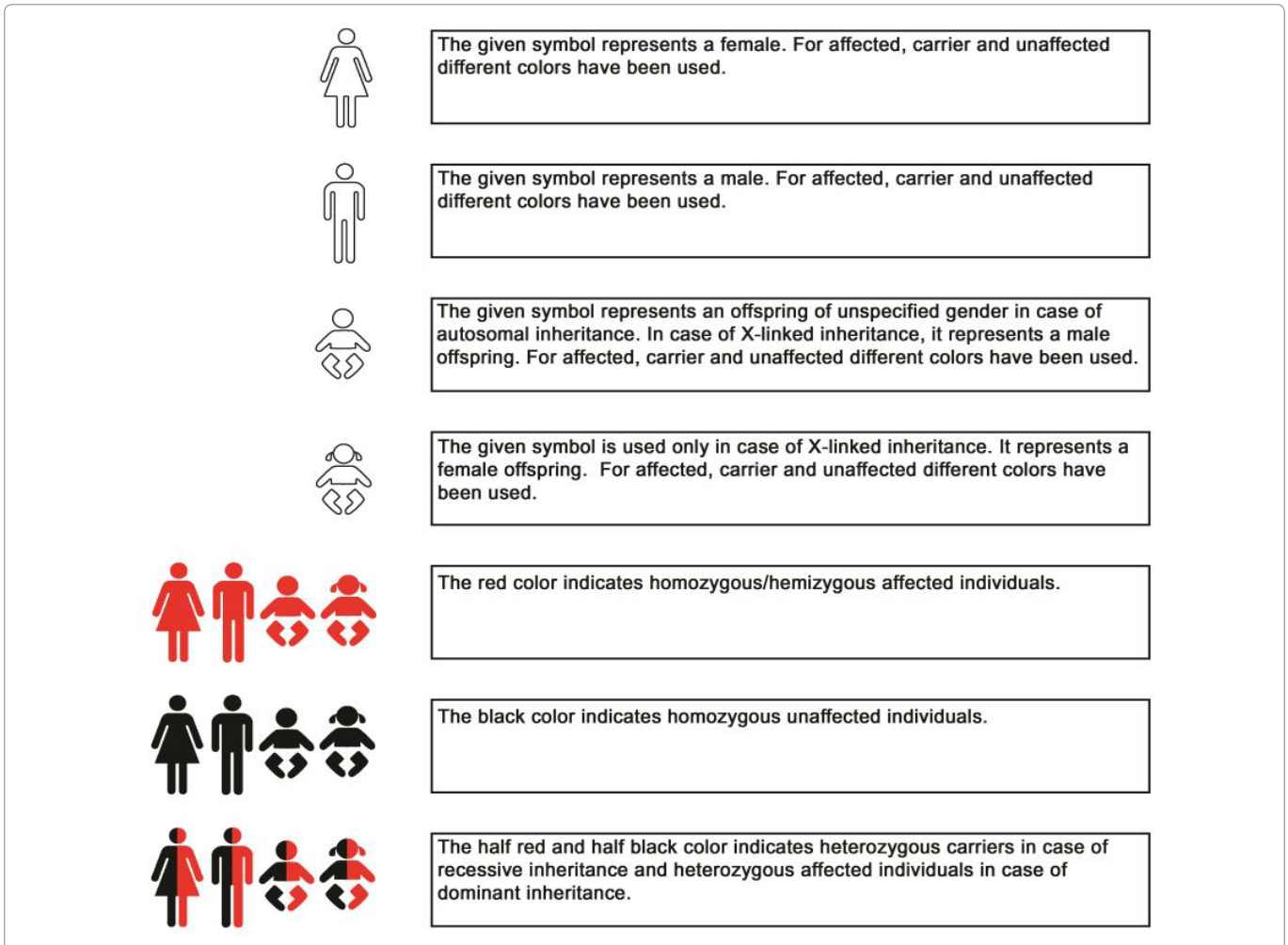

Figure 1: Symbols used for the pictorial representation of an individual's mode of inheritance.

