

Original Research Article

Genetics of multiplex familial vitiligo as cases and controls: a preliminary report

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ABSTRACT

Background: Studies on etiology of generalized vitiligo have established that it is a genetically determined, autoimmune, auto inflammatory diathesis. Polymorphism in myriad genes is associated with vitiligo and newer ones are being identified with advancement in research methods. This study enabled inheritance pattern recognition in multiplex familial cases of vitiligo and subject members to whole exome sequencing (WES) to find out the etiological gene defect in a particular family.

Methods: 7 multiplex families with at least two cases and two healthy counterparts, spread across generations were enrolled. Demographic data, clinical history and familial data were collected for pedigree analysis and blood samples were collected for extraction of genomic DNA.

Results: Multiple possible modes of Mendelian inheritance could be contemplated to cause disease expression in probands. Autosomal recessive pattern was observed to be the most likely amongst various possible modes, followed closely by autosomal dominant, with X-linked recessive, and X-linked dominant occurring less frequently. WES for one of the multiplex families is desirable and planned at an apex institute of genetic studies which will reveal the etiologic gene defect on conclusion of analysis.

Conclusions: In this study we have used to our advantage the familial occurrence of the disease to seek for genes which occurred with a greater frequency in affected members of a family than their healthy counterparts, assuming that the same mutated gene should be universally present in all affected members across generations in the same family, enabling a better understanding of genetic predisposition in familial vitiligo.

Keywords: Familial vitiligo, Multiplex vitiligo families, Whole exome sequencing

INTRODUCTION

Vitiligo is a depigmenting disorder characterized by progressive, patchy, multifocal loss of pigmentation of skin, overlying hair, and often mucous membranes resulting from loss of melanocytes from the involved areas. It is a cause of social stigma; as sufferers, especially from the darker races, are easily recognized as deviation from the norm. It has a worldwide prevalence of about 1% and affects both sexes equally.¹ In a classical case, diagnosis can be made by mere clinical

examination, although a Wood's lamp examination might aid in diagnosis in the fair-skinned. Vitiligo is broadly classified into three types (Bordeaux VGICC (Vitiligo Global Issues Consensus Conference) classification and consensus nomenclature): nonsegmental vitiligo (NSV), segmental vitiligo (SV) and undetermined/unclassified vitiligo. Nonsegmental vitiligo has subtypes including acrofacial, mucosal, generalized (also used interchangeably with vitiligo hereafter), universal, mixed (associated with segmental vitiligo) and rare variants. Segmental vitiligo can be uni-, bi- or plurisegmental.

Undetermined/unclassified vitiligo includes focal and mucosal (one site in isolation) varieties.²

Vitiligo is a multifactorial, polygenic disorder, with a complex pathogenesis that is not yet well understood. Of various theories of disease pathogenesis, the most accepted is that genetic and non-genetic factors interact to influence melanocyte function and survival, eventually leading to autoimmune destruction of melanocytes where the rate of loss exceeds the rate of replacement.³ It is known to be associated with other autoimmune disorders. Also, cases of vitiligo within families have elevated frequencies of other autoimmune diseases, suggesting that they possess susceptibility factors for autoimmune diseases which are most likely mediated genetically.⁴

The inability to delineate a single etiological factor and with the ever-growing knowledge from molecular studies it has been proposed that genetic susceptibility is a pre-requisite, in addition to environmental factors for causing vitiligo. The genes and their different combinations involved in pathogenesis of generalized vitiligo are not known, but a few susceptibility genes have been identified. Strong evidence exists that polymorphisms in genes like *HLA*, *PTPN22*, *NALP1*, *CTLA4*, *IKZF4*, *IL2RA*, *BTNL2*, *ZMIZ1*, *PMEL*, *CLNK*, *RNASET2* and *TRAF2* may predispose to acquiring vitiligo.⁵

A genetic mutation causing vitiligo in an individual may, or may not be the cause in other individuals. The familial occurrence of the disease could be used to our advantage to seek for genes which occurred with a greater frequency in affected members of the same family than their healthy counterparts, assuming that the same mutated gene should be universally present in all affected members across generations in a family. So, the contribution of the predisposing genetic factors to vitiligo could be more clearly investigated in familial cases of vitiligo, leading to a better understanding of the genetic predisposition theory.

