Chapter 4
Analysis of single nucleotide variants (SNVs) in DAZ and CDY1 specific gene copies and its affect on spermatogenic impairment and male infertility

4.1 Introduction

The primary candidate gene to be segregated from the Y chromosome AZFc region is DAZ (deleted in azoospermia) (Rejio et al., 1995). Moreover, DAZ originated on the Y chromosome as a transposition of a DNA segment including the autosomal germ cell-specific gene DAZL (deleted in azoospermia like) in chromosome 3. However, the comparative analysis in primates illustrates that the DAZ transposition arise 35 million years ago in a common ancestor of old world monkeys and in humans (Seboun et al., 1997). On contrary, there is at least one other functional gene surrounding the AZFc interval namely CDY1, a retroposon that encodes for histone acetyltransferase protein, which is localized in the nuclei of maturing spermatids and has a strong preference for histone H4 (Lahn et al., 2002). Histone hyperacetylation in late spermatids results in a more open chromatin structure, which assist not only the spermatogenic histone replacement but also offer an easier access of the transcriptional machinery to the postmeiotic sperm DNA (Giachini et al., 2008). On the other hand, CDY1 is believed to retropose directly into MSY (male specific region of the Y chromosome) from a transcript of the autosomal gene CDYL on chromosome 6 above 150 million years ago even though it is found
only in primates, creating it one of the oldest genes on the Y chromosome (Dorus et al., 2003). Presently, DAZ and CDY1 are the only transcription units in the AZFc region for which there are evidences that function has been selectively sustained on the Y chromosome.

The DAZ gene family is located in the distal euchromatic part of the long arm of human Y chromosome (Yq11.23) in the AZFc region (Vogt, 1998; Saxena et al., 2000). The exact functional role of the DAZ genes in germ cells is not yet known. Human DAZ proteins are found in late spermatids, sperm tails, spermatogonia and spermatocytes (Habermann et al., 1998; Rejio et al., 2000). There are four copies of the DAZ in Y chromosome, which are almost identical and 2 clusters of these genes are inverted pairs of DAZ1/2 and DAZ3/4 (McElreavey et al., 2006). The deletion of each member of DAZ may have a differential effect on fertility. Deletions in DAZ2, DAZ3, and DAZ4 copies are found in both fertile and infertile men and are described as familial variants inherited from father to son. However, DAZ1/2 deletions are reported to be restricted only to infertile men. Expression of DAZ1 seems to be essential for spermatogenesis, but recent studies have reported the fertile individuals with DAZ1 deletions (Machev et al., 2004; Fernandes et al., 2006). The most important AZFc candidate infertility genes are believed to be DAZ and CDY1 (Fernandes et al., 2002; Lahn et al., 2002). Krausz et al., (2009) detected four major combinations of deletions namely, DAZ1/2 + CDY1a, DAZ1/2 + CDY1b, DAZ3/4 + CDY1a and DAZ3/4 + CDY1b. Repping and his colleagues (2003) have identified three types of deletions, not all of which included the DAZ1/2 cluster or the DAZ3/4 cluster. Later, Machev and colleagues (2004) found four types of deletions and showed that only deletions containing DAZ3/4 are linked with infertility.
Previously, few studies have suggested that the $DAZ1/2$ deletion represents an important genetic defect leading to impaired spermatogenesis in Caucasians. In contrast, a recent study observed 1.4% $DAZ1/2$ deletion in normozoospermic Chinese men with no significant differences found in frequencies between case and control groups suggesting that the $DAZ1/2$ deletion on its own may be insufficient to result in spermatogenic impairment. The reason for diverse observations in different studies could be manifold, such as genetic modification, Y chromosome haplogroups in different ethnic populations (Vogt, 2005). A recent multicenter study that examined the impact of different deletions of $DAZ$ and $CDY1$ gene copies in Europe concluded that the presence or absence of specific $DAZ$ or $CDY1$ copies are not associated with the fertility problems of the subjects (Krausz et al., 2009). In another study Yang et al., (2010) reported a link between haplogroups in Han Chinese men and the loss of $CDY1a$ or $CDY1b$ in gr/gr deletion carriers. They also observed that both deletions of $CDY1a$ and $CDY1b$ are common in the patient group and found a correlation between deletion of $DAZ1/2$ and spermatogenic failure.

