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ASCERTAINING THE GENETIC STATUS OF THE CHROMIUM EXPOSED HUMAN Y CHROMOSOME

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ABSTRACT

The human Y chromosome provides an ideal setting for studying genetic effects of genotoxic agents owing to its small size, limited number of genes and haploid status. We analyzed the Y linked loci in males exposed to chromium from Kanpur, India. This study would help to comprehend the male specific targets of chromium genotoxicity, if any.

A total of 101 individuals were screened for recombination events in the Y chromosome: P5-P1 proximal; P5-P1 distal; gr/gr; TSPY-TSPY, b1/b3 and b2/b3. The single nucleotide variants of candidate genes (DAZ, TTY4, BPY2, GOLGA2LY) and amplicons of the AZFc region were also analyzed.

No known recombination was detected in the samples with respect to any of the above mentioned loci. The AZFa region showed a few deletions. SNV analysis of the AZFc region in 38 exposed samples showed 69 variations compared to that of normal ones. Overall, the human Y chromosome seems to be well protected from the effects of chromium.

Keywords: Y chromosome; Chromium exposure; Single nucleotide variants

I INTRODUCTION

The human Y chromosome harbours three regions: AZFa, AZFb, and AZFc (Azoospermia factors a, b, and c), essential for spermatogenesis (Vogt et al., 1996) by the virtue of presence of candidate male infertility genes like *DAZ, RBMY, DBY and USP9Y* (Foresta et al., 2001). Moreover, the human Y chromosome is an ideal candidate to explore genetic alterations due to its small size, haploid status and few genes. We reported earlier that males exposed to arsenic showed random microdeletions in the AZFa and c regions of the Y chromosome. Also, analysis of single nucleotide variants reflected variations across different copies of genes and amplicons of the AZFc region [Ali and Ali 2010]. In the present study, we studied Y linked loci in the context of exposure to chromium.

Chromium is listed amongst the top 20 hazardous substances by the Agency for Toxic Substances and Disease Registry/Environmental Protection Agency priority list (CERCLA 2002). Chromium exists predominantly in two states: Cr(III) and Cr(VI). Cr(III) is the stable form which is known to be essential for sugar and fat metabolism (Cefalu et al 2002). Contrastingly, Cr(VI) is a highly potent human carcinogen, chiefly originating from the welding and leather industries (Dayan et al 2001). Cr(VI) enters the cell through the sulfate anion

transporter and is subsequently reduced to stable Cr(III) through intermediate stages of Cr(V) and Cr(IV). The toxicity to the body is caused by the DNA damage due to the reactive oxidative intermediates and by binding of Cr(III) to DNA (Wetterhalm et al 1989). This binding leads to Cr(III)-mediated DNA interstand crosslink, Cr(III)-mediated protein-DNA crosslink or Cr(III)-DNA monoadduct (O'Brien et al 2003). The biological implications of these interactions are not yet fully understood. Till date Cr(III) has not been reported to show any carcinogenesis effects on the animal models though its interactions are known to interfere with transcriptional activator-coactivator complexes, thereby affecting the expression of corresponding genes (Klein et al. 1996). Interestingly, the target genes of chromium toxicity exclude the housekeeping genes, possibly due to the differences in their chromatin structure as to that of inducible genes. It has been suggested that the open chromatin structure of inducible genes makes it a better candidate for Cr(III) binding (Manning et al 2002).

There are several leather and tanning industries located in and around the city of Kanpur, UP, India. People residing in these areas and workers of the factories are often exposed to high levels of chromium. Though expected but the genetic effects on these people are yet to be elucidated. In the present study, we collected samples from Kanpur, UP, India from the factory workers and analyzed for anomalies at the genetic level focusing on the Y chromosome.