This study was undertaken with the specific aims of (1) Identification of multigenerational families of vitiligo vulgaris with at least two affected members in the family (cases) and at least two unaffected members of the family (controls) for their pedigree charting, analysis of the inheritance pattern and collection of blood samples for harvesting gDNA (genomic DNA); and (2) Identification of gene sequences, if any, occurring exclusively or with a greater frequency or in a mutated form in patients of vitiligo when compared to their healthy counterparts using genomics approach (whole exome sequencing). The present article reports our findings regarding specific aim (1).

METHODS

Families with a minimum of two affected members of generalized vitiligo (cases) spanning at least two generations, and two healthy members (controls) were

recruited for the study from Dermatology OPD at a tertiary care centre in Pune, between July 2014 to November 2017. Seven families were enrolled including both male and female cases with ages ranging from 9 years to 70 years.

Patients with segmental, unclassified or focal forms of vitiligo were excluded from the study. Patients with other Mendelian hypopigmentary spotting disorders, such as piebaldism, albinism and the various forms of Waardenburg syndrome, which result from mutations in specific single genes and mimic vitiligo were excluded. Patients presenting with depigmentation resulting from contact exposure to known depigmenting agents (phenols, catechols, quinines etc.); that occurring secondary to chronic inflammation, psoriasis, other forms of dermatitis; and depigmentation secondary to infection, scars, burns and various other skin insults were excluded.

A written informed consent was obtained from all the subjects for genetic analysis. Sociodemographic and clinical information was obtained from the subjects through questionnaires. A pedigree was charted (Figure 1, Figure 2) for each family to study the possible modes of inheritance in each case. 5 mL of blood from affected (cases) and unaffected (controls) members of a family was collected in heparinized tubes and an apex institute in Delhi has been approached to conduct extraction of genomic DNA.

One of the seven families (Figure 2) was selected for carrying out whole exome sequencing as positive history of vitiligo could be elucidated across 4 generations and a multitude of cases were present for sample collection which increased the chances of pin-pointing the causal mutation- occurring with a greater frequency in affected individuals than in their healthy counterparts. The study was approved by the Institute's Ethics Committee.

RESULTS

In the conducted study, seven families were enrolled containing vitiligo patients (6 males and 13 females) spanning more than one generation, and the largest included five vitiligo cases across four generations. Amongst multigeneration kindreds there were examples of male to female (Figure 1a, 1e, 1f), male to male (Figure 1d), and female to female (Figure 1c) transmission but no female to male transmission. There were no instances of parental consanguinity. Evidence for transmission through successive generation was observed in each case. The youngest patient enrolled in the study was a 9-year-old female who was suffering from generalized vitiligo, while the oldest was a 70-year-old male whose disease started as generalised vitiligo but presented to us with vitiligo universalis. Children of parents suffering from vitiligo were genetically susceptible to acquiring the disease, in whom physical trauma incited the first vitiliginous lesion (cases in family 1, 3, 6 and 7).