The precise biological role of $DAZ$ and $CDY$ gene families are still questionable, however, the expression levels and patterns indicate that they are essential for spermatogenesis. Based on the expression data, much research has focused on deletion frequency as well as the types of loss of $DAZ$ and $CDY$ families and relationship to male infertility (Giachini et al., 2005). Further understanding of these deletions would be useful in elucidating the role of $DAZ$ and $CDY$ gene families in spermatogenesis. As stated earlier, the deletions are classified in to 4 types by the gene copy loss of $DAZ$ and $CDY$. All these development together with the potential geographical differences in copy deletion and distribution with Y haplogroup suggest
that further investigation of the deletion rearrangement, its relationship with Y chromosome and spermatogenic impairment necessitate the investigations in population with different origin (Machev et al., 2004; Yang et al., 2008a; Krausz et al., 2009). Thus, the current study has been initiated as to the best of our knowledge the association of DAZ and CDY1 gene copy deletion with phenotypic variants of AZFc subdeletions are not thoroughly examined in Indian population. Having said that there are only two reports from Northern India and West coast of India (Shahid et al., 2011; Sen et al., 2015), whereas there is no data available from Southern part of Indian population.

Since most of the individuals with AZFc deletions exhibit poor semen quality, in our present investigation we aimed to determine the frequency of deletion in DAZ and CDY1 specific gene copy from South Indian admixture population. Further, our study focused to identify whether DAZ and CDY1 specific gene copy deletions are establishment of the phenotypic expression in AZFc partial deletions through single nucleotide variant analysis (SNVs) or single family variants (SFVs). Finally, whether these observed SNVs have any significant association with spermatogenic impairment and male infertility among infertile and normozoospermic controls.

4.2 Materials and Method

4.2.1 IHEC clearance statement: As mentioned in the chapter 1 and 2 the current research work was approved by IHEC. In addition, informed consent forms were obtained from the all subjects after briefing the purpose of the present study.
4.2.2 Study population and geographic location details

**Group 1 - Urban fertile individuals (Normozoospermic controls):** Out of 244 normozoospermic controls, who were screened for Y chromosome AZFc partial deletions, 27 individuals exhibited random deletions for STS’s sY1291, sY1191 and sY1197. Later, DAZ and CDY1 SNV analysis was carried out by recruiting these 27 subjects with Y chromosome AZFc partial deletions.

**Group 2 - Urban infertile:** A total of 239 infertile individuals were subjected for Y chromosome AZFc partial deletion analysis. However, a total of 44 individuals showed deletions randomly for STS’s sY1291, sY1191 and sY1197 combinations. Further, to fine tune the deletion, we analyzed whether gene dosage (DAZ and CDY) reduction represents a risk factor for spermatogenic failure by recruiting the 44 infertile subjects for SNV analysis.

**Group 3 - Siddi tribes:** As mentioned in the previous chapter, out of 200 Siddi tribal men only one subject showed Y chromosome AZFc partial deletions for b2/b3 deletion. However, for the SNV analysis of DAZ and CDY1 copies, total number was increased to 10 men and the present investigation was carried out for gene dosage reduction.

4.2.3 Analysis of SNVs / SFVs: In order to investigate SNVs or SFVs in DAZ and CDY1 genes, previously described protocol has been employed with minor modifications (Machev et al., 2004). For DAZ SNV analysis, the SNV at STS - sY587 in the intron 10, also known as DAZ-SNV V was employed that distinguishes DAZ1/2 (A) from DAZ3/4 (G). However, for CDY1 analysis since the Y chromosome
reference sequence illustrates the lack of SNVs within the transcribed segment of 
\textit{CDY1} gene, the presence of \textit{CDY1a} and \textit{CDY1b} was tested for the C/A by employing 
SNVs situated at 7750bp 5’ of the \textit{CDY1} translation start codon.