II MATERIALS AND METHODS

2.1 Collection of blood samples and genomic DNA isolation

Human blood samples were collected from 101 males with their informed consent, strictly in accordance with the guidelines of Institutes Ethical and Biosafety Committee. They were in the age group of 19-80 years. Blood samples were collected from 80 unexposed males from New Delhi to be used as controls. These people have more or less similar life style compared to chromium exposed population of Kanpur. They were in the age group of 22-60 years. A comparative age wise distribution of exposed and unexposed samples is shown in figure 1. The level of chromium in the blood was estimated through atomic absorption spectrophotometry (Christensen et al 1993). This was found to be in the range of 130 to 150µg/L of blood in the exposed samples which is ten times higher than ~15 µg/L of blood in the unexposed normal samples. Genomic DNA was isolated using standard protocols.

2.2 STS screening and SNV analysis

PCR reactions in 20 µl volume were carried out using Go Taq polymerase and 5x reaction buffer (Promega, Madison, USA), 200 µM dNTPs and 100 ng of template DNA. The reaction was conducted for 30 cycles, each involving denaturation at 95^0C for 1 minute, annealing at requisite temperature for 1 minute and extension at 72^0C for 1 minute besides initial denaturation at 95^0C for 5 minutes and final extension at 72^0C for 10 minutes. The amplified products were resolved on appropriate agarose gels. β-actin was used as positive control (Premi et al 2007).

STS spanning all the known regions of Y chromosome showing recombination deletions were amplified using end point PCR (Ali and Ali 2010). These are based on established results (Repping et al 2002; Repping et

al2003; Repping et al 2004; Kuroda-Kawaguchi et al 2001; Ferlin et al 2003; Sun et al 2000; Kamp et al 2000; Blanco et al 2000; Jobling et al 2007) and cover the three AZF regions. The fate of SNVs across four genes and there amplicons were analyzed as reported earlier (Ali and Ali 2010).

2.3 Sequencing of SNV amplicons

The reactions in 20µl volume were carried out using *Pfu* DNA polymerase (NEB), 200 µM dNTPs and 100ng of template DNA as per conditions mentioned above. The products were resolved on agarose gel, fragments were eluted and sequencing was done on ABI 3130xl genetic analyzer using Big dye terminator v3.1 chemistry. The sequences were analyzed on sequencing analysis software v5.3.1.

III RESULTS

3.1 Overall STS analysis for recombination events and chromosome intactness

None of the studied samples exhibited any of the signature STS profiles for the known recombination events. A representative illustration of the analysis of STSs in the three AZF regions is given in figure 2

AZFa region

The AZFa region was analyzed for HERV mediated recombination as per established protocols (Sun et al 2000). We did not find any of the representative provirus A or B mediated recombination but several samples showed some deletions confined to the provirus B region (figure 3).

AZFb region

The AZFb region was analyzed by multiplex PCR using standard STS markers. All the samples were found to be positive for these STSs (figure 2) indicating the intactness of AZFb region.

AZFc region

The AZFc region was analyzed for its intactness and occurrence of different recombination events (P5-P1 proximal; P5-P1 distal; gr/gr; b1/b3 and b2/b3) as per established STS markers (Repping et al 2002; Repping et al 2003; Repping et al 2004; Kuroda-Kawaguchi et al 2001; Blanco et al 2000; Jobling et al 2007). Further, the samples were also screened for TSPY-TSPY recombination (sY1240, sY1250 positive and sY276, sY1238, sY637, sY1319 all negative). No sample exhibited any of the recombination events. However, there were few random microdeletions in some samples. Representative results of STS mapping of the AZFc region are shown in figure 2.

3.2 SNV/SVF analysis

A total of 17 SNVs were analyzed encompassing 4 genes (DAZ, BPY2, GOLGA2LY, and TTY4) and 3 amplicons (blue, green and gr) as per standard protocols (Navarro-Costa et al 2007; Fernandes et al 2004; Repping et al 2004). Representative gel pictures of analysis of SNVs are given in figure 4.

The exposed and normal samples showed similar results in the 7 DAZ SNVs as summarized in table 1. However, the SNVs localized in amplicons and other AZFc genes (BPY2, TTY4 and GOLGA2LY) did show

variations (figure 5). Overall, the SNV analysis showed 69 variations in 38 exposed samples compared to that of normal ones.