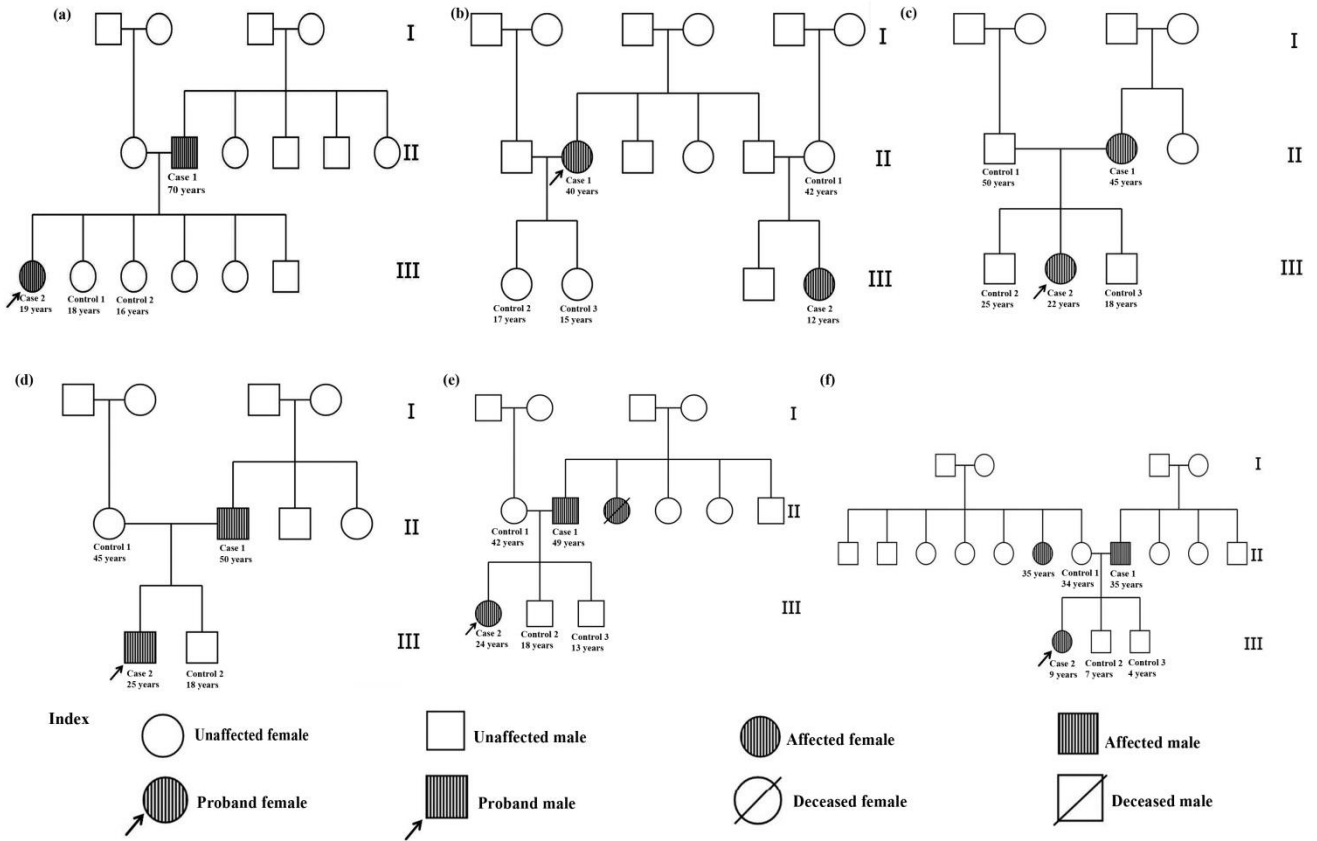


Figure 1: Pedigree analyses; (a)- Family 1, (b)- Family 2, (c)- Family 3, (d)- Family 4, (e)- Family 5, (f)- Family 6.