\textbf{4.2.4 Primer sequence information:} Using gradient PCR (Super Cycler, Kyratec, 
Australia) method, each set of primers that were synthesised and obtained from IDT, 
USA were further optimized and validated. The primer details are mentioned below:

\textbf{i. DAZ gene specific copies}

sY587 – o912 Forward Primer: TGTATTTAAAATGTGCACTTCACTGT 
sY587 – o913 Reverse Primer: CCAGTCACAAAAATGCCACAT

\textbf{ii. CDY1 gene specific copies}

\textit{CDY1} 7750 – o1025 Forward Primer: GAAATGCCATAATGTGCTAACACTG 
\textit{CDY1} 7750 – o1026 Reverse Primer: AAGGAGAGTGTTAATACATACCCTG

\textbf{4.2.5 Reaction mixture and reaction conditions for PCR:} Genomic DNA was 
extracted from the peripheral blood sample using QiAmp® Blood DNA mini kit 
(Qiagen, Germany) as per the manufacturer’s standard protocol (Refer section 2.2.4). 
While, the genomic DNA from fertile men (positive control) provide the sensitivity 
and specificity of each PCR reaction performed, the female DNA sample (negative 
control) offer the specificity of the primer pairs employed for Y chromosome. Further, 
a water sample (blank) containing all components except the genomic DNA was run 
with each set of primers in order to detect any possible reagent contamination.

PCR components for reaction mixture:

\textbf{i.} 12µL of PCR ready Go Taq colourless Master Mix (Promega, USA)

\textbf{ii.} For sY587, 0.65µL of forward and reverse primer (IDT, USA)
For CDY1-7750, 0.55µL of forward and reverse primer (IDT, USA)

iii. 0.30µL of bovine serum albumin solution 
iv. 9.7µL of triple distilled water 
v. 2.0µL of template DNA

The Restriction Length Fragment Polymorphism (RFLP)-PCR reaction conditions are as follows:

Step i. Initial denaturation - 94°C for 3 minutes
Step ii. Denaturation - 94°C for 30 seconds
Step iii. Annealing for sY587 & CDY1-7750 - 57°C for 30 seconds
Step iv. Extension - 72°C for 30 seconds
Step v. Final extension - 72°C for 5 minutes
Step vi. Hold - 10°C for infinite time

For DNA amplification, PCR reaction from denaturation to extension step was repeated 35 times (35 cycles) before holding the reaction mixture at 10°C.

4.2.6 RFLP based SNVs/SFVs analysis: The PCR amplified product for sY587 marker was digested using DraI restriction endonuclease that discriminates DAZ1/2 from DAZ3/4. Similarly, for recognition of CDY1a and/or CDY1b deletion the reaction mixture was treated with restriction endonuclease, PvuII. Finally, the observed deletion pattern was categorized into four different groups, namely:

i. DAZ1/2 + CDY1a
ii. DAZ1/2 + CDY1b
iii. DAZ3/4 + CDY1a
iv. DAZ3/4 + CDY1b
a. **Restriction Digestion:** 10µL of amplified PCR product was utilized for restriction digestion using the *Dra*I and *Pvu*II restriction endonuclease (Thermo Scientific, USA), which discriminates *DAZ1/2* from *DAZ3/4* and *CDY1a* from *CDY1b* respectively. The reaction components for restrictive digestion are mentioned below:

i. PCR reaction mixture - 10µL  
ii. Nuclease free water - 18µL  
iii. 10X Buffer tango/G - 2µL  
iv. *Dra*I and *Pvu*II (2units/reaction) - 1-2µL

The reaction mixture was incubated at 37°C for 3 hours before analysing the digested products on agarose gel stained with EtBr.