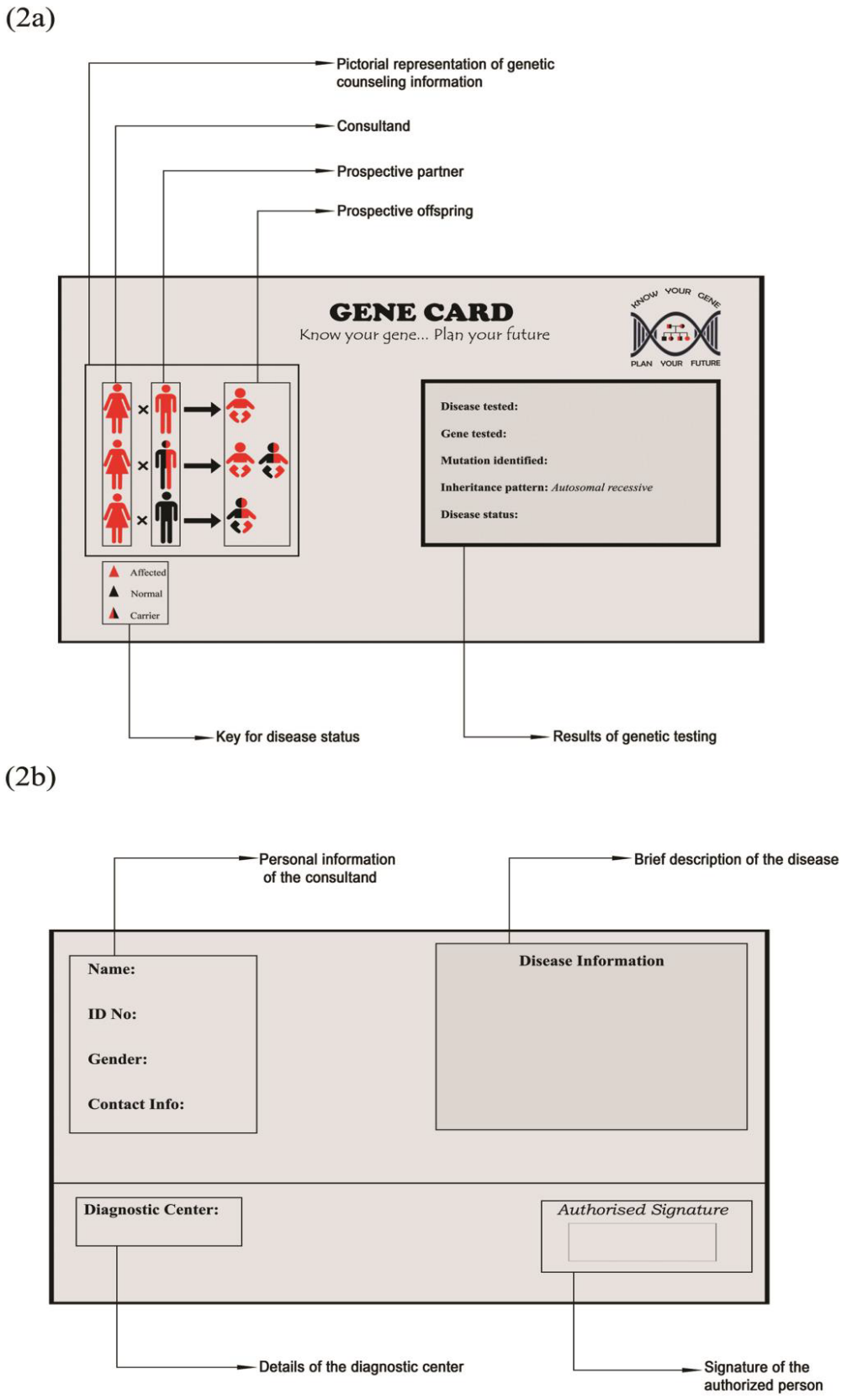

Figure 2: a) General layout of the front side of the gene card. **b)** General layout of the back side of the gene card.

Gene cards for Mendelian inheritance

Autosomal dominant and recessive diseases are not gender-specific, and affect both males and females equally. Therefore, in the gene cards, the offspring were represented without specifying their gender. Three different gene cards were made for the affected homozygote, affected heterozygote, and normal consultand with autosomal dominant diseases as shown in Figures 3d, 3e, 3f. Likewise three different gene cards were made for the carrier, affected, and normal consultands with autosomal recessive diseases also shown in Figures 3a, 3b, 3c.