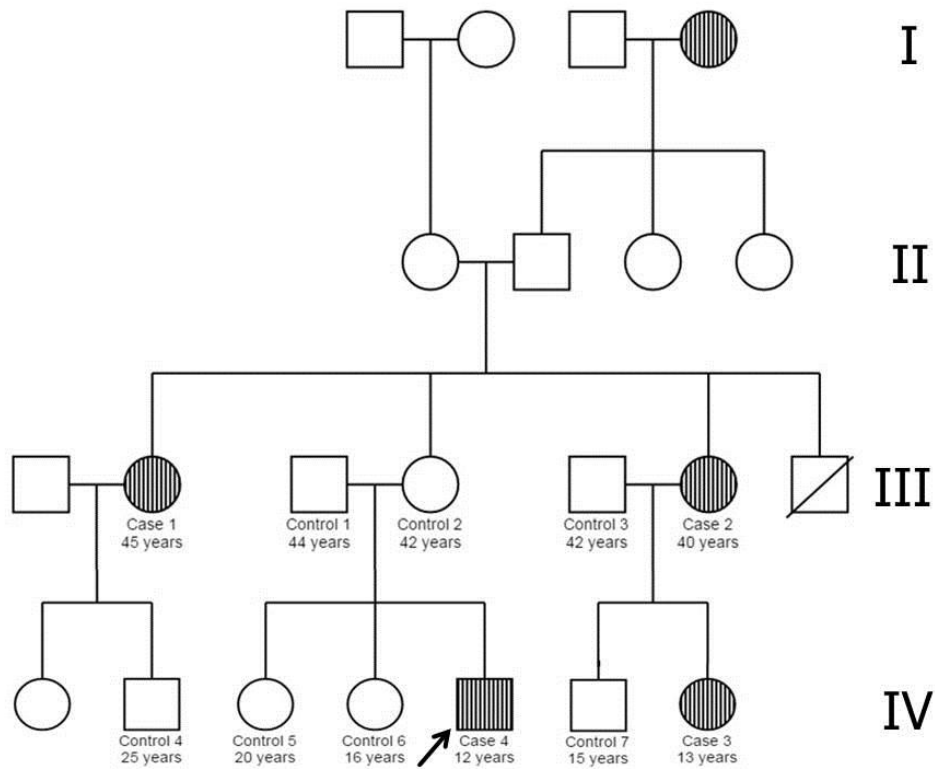


Figure 2: Pedigree of Family 7- selected for whole exome sequencing.

The results of segregational analysis of pedigree charts and analysis are as follows.

In family 1 (Figure 1a) cases were spread across two generations including a 70-year-old male who developed his first vitiliginous patch spontaneously at the age of 15 years and progressing to generalized vitiligo at the time of presentation. He fathered 5 daughters and a son, amongst whom only 1 daughter was affected. His eldest daughter- aged 19 years developed her first lesion following a trauma at the age of 5 years. Her mother was unaffected.

Cases of family 2 (Figure 1b) span two generations; a female— aged 40 years, affected by vitiligo since the age of 15, but neither her parents, siblings or children were affected. However, her niece aged 12 years was affected by vitiligo since the age of 9 years. Neither her parents nor her siblings suffered from vitiligo.

In family 3 (Figure 1c) two generations were affected by vitiligo. The proband, a 22-year-old female acquired her first vitiliginous patch after an injury at the age of 19 years. Her siblings were not affected by vitiligo. Her mother, aged 45, was suffering from vitiligo since the age of 30. Her father however was disease-free.

In family 4 (Figure 1d), two sequential generations were affected by vitiligo. The proband- a 25-year-old male suffered from vitiligo since the age of 15 years. His sibling and mother were unaffected, however his father, aged 50 years was suffering from vitiligo since the age of 20 years. No positive history of any other family member affected by vitiligo could be illicited.

Family 5 (Figure 1e) had three cases spread across two generations. A female, aged 24 years suffered from vitiligo since the age of 8 years. Her mother and siblings

were unaffected. Her father, aged 49 years, also suffered from vitiligo who acquired the disease at the age of 34 years. Neither of his parents were affected, but one of his younger sister, amongst four siblings, was affected by vitiligo.

In family 6 (Figure 1f), the cases were spread across two generations. The proband- a female aged 9 years was suffering from vitiligo since a year- which occurred at a site of injury. None of her siblings or mother were affected, but her father, aged 35 years, acquired vitiligo at the age of 11 years and was the only affected child amongst the four children of his parents. None of his parents were affected. One of the maternal aunts of the proband was also affected by vitiligo. However, none of the aunt's siblings or parents were affected by vitiligo.

Family 7 (Figure 2) had cases spanning 4 generations. The proband, a 12-year-old male was suffering from vitiligo since 2 years. None of his siblings or parents were affected. However, his great grandmother (deceased), two of his maternal aunts (aged 45 and 40 years), and one of his first cousins (a female, aged 13 years) were affected by vitiligo. This further proband, a 13-year-old female had started to show vitiliginous patches since 4 months who is the daughter of one of the first proband's maternal aunt (aged 40 years). Only one amongst the 3 siblings of her mother is affected by vitiligo. None of the children of the second proband's maternal aunt (female, aged 45 years) is suffering from vitiligo. Interestingly, none of the members in the second generation exhibited features of vitiligo. Both cases of IV generation acquired vitiligo following trauma.

In all the families, although not universally similar, vitiligo could be contemplated to exhibit multiple possible patterns of inheritance and a few patterns could be refuted (Table 1).

Table 1: Possible patterns of inheritance in families under study.

	Autosomal dominant (AD)	Autosomal recessive (AR)	X-linked dominant	X-linked recessive	Sporadic
Family 1	Y	Y	N	N	Y
Family 2	N	Y	N	N	Y
Family 3	Y	Y	Y	N	Y
Family 4	Y	Y	N	N	Y
Family 5	Y	Y	Y	N	Y
Family 6	Y	Y	Y	Y	Y
Family 7	Y	Y	Y	Y	Y

Key: Y- Possible; N- Not possible.