b. **Agarose gel electrophoresis:** To prepare 2% gel, 2gms of agarose (SRL, Mumbai, India) was dissolved in 2mL of 40X TAE buffer (Promega, USA) and total volume was made up to 100mL using double distilled water. The solution containing conical flask was heated in a microwave for 3-5 minutes by swirling the solution. After cooling down 6µL of EtBr (SRL, Mumbai, India) was added to the conical flask. Finally, the gel was poured in to a casting tray and allowed to solidify for 20-30 minutes. Later, 2µL of gel loading dye was added to all the digested products before loading the samples in to the casted gel. In order to estimate the PCR product size, along with the samples 100bp DNA ladder (Merck Millipore GeNeI, India) was loaded in a separate lane. Subsequently, the PCR products were separated at 80V constant voltage until the loading dye migrates three by fourth of the gel. At last, the DNA bands were visualized and image was documented using software based gel documentation system (UVItec, Cambridge).
4.2.7 **Statistical analysis:** The obtained data was analyzed using the statistical software SPSS version 21. The mean ± SD was calculated for continues variables between normozoospermic controls and infertile group. The Chi-square test (Fisher Exact test) was applied between the case and control groups to calculate the significant difference in frequency. Independent *t*-test analysis employed to calculate the significance difference between SNV pattern and variation in semen profile and sperm function test among case and control group. Further, odds ratio and 95% confidence of interval was calculated using Binary logistic regression analysis. For all the statistical tests, *p* < 0.05 was considered as significant.

4.3 Results

The current study involves a total of 10 Siddi tribal men, 27 normozoospermic controls and 44 infertile individuals for SNVs analysis. In order to characterize the type of AZFc partial deletion, the screening *DAZ* and *CDY1* specific gene copies and their functional importance on spermatogenesis are elucidated. Present study has documented four different types of partial deletion pattern namely, *DAZ1/2 + CDY1a*, *DAZ1/2 + CDY1b*, *DAZ3/4 + CDY1a* and *DAZ3/4+CDY1b* in our study subjects (Figure 4.1 - 4.2). Interestingly, while investigating the *DAZ* and *CDY1* gene copy dosage the tribal subject who showed AZFc partial deletion for b2/b3 deletion, also displayed *DAZ3/4 + CDY1a* deletion pattern, whereas the rest of the Siddi’s are negative for any pattern of SNV deletions (Figure 4.3 and Table 4.1). As stated in the previous chapter, this specific individual with SNV deletion is married and fathered children. Further, 29 individuals out of 44 infertile men (65.9%) and 11 subjects out of 27 normozoospermic controls (40.7%) are positive for *DAZ* and *CDY1* deletions.
accounting for 11.5% and 7.69% $DAZ1/2 + CDY1a$ deletion and 3.84% and 7.69% $DAZ1/2 + CDY1b$ deletion respectively in infertile and normozoospermic control samples (Table 4.1). Additionally, $DAZ3/4 + CDY1a$ is the major sub-pattern of deletion observed among infertile (61.5%) and normozoospermic controls (61.5%) (Figure 4.3 and Table 4.1). Moreover, the $DAZ3/4 + CDY1a$ pattern of subdeletion is predominant among subjects with AZFc gr/gr partial deletion in infertile (46.1%) as well as in normozoospermic controls (61.5%) (Figure 4.8) and specifically, azoospermic and oligozoospermic subjects in infertile group showed a higher incidence of $DAZ3/4 + CDY1a$ subdeletions. Finally, the subdeletion pattern of $DAZ3/4 + CDY1b$ shared similar distribution among case (23%) and control (23%) scoring second highest in which again gr/gr deletion pattern is more prominent (Figure 4.3 and Table 4.1).