To rule out the possibility of mutations affecting the restriction fragment profile of the samples, PCR products of all the SNVs were sequenced. None of the samples showed any mutations and the sequencing results corroborated with the restriction profiles (figure 6).

IV DISCUSSION

Chromium is an established human carcinogen but its genetic effects beyond carcinogenesis are not yet fully understood. Moreover, the threshold level of exposure that may lead to cancer is also not established. According to an epidemiological study, carcinogenesis caused due to Cr(VI), requires prolong exposure (De Flora 2000). Contrary to this, reports are there stating 25% life time risk of death caused due to lung cancer even with the permissible exposure limit of 52µg/m^3 (Gibb et al 2000; Park et al 2004). Consequently, Occupational Society and Health Administration (OSHA) reduced the permissible exposure limit to 5µg/m^3 in 2006 (OSHA 2006). This highlights our minimal understanding of chromium toxicity to the human population. Moreover, the reduction of Cr(VI) to Cr(III) resulting in the toxic intermediates is not dependent on any enzymes. Instead, it happens by direct electron transfer from ascorbic acid and non protein thiol-groups (Zhitkovich 2005). This is indicative of the potency of Cr as toxic element in the human system.

From the present data, we infer that of the studied Y-linked genes and loci, AZFa region showed few microdeletions but it is difficult to fathom that the same is caused due to chromium exposure. Thus, the Y chromosome seems to be protected by some innate mechanisms. Studies on more number of samples with different levels of chromium exposure from across different populations would help understand its genotoxicity.

Competing Interests

The authors declare that they have no competing interests.