DISCUSSION

Vitiligo is a depigmentary disorder of the skin and hair that results from selective destruction of melanocytes. To date, the pathogenesis of vitiligo is unknown, although there are many theories about its etiology including self-

destructive, biochemical, neural, autoimmune, and genetic hypotheses.⁴

Familial clustering of cases of vitiligo was noted as early as 1933, although any simple Mendelian pattern of segregation was not apparent.⁶⁻⁹ However, the fact that

30% of vitiligo patients have a family history suggests that genetic factors might have an important effect on the development of vitiligo. The existence of familial aggregation and the cases of vitiligo in monozygotic twins further indicates the possible role of genetic factors involved in the development of vitiligo.^{7,8} In the past, vitiligo was perceived to be inherited by autosomal dominant, or autosomal recessive mechanisms, however recent analysis suggests that it is determined by a multifactorial inheritance.^{8,10} In concordance with other studies, we report transmission of vitiligo from father to son (as observed in family 4) in the current study- indicating that vitiligo is not transmitted by X-linked inheritance.⁸

The advantage of selecting a multiplex family- A number of possible mutations have been suspected to cause vitiligo. Of the seven recruited families, the cause of vitiligo across all families might not necessarily be the same. However, in a multiplex family- with cases spanning multiple generations and involving first and second degree relatives, the gene causing vitiligo- in all probability will be the same in all individuals, and will be expressed more frequently in affected cases than their healthy counterparts. In the present study, amongst various possible modes of inheritance, it was observed that autosomal recessive pattern was the most likely pattern, followed closely by autosomal dominant, with X-linked recessive and X-linked dominant occurring less frequently (Table 1).

As with earlier studies, lack of multiplex families poses a major hurdle in the application of concrete genetic models to the acquired data set from familial cases of vitiligo. Only ~10% of the families are multiplex, and, even among multiplex families, most families have only one affected relative of the proband. The parents of affected children are, most often, both unaffected.^{10,11}

Amongst the recruited families, family 7 had maximum number of affected cases. Family history established a confirmatory diagnosis of vitiligo in a female of I generation. Interestingly, none of the members in II generation exhibited features of vitiligo. III and IV generations included cases with both first and second degree relatives. Since blood samples from each of the four affected (cases 1-4) and seven non-affected individuals (controls 1-7) could be collected there were maximum chances of being able to locate the causal mutation leading to vitiligo in this particular family.

A variety of approaches have been used to identify genes that mediate susceptibility to vitiligo. Gene expression analyses can be used to identify genes in case control studies, in a way that certain (etiologic) genes would be differentially expressed in cells or tissue from vitiligo patients versus controls; or from diseased tissue versus normal tissue from the same patient.

Such studies can generate lists of candidate genes, but they cannot distinguish genes exhibiting the primary effect from the many more genes that show variation because of individual variation brought about by the outbred genetic background among humans. On the basis of biological hypothesis, suspected candidate gene/s for vitiligo can be tested using genetic association studies. Since majority of cases occur without involvement of other family members, this approach can be utilized to study specific candidate genes in singletons. However, such an approach has been limited to test already known gene candidates. Furthermore, population admixture and population variability subjects genetic analyses to both false-positive and false-negative errors. As opposed to the aforementioned studies, genetic linkage studies scan the entire human genome for chromosomal regions that nonrandomly segregate with vitiligo in multiplex families. This approach can identify genes which were not known previously to be associated with vitiligo, but is limited to the analysis of multiplex families only whose underlying etiologic gene defect may, or may not be the same as that of majority of cases without familial history.⁴ Exome sequencing is a relatively new technique which involves sequencing of just the protein coding regions of the genome, which form the major workhorse in the cell modulating the biological functions and outcome. Whole exome sequencing for Family 7 is planned but may get delayed due to financial hurdles and time constraints.

Considering that most of the genetic associations are restricted to moderate effects, large sample size studies are required in future investigations in order for the subtle variations to be detected. To be able to pin-point and learn about the most commonly occurring mutation, a large number of multiplex families are required with cases spread across multiple generations. Lack of cases (multiplex families), cost consideration for exome sequencing and time permissibility were the main limitations of this study. Larger studies in future are required that may yield novel insights into the pathogenesis of vitiligo, leading to both improved treatment and its prevention.

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