Furthermore, the effects of SNVs over different semen parameters are also examined by employing independent $t$-test. The analysis revealed that $DAZ1/2 + CDY1a$ subdeletion pattern showed a significant decline only in sperm count ($p = 0.04; 95\% \text{ CI} = 4.80 – 134.6$), but not for other semen variables and sperm functional assay (Figure 4.4 and Table 4.2). Given that only one individual in control and infertile group displayed $DAZ1/2 + CDY1b$ deletion (Figure 4.5), the independent $t$-test analysis has not been performed. However, for $DAZ3/4 + CDY1a$ deletion statistical analysis revealed significant differences only for sperm count ($p = 0.01; 95\% \text{ CI} = 13.6 – 95.2$), not for other variables including sperm function tests (Figure 4.6 and Table 4.2). Interestingly, $DAZ3/4 + CDY1b$ subdeletion pattern showed statistical significance for all semen variables. Compared to normozoospermic controls, sperm morphology ($p = 0.02; 95\% \text{ CI} = 2.3 – 21.9$), vitality ($p = 0.01; 95\% \text{ CI} = 2.3 – 21.9$),
CI = 10.2 – 74.8), count ($p = 0.002; 95\% \text{ CI} = 21.3 – 69.6$) and motility ($p = 0.003; 95\% \text{ CI} = 15.9 – 58.2$) values are reduced in infertile samples (Figure 4.7A and Table 4.2). Additionally, sperm function test for HOS also exhibited statistical significance differences ($p = 0.01; 95\% \text{ CI} = 5.75 – 52.1$), but not NCD and AIT (Figure 4.7B and Table 4.2). Taken together, independent $t$-test analysis unraveled that, compared to $DAZ1/2 + CDY1a$ and $DAZ3/4 + CDY1a$, the $DAZ3/4 + CDY1b$ SNV pattern is strongly associated with impaired spermatogenesis and sperm dysfunction.

To further investigate the effect of $DAZ$ and $CDY1$ SNV on genotype-phenotype correlation and risk factor for spermatogenic impairment, odds ratio and 95\% CI are calculated between the case and control groups. The analysis showed significance between case and control for $DAZ3/4 + CDY1a$ and $DAZ3/4 + CDY1b$ SNV patterns, but not for $DAZ1/2 + CDY1a$ and $DAZ1/2 + CDY1b$ (Table 4.3). Compared to $DAZ3/4 + CDY1b$ deletion pattern ($OR = 1.0; 95\% \text{ CI} = 0.20 – 4.85; p = 0.05$), $DAZ3/4+CDY1a$ SNV showed higher significance values ($OR = 1.0; 95\% \text{ CI} = 0.25 – 3.92; p = 0.0001$) in our study subjects, thereby exhibiting a significant association for spermatogenic impairment and possible risk factor for male infertility (Table 4.3).

Next, the frequency of SNV patterns in different types AZFc partial deletions that investigated by employing various STS markers (gr/gr, b1/b3 and b2/b3) are correlated. As stated earlier, the individuals who carried gr/gr deletion also showed higher incidence of SNV pattern for $DAZ3/4 + CDY1a$ compared to b1/b3 and b2/b3 partial deletions (Figure 4.8). Further, odds ratio analysis at 95\% CI determined by binary logistic regression analysis revealed significant difference for infertile
individuals with gr/gr deletion, implying it as a risk factor. However, the risk factors associated with b1/b3 and b2/b3 deletions are relatively lesser in comparison to gr/gr deletion (Table 4.4). Thus, our data suggests that the individuals carrying gr/gr deletion possibly have higher chance of exhibiting $DAZ3/4 + CDY1a$ SNV pattern and in addition, the subjects who carry this specific deletion and SNV pattern combination may be associated with increased risk of spermatogenic impairment and male infertility.

4.4 Discussion

In the present study, systematic analyses of individuals with AZFc deletions are screened for $DAZ$ and $CDY1$ specific gene copy deletion. Our study demonstrate that the men with AZFc partial deletions showing two copies of $DAZ$ with one copy of $CDY1$ gene are highly predisposed for spermatogenic impairment with increase number of azoospermia and oligospermia.