Since females have two X chromosomes and males have only one, X-linked dominant and recessive diseases manifest in a gender-specific manner. Therefore, the offspring in the gene cards were represented with appropriate genders. Three gene cards were made for the carrier, affected, and normal female consultands shown in Figures 4a, 4b, 4c and two gene cards were made for the affected, and normal male consultands as shown in Figures 4d, 4e with X-linked recessive disease. Similarly three gene cards were made for the affected homozygote, affected heterozygote, and normal female consultands as shown in Figures 4f, 4g, 4h and two gene cards were made for the affected and normal male consultands as Figures 4i, 4j with X-linked dominant disease. The phenotype severity of X-linked diseases in females may vary due to X chromosome inactivation, which randomly inactivates either of the X chromosomes. Therefore, a footnote to this effect was added in the gene cards for X-linked diseases.

The Y chromosome is present and inherited only in males and as a result Y-linked diseases manifest only in males. Therefore, the offspring in the gene card were represented with appropriate genders. Gene cards were made for the affected and normal male consultands tested for Y-linked diseases shown in Figure 5.

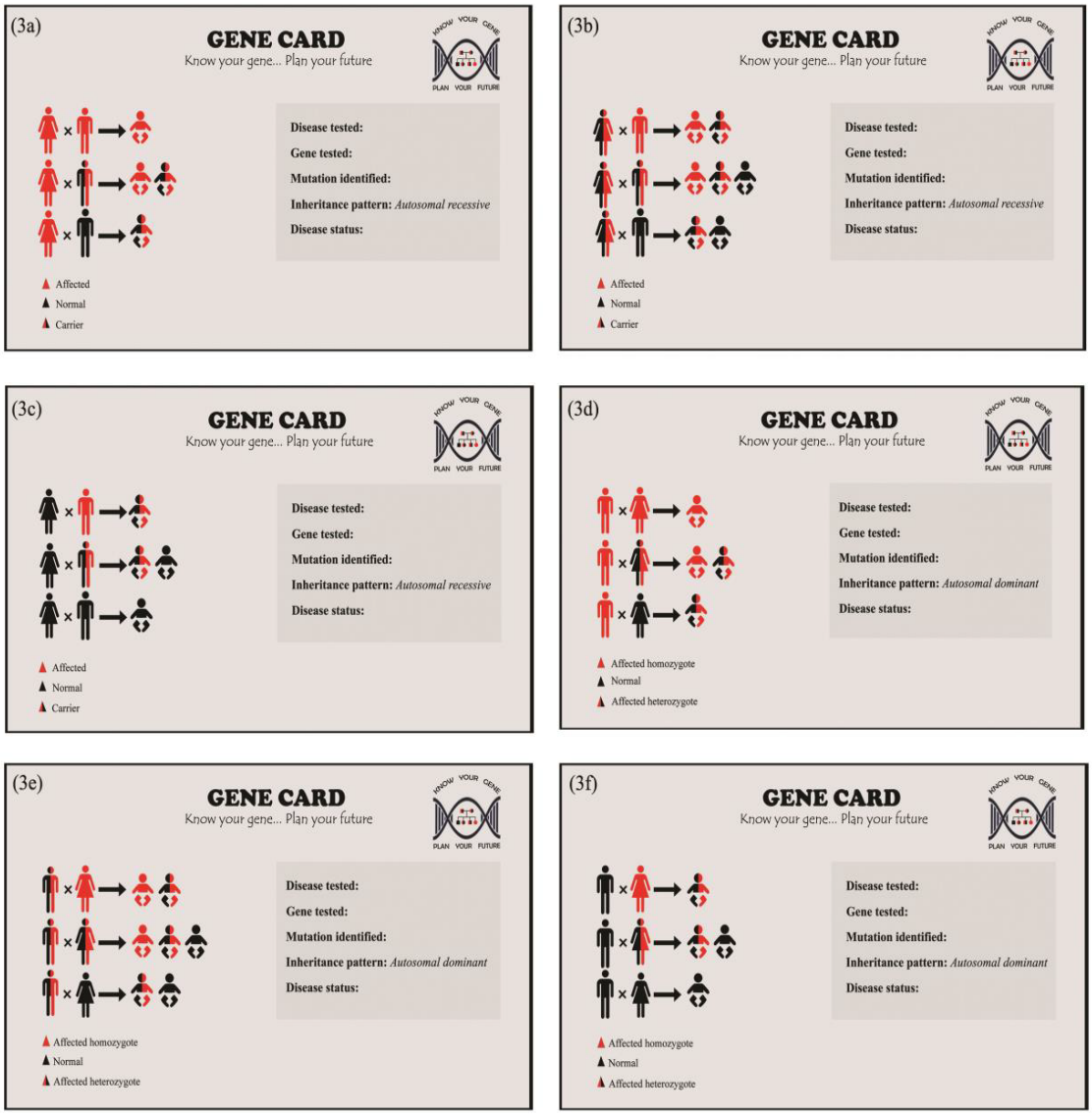

Figure 3: Sample gene cards designed to be issued in case of autosomal recessive disease. **a)** Affected females. **b)** Carrier females. **c)** Unaffected females. Sample gene cards designed to be issued in case of autosomal dominant disease. **d)** Affected homozygous males. **e)** Affected heterozygous males. **f)** Unaffected males.