Figures

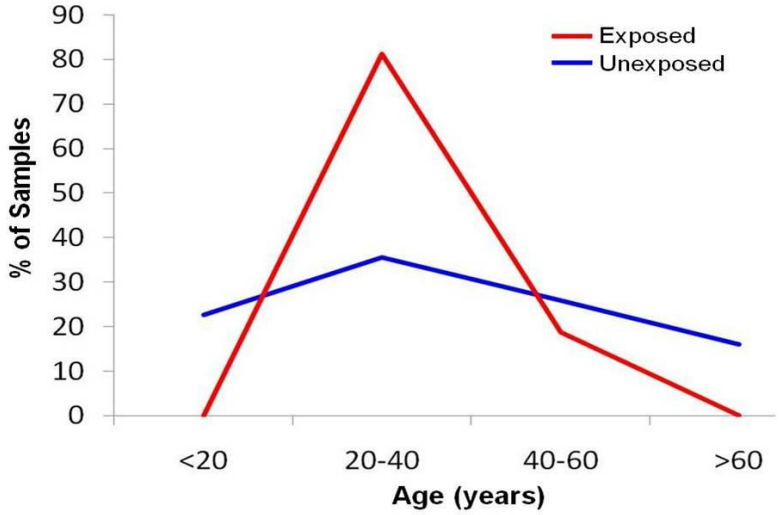

Fig 1 Age wise distribution of exposed and unexposed samples used in the study

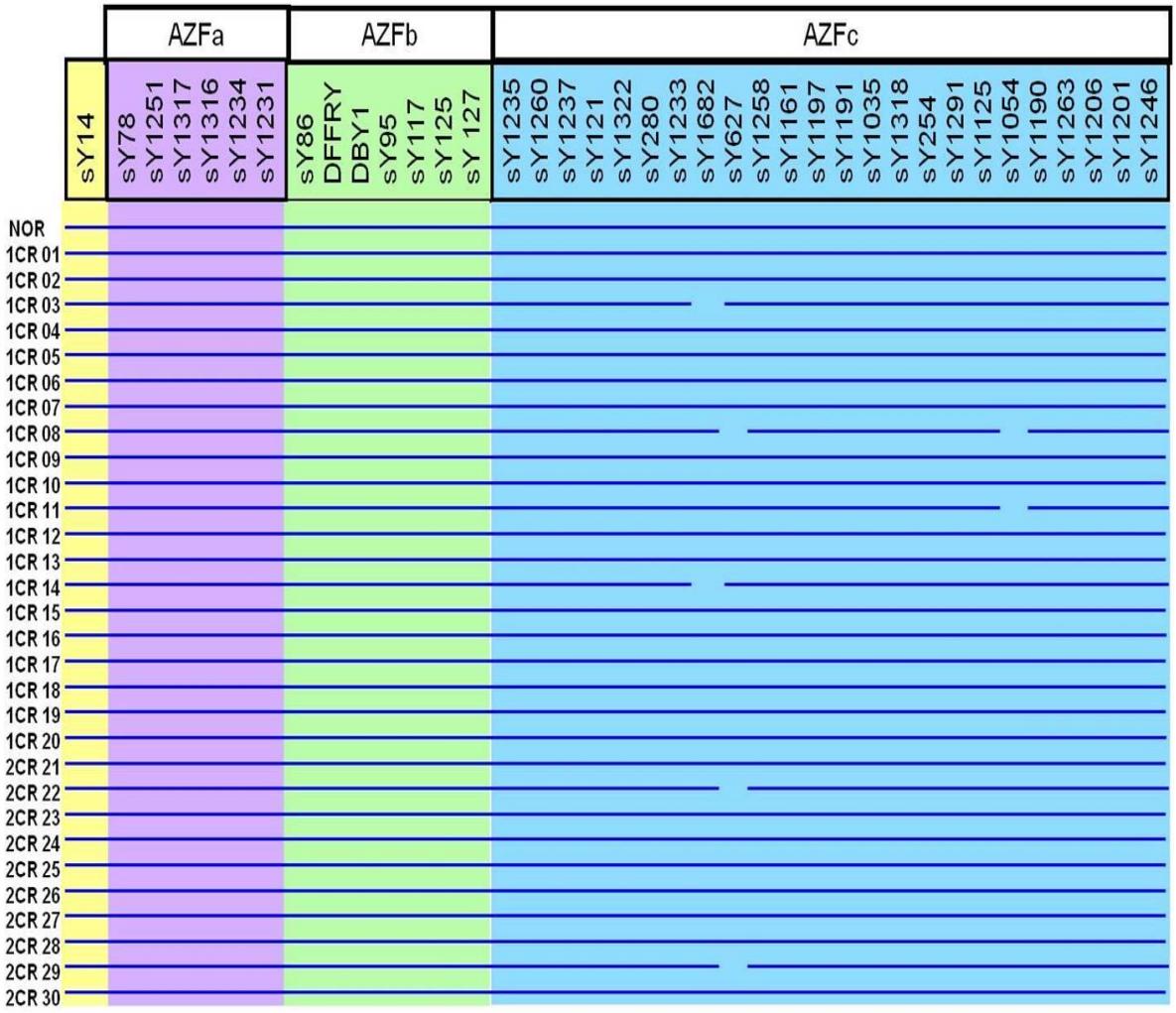

Fig 2 Screening of STSs across the AZF regions of Y chromosome Diagrammatic representation summarizing STS analysis in the representative samples for the presence of AZFa, AZFb and for different recombination deletions in the AZFc region. These included P5-Proximal P1, P5-Distal P1, gr/gr, b1/b3, b2/b3, TSPY-TSPY. sY14 located in SRY gene was used as positive control. The sample IDs are given on the left side while the STSs analyzed are on top. Continuous blue line is indicative of intactness of the STSs screened while the breaks in the lines reflect absence of the same.