Krausz et al., (2009) reported that the individuals who had $DAZ$ and/or $CDY1$ gene copies intact despite having gr/gr, b1/b3 or b2/b3 deletion. So, in order to understand the impact of $DAZ$ and $CDY1$ copy deletions, we categorized our study subjects with AZFc partial deletions namely, gr/gr, b1/b3 and b2/b3 based on the $DAZ$ and $CDY1$ gene copy deletion. In our study four types of classical SNV patterns, which consisted of eliminating two $DAZ$ gene copies and one $CDY1$ gene copy that are observed to be strongly associated with spermatogenic impairment, wherein azoospermia and oligospermia conditions are comparatively on higher side. However, individuals with $DAZ3/4 + CDY1a$ and $CDY1b$ deletions are in higher frequency with significantly poor semen variables compared to $DAZ1/2 + CDY1a$ and $CDY1b$
deletion pattern. The frequency of $DAZ3/4 + CDY1a$ and $CDY1b$ deletions is comparable between both infertile and control groups. Thus, the findings suggest that in subjects with AZFc partial deletions and in combination loss of copies of either $DAZ$ and/or $CDY1$ genes are associated with higher risk factor for spermatogenic impairment, which results in male infertility. This observation is in consistence with a report from West coast of India (Sen et al., 2015).

High frequency of the $DAZ1/2$ deletion has been observed in patients with spermatogenic failure in Europe (Fernandes et al., 2002; Ferlin et al., 2002, 2005). However, the deletion of $DAZ3/4$ is fixed in haplogroup N, which is common among northern Eurasians. Further, the presence of $DAZ1/2$ copy, but not $DAZ3/4$, is solely responsible for normal spermatogenesis in Eurasian population (Fernandes et al., 2004; Repping et al., 2004b). Interestingly, in our study relatively incidence of $DAZ1/2 + CDY1a$ and $CDY1b$ deletions may be attributable to reduced number of deletion carriers. Nevertheless, $DAZ1/2 + CDY1a$ gene copy deletion showed significant association for low sperm count ($p = 0.04$) even in lower sample number.

Fernandes et al., (2002) reported the prevalence of gr/gr deletions removing $DAZ1/2$ copies in infertile men of Caucasian origin and other groups. Another study by, Machev et al., (2004) illustrated that the $DAZ3/4 + CDY1a$ deletion and the variant loss of $CDY1a$ are associated with infertility. However, studies from Han Chinese population illustrated that only gr/gr based $DAZ1/2 + CDY1a$ and $CDY1b$ showed higher frequency of deletion and significant risk factor for spermatogenic impairment instead of $DAZ3/4 + CDY1a$ and $CDY1b$, due to low number deletion carriers that exhibited limited role in spermatogenic damage (Yang et al., 2008). On
the contrary, in our study the frequency of $DAZ3/4 + CDY1a$ and $CDY1b$ deletions are higher with similar distribution among infertile and control groups. In addition, our striking observation that $DAZ3/4 + CDY1a$ deletion is associated with significant reduction in, whereas $DAZ3/4 + CDY1b$ SNV pattern showed significant reduction for all the semen variables and HOS test.

A most recent study from Northern part of India examined types of $DAZ$ gene copy deletions and indicated their differential influence in azoospermic patients (Shahid et al., 2011). The group observed that seritoli cell only syndrome (SCOS) with $DAZ1/2$ deletions, whereas spermatogenic arrest (SA) with missing $DAZ3/4$ gene copy. Later, they demonstrated that patients with SA and carry gr/gr deletions involving $DAZ3/4$ genes might be the possible risk factor for the spermatogenic impairment. Consistently, in the present study individuals with gr/gr deletions carrying $DAZ3/4 + CDY1a$ and/or $CDY1b$ deletion patterns are in higher frequency compared to b1/b3 or b2/b3 deletion combination. The odds ratio calculation for risk factor assessment showed statistical significant difference for gr/gr - $DAZ3/4 + CDY1a$ combination.