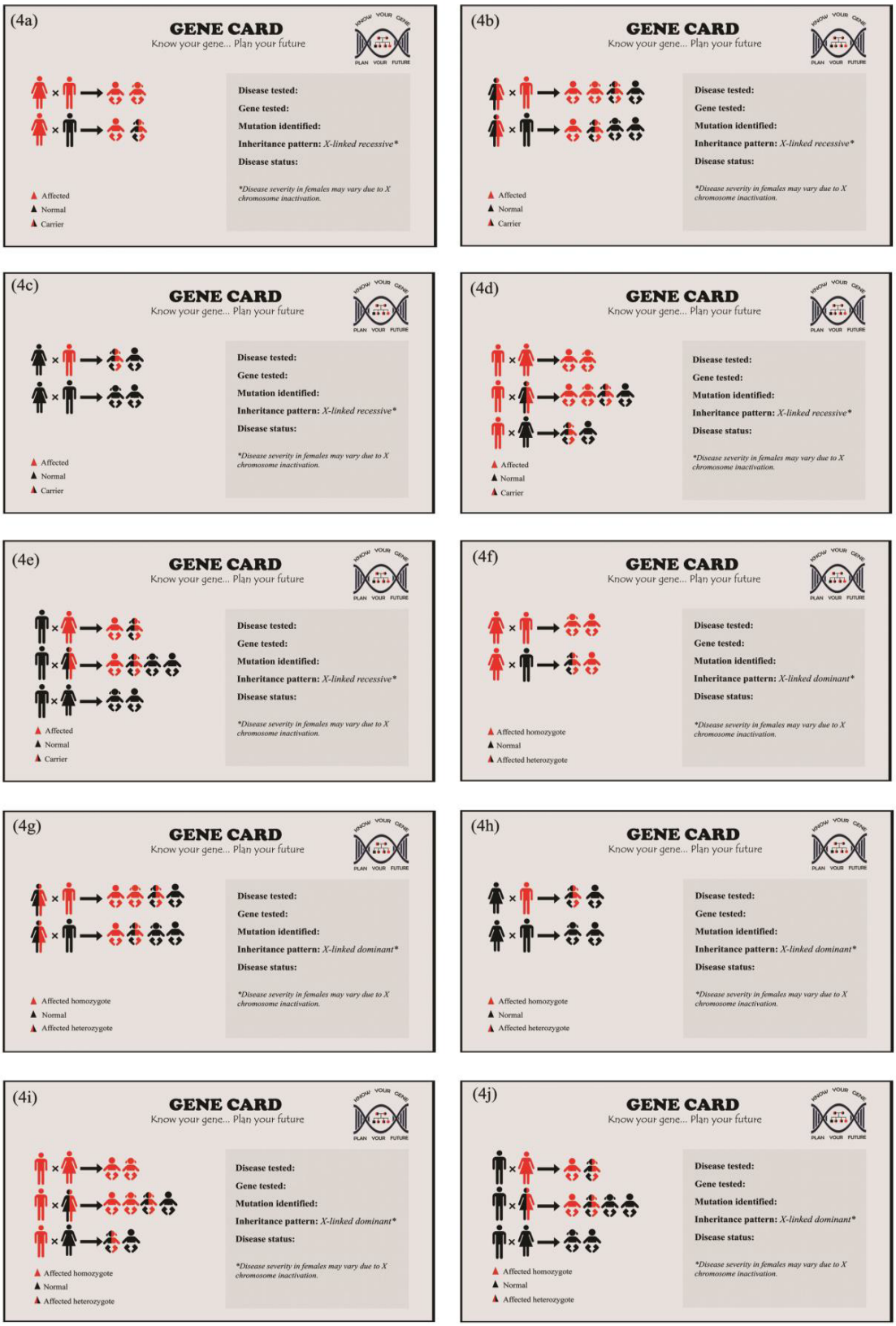

Figure 4: Sample gene cards designed to be issued in case of X-linked recessive disease. **a)** Affected females. **b)** Carrier females. **c)** Unaffected females. **d)** Affected males. **e)** Unaffected males. Sample gene cards designed to be issued in case X-linked dominant disease. **f)** Affected homozygous females. **g)** Affected heterozygous females. **h)** Unaffected females. **i)** Affected males. **j)** Unaffected males.