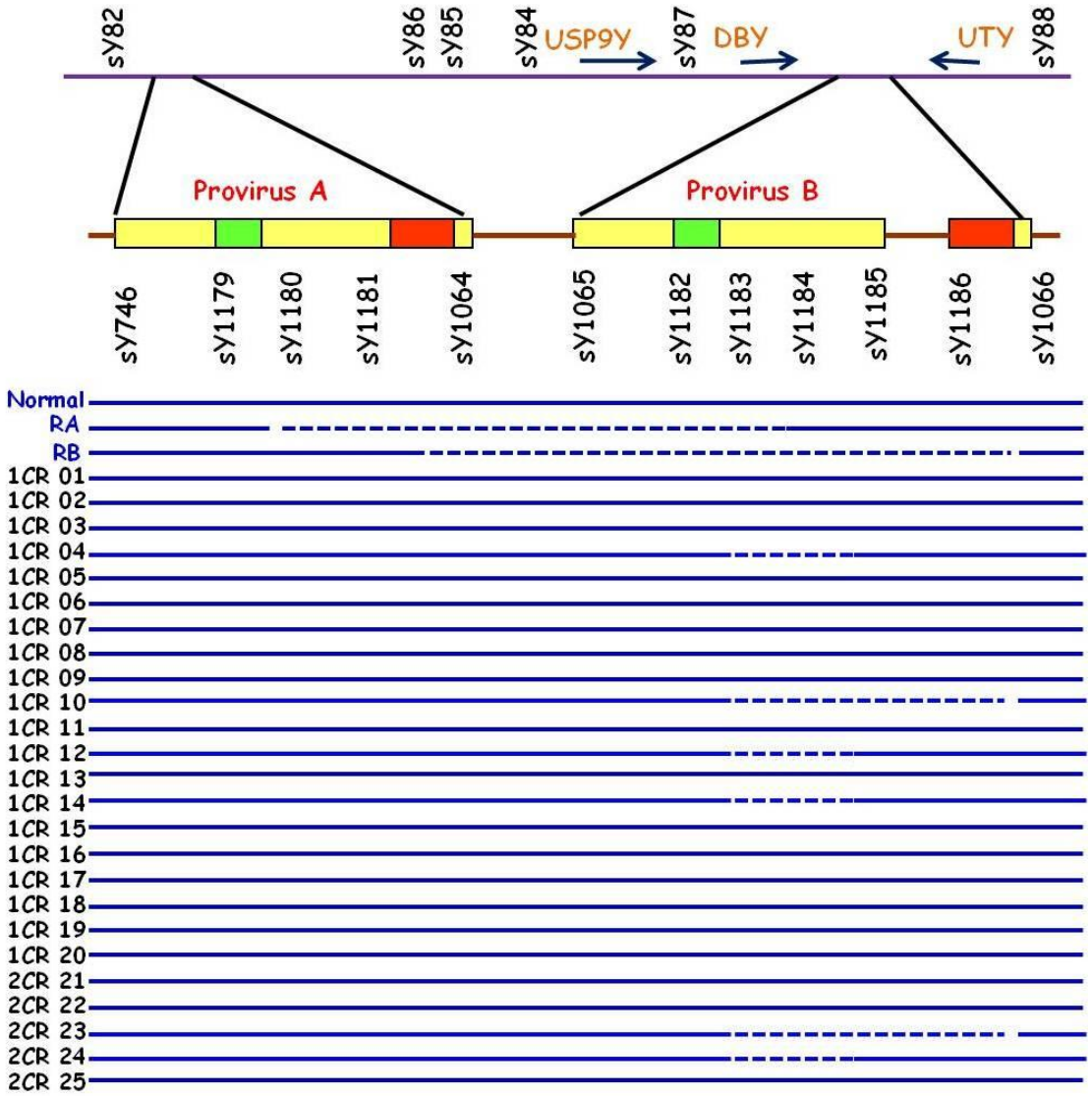

Fig 3 STS analysis of AZFa region for HERV mediated recombination A graphical illustration summarizing the screening of recombination due to HERV provirus sequences in the AZFa region. The green and red bars are the location of homologous sequences responsible for recombination. The STSs analyzed have been mentioned. The location of genes in the region is indicated on top. Intact line indicates presence of STSs while dotted line reflects its deletion. Normal samples represent no recombination event; RA represents deletion pattern expected due to recombination of sequences of

the green bar while RB represents pattern due to that of red bar. The STSs are given on top while sample IDs are given on the left.

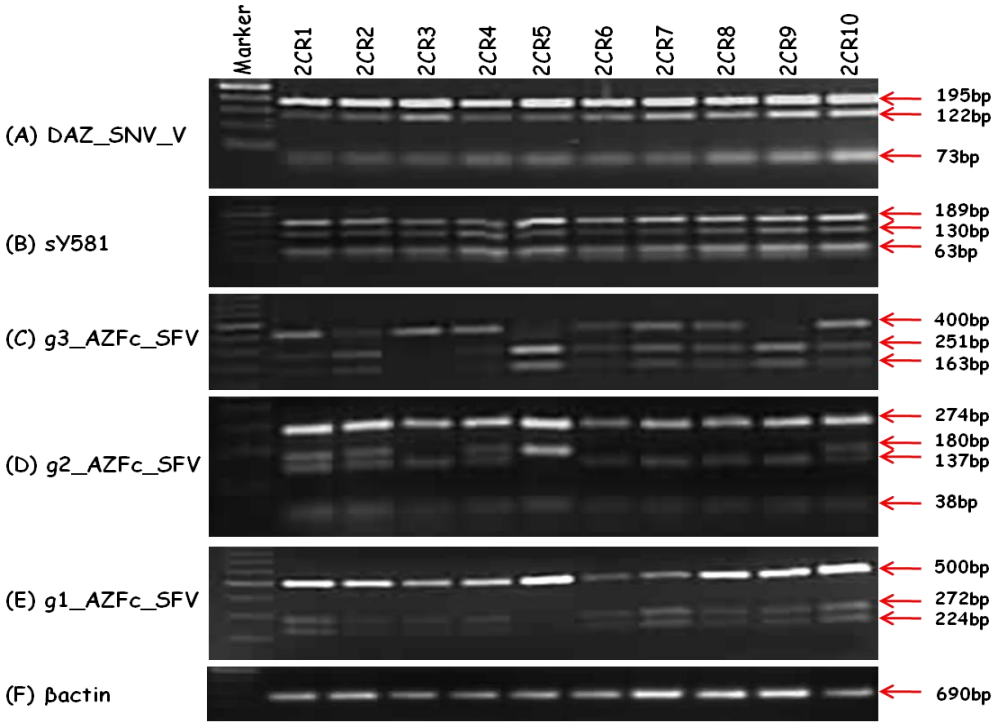

Fig 4 SNV analyses of the genes and amplicons Gel pictures of representative SNVs are shown. The target SNV is mentioned on the left, sample IDs are on top while the fragment lengths on right. Note the absence of certain fragments in **c, d** and **e** representing g3, g2 and g1 amplicons, respectively. **(f)** β-actin was taken as positive control.