Case study

We have studied the A>T mutation in the 6th codon of HBB gene, which is the only known disease causing mutation for sickle cell anemia. A 614 bp fragment of HBB gene flanking the A>T mutation was amplified by PCR, and restricted with the *Eco*81I enzyme to generate diagnostic PCR-RFLP profiles [12,13]. In the wild-type allele, there are two *Eco*81I sites but in the mutant allele there is only one site because the other is lost due to the A>T mutation. As a result, the PCR-RFLP of wild type (+/+), carrier (+/-), and affected (-/-) individuals showed three, four and two bands, respectively. The PCR-RFLP profiles of nine consultands are shown in Figure 6; their classification based on marital status, and the number of consultands with homozygous mutant, homozygous wild type and heterozygous genotypes are shown in Figure 7. Based on the results obtained from genetic testing, appropriate gene cards were issued to all the consultands. The gene cards issued to five consultands are shown in Figure 8.

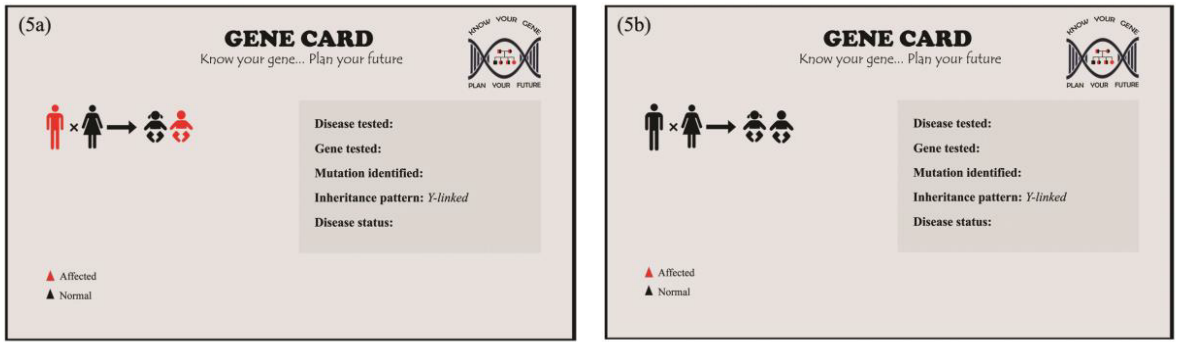

Figure 5: Sample gene cards designed to be issued in case of for Y-linked diseases. **a)** Affected males. **b)** Unaffected males.

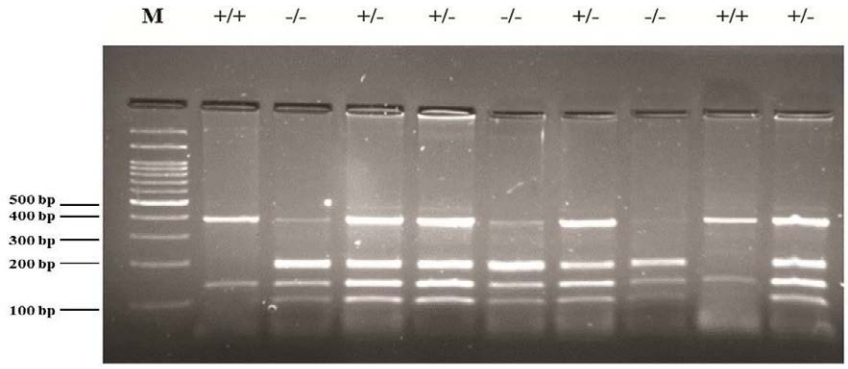

Figure 6: The figure represents PCR-RFLP profiles of nine patients obtained by PCR amplification of a 614 bp fragment from the (HBB) gene flanking the A>T mutation in the 6th codon and restriction digestion with *Eco*81I enzyme. M: 100 bp ladder. (+/+): Homozygous wild-type. (+/-): Heterozygous mutant. (-/-): Homozygous mutant.