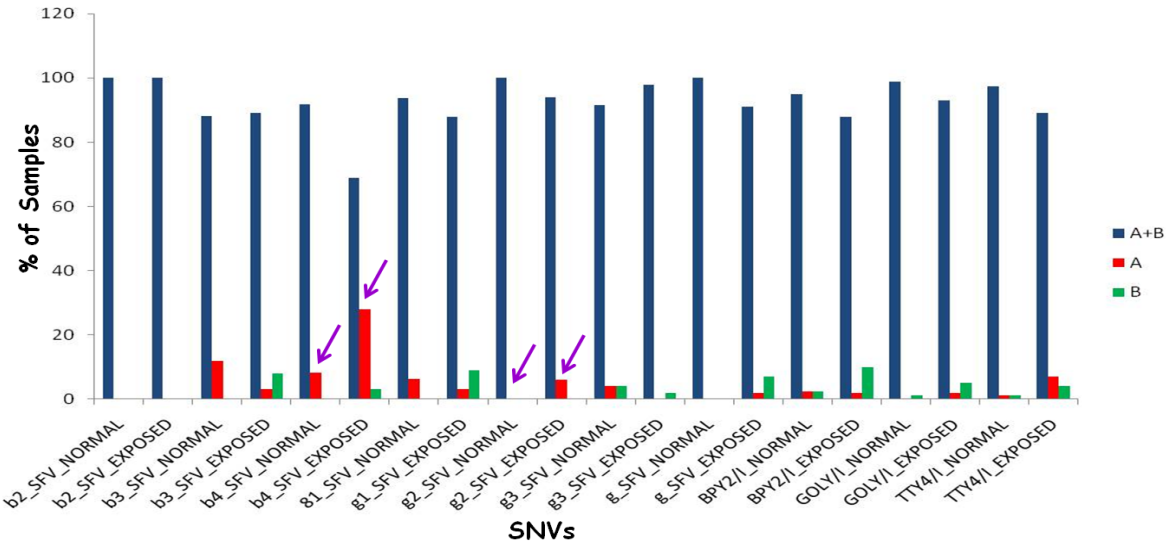

Fig 5 Variation in SNV profiles across exposed and normal samples Comparative analysis of the results of SNVs present in the amplicons and genes (BPY2, GOLGA2LY and TTY4) of the AZFc region between the unexposed and exposed samples. The arrows indicate profiles of b4 and g2 amplicons where significant differences were observed in the two sample sets.

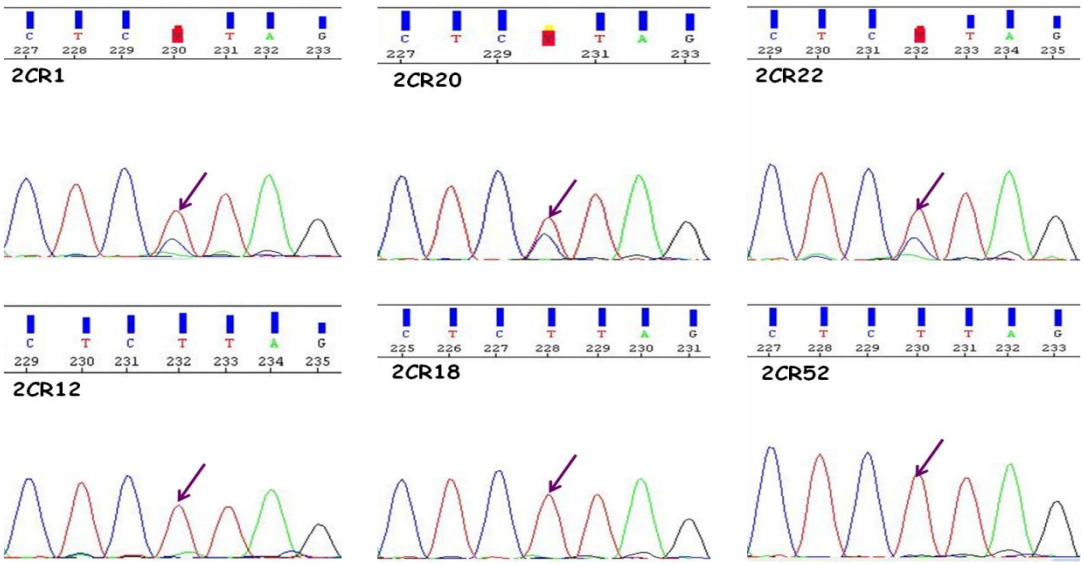

Fig 6 Sequencing of b3 SNV in the AZFc region XmaJI enzyme with recognition site CCTAGG was used for this SNV. The variant sequence was CTTAGG. Samples having normal restriction profile (2C1, 2C20, 2C22) showed dual peaks corresponding to Y nomenclature (C/T) suggesting the presence of both the sequences in b3 amplicon. However, samples (2C12, 2C18, 2C52) with varying restriction profile showed only one peak corresponding to T indicating the absence of restriction site with result, amplicons remained undigested. The arrows highlight the site of variation.

Table 1: Summary of DAZ SNVs in the samples

Target	SNV	Profile	Exposed (%)	Unexposed (%)
DAZ	I	A+B	97	100
		A	3	0
		B	-	0
	II	A+B	98	100
		A	1	0
		B	1	0
	III	A+B	100	100
		A	-	0
		B	-	0
	IV	A+B	100	100
		A	-	0
		B	-	0
	V	A+B	97	100
		A	1	0
		B	2	0

	VI	A+B	100	100
		A	-	0
		B	-	0
	sY581	A+B	100	100
		A	-	0
		B	-	0

V ACKNOWLEDGEMENT

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