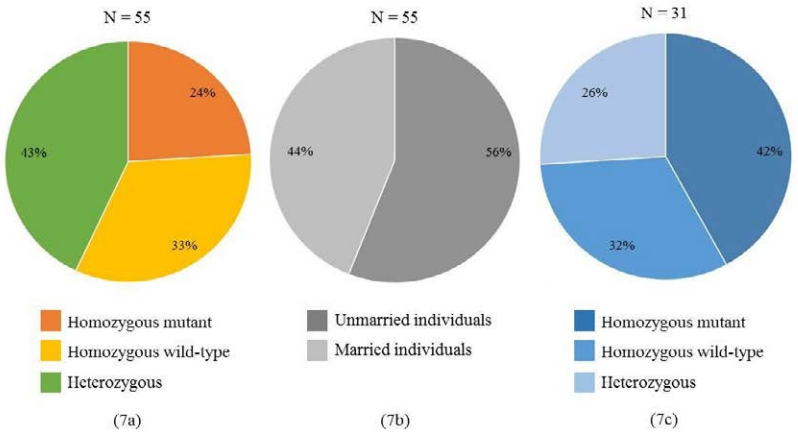

Figure 7: a) Pie chart representing the total number of consultands is shown in this figure with homozygous mutant, homozygous wild-type, and heterozygous genotypes. **b)** Classification of the consultands based on their marital status. **c)** The number of unmarried consultands with homozygous mutant, homozygous wild type, and heterozygous genotypes.

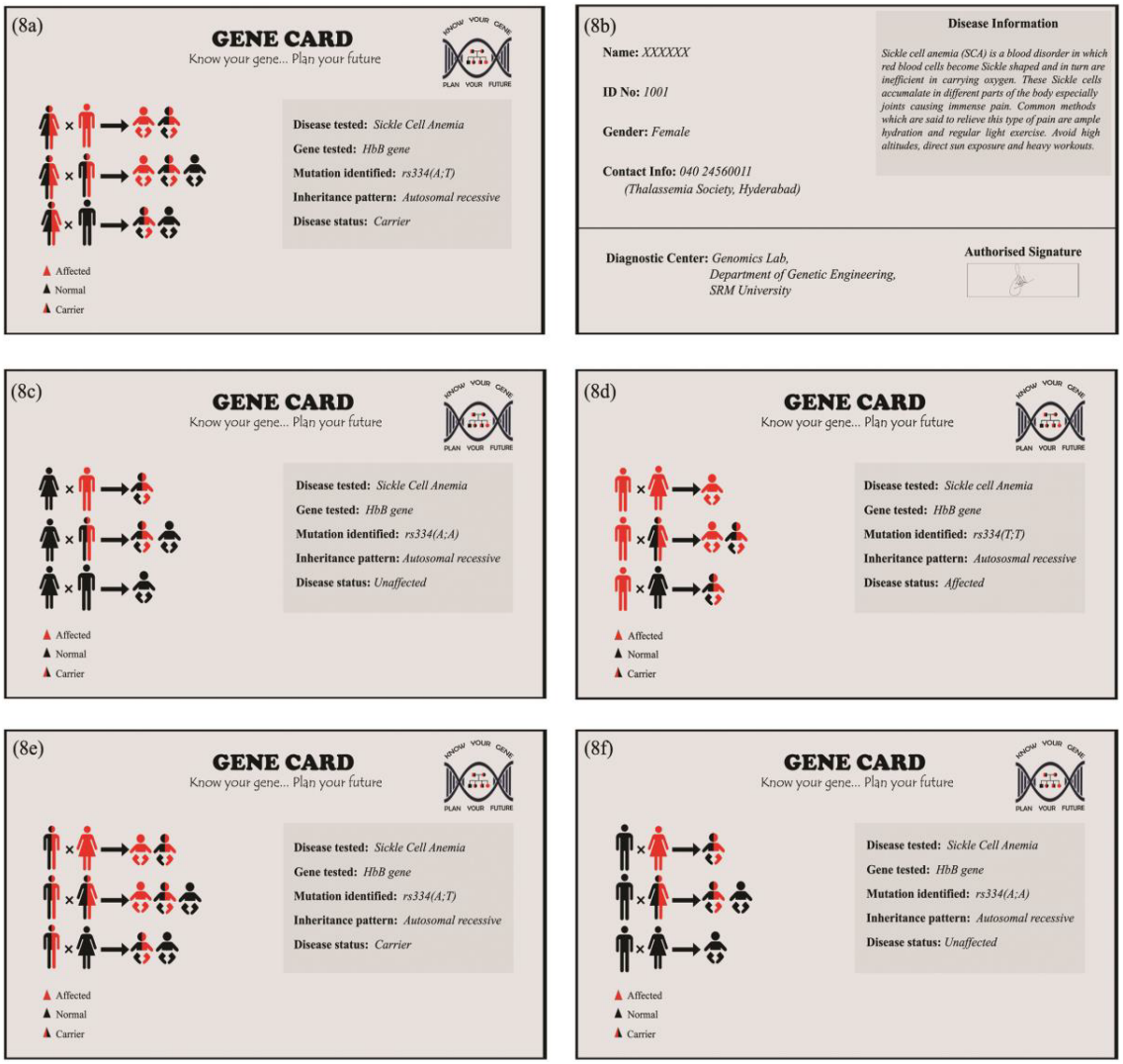

Figure 8: Gene cards issued to the carrier females who were tested for mutation in HBB for Sickle cell anemia. **a)** Front side of the card. **b)** Back side of the card. **c)** Unaffected females. **d)** Affected males. **e)** Carrier males. **f)** Unaffected males.

Discussion

Gene cards were designed to be specific to each consultand. The cards highlight the probability that the consultand's offspring will inherit the disease by considering the genotypes of the consultand and the prospective partner. In the same way, gene cards were designed for all the five Mendelian inheritance patterns.

Since most genetic diseases do not have a cure and the treatment options are expensive, prevention becomes a necessity rather than an option. In low-resource countries, where the public cannot afford long term treatment, preventive measures become all the more important for the survival and sustenance of the affected families [14]. In this context, gene cards, which explain the mode of inheritance of a disease, and act as a guide to prevent the inheritance of faulty genes, can play a significant role. In the current study, the effectiveness of the gene cards was tested in the genetic counseling of families affected with SCA, an autosomal recessive disease. Genetic testing was done to identify the disease-causing mutation and gene cards for the autosomal recessive inheritance pattern were issued based on the genotype of the consultand. During genetic counseling, the gene cards were found to be extremely useful to the consultands for understanding the inheritance pattern and the implications of his/her genetic condition for present and future generations. When a pedigree was used to explain the inheritance of SCA to a family, they found it difficult to understand what the different signs on the pedigree represented the inheritance pattern of the disease, and its implications; however, when the same family was provided with a gene card, they found it easier to understand these aspects.

The gene cards were most useful to the unmarried consultands who comprised 56% of the study group. This group found the gene cards very informative, as they could use the genetic information for selecting a partner and pregnancy planning. This also led them to realize how important it is that their prospective partner also undergoes genetic testing. Consultands who were already married and planning for pregnancy found their gene cards helpful in understanding the probability of their offspring inheriting the genetic disease and to decide about prenatal testing as needed.

Gene cards, if used effectively, can be a tool to spread awareness about genetic testing and counseling. However, the efficiency of the tool is limited due to certain factors. Primarily, the cards are limited to Mendelian disorders, which occur only in 10 out of 1000 births worldwide; however, this means that millions of people would reap the benefits of gene cards. Second, while the gene cards provide general inheritance pattern and counseling information, other factors like penetrance, expressivity and X-chromosome inactivation, were not taken into account in forecasting the manifestation of the disease. However, the gene cards provide basic genetic information based on which more elaborate genetic and clinical investigations may be undertaken, to determine the impact of the above-mentioned factors on the manifestation of the disease in a particular individual.

Conclusion

The gene cards are not meant to bring about a revolution in healthcare but to alter an individual's and a community's approach towards the management of genetic diseases. Effective counseling, combined with accurate genetic testing, has the potential to eliminate inherited diseases in the future generations of the affected families. Through the addition of this concept, we hope to reach even the deepest layer of society for whom prevention is the only means of curing these genetic diseases.

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Ethical Approval and Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 1983. Informed consent was obtained from all individual participants included in the study.

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Author Affiliation

Top

[1]*Genomics Laboratory, Department of Genetic Engineering, SRM University, Kattankulathur 603203, Kancheepuram District, Tamil Nadu, India*

[2]*Thalassemia & Sickle Cell Society, Purani Haveli, Hyderabad, India*