Nevertheless, environmental factors, genetic modifications and probably the Y chromosome haplogroups may play an important role in variation in SNV patterns observed across different population. Unexpectedly, the gr/gr partial deletion with either $DAZ1/2$ or $DAZ3/4$ genes elimination has been observed in two normozoospermic individuals, which is found to be fully compatible with proven fertility. Similarly, normozoospermic controls in our study also showed different SNV deletion patterns with proven fertility. Meanwhile, our findings are not in agreement
with the observation that gr/gr partial deletions along with DAZ1/2 gene deletions are significant risk factor for spermatogenic impairment and associated with male infertility (Repping et al., 2003; Fernandes et al., 2002; Ferlin et al., 2005).

In parallel, a study demonstrates that the b1/b3 deletion is linked with DAZ1/2 gene deletions and it appears to be a risk factor for spermatogenic damage (Shahid et al., 2011). Findings by Lu et al., (2009) indicated that in b2/b3 deleted group of Han Chinese population, the frequency of DAZ3/4 + CDY1a deletion pattern is relatively higher in azoospermia and oligospermia cases, suggesting significant association in both infertile conditions. Further, the study showed that DAZ3/4 + CDY1a deletion pattern is likely to be associated with spermatogenic impairment. Similarly, individuals with b2/b3 deletion carrying DAZ3/4 + CDY1a was next to the gr/gr deletion carriers and showed slight variation in the semen variables between infertile and controls causing spermatogenic damage.

From clinical point of view, in order to assess the risk we have addressed whether the deletion pattern and sperm variables can be employed as a prognostic marker for occurrence of AZFc partial deletions and DAZ/CDY1 SNVs. Interestingly, the risk assessment analysis for DAZ/CDY1 SNVs revealed that, individuals carrying AZFc partial deletion, especially gr/gr and b2/b3 deletion combination with DAZ3/4+CDY1a and CDY1b pattern shows higher risk factor for spermatogenic impairment leading to male infertility. Based on our current observation, we suggest that AZFc partial deletion screening followed by DAZ and CDY1 gene copy deletion analysis may be offered in a clinical investigation. As we know, most men with azoospermia and severe oligozoospermia opt intra cytoplasmic sperm injection (ICSI)
for biological parenthood, it is clear evident that these AZFc partial deletions will also be vertically transmitted to their sons. Knowing the fact that AZFc subdeletions by themselves confer high risk ofazoospermia and oligozoospermia. Further, these AZFc partial have a susceptibility to extend into full AZFc deletions in coming generation (Zhang et al., 2007). In this situation it is necessary to recommend this testing in clinical investigation of infertile male and also counsel these subjects for the risk assessment prior undergoing to ICSI.
Figure 4.1: (A) Agarose gel shows the undigested product of $DAZ$ SNV V (sY587) among study cohort. IF – infertile; NCS - normozoospermic control, TB - Siddi tribe. (B, C, and D) Representation of $DAZ$ SNV V (sY587) digested products with $DraI$ restriction endonucleases, which distinguishes $DAZ3/4$ and $DAZ1/2$ at A/G site. White arrow indicates absence of $DAZ$ specific copy deletion in the study subjects. First lane in all the image corresponds to 100bp marker and product size are 270bp, 195bp, 49bp and 26bp.
Figure 4.2: (A) Agarose gel shows the undigested product of CDY1-7750’ among study cohort. IF – infertile; NCS - Normozoospermic control, TB - Siddi tribe. (B, C, and D) Representation of CDY-7750’ digested products with PvuII restriction endonucleases that differentiates CDY1a and CDY1b at C/A site. White arrow indicates absence of CDY1 specific copy deletion in the study subjects. First lane in all the image corresponds to 100bp marker and product size are 201bp, 119bp and 82bp.
**Figure 4.3:** Distribution of different types of DAZ and CDY1 SNV deletion combination in Siddi tribal men, normozoospermic controls and infertile men.

**Figure 4.4:** Illustration of average scores for (A) semen variables and (B) sperm function assay in control and infertile group with DAZ1/2 + CDY1a SNV pattern. Error bars indicate mean ± SE.
Figure 4.5: Histogram shows the mean scores for (A) semen variables and (B) sperm function assay in control and infertile group with $DAZ1/2 + CDY1b$ SNV pattern. Error bars indicate mean ± SE.

Figure 4.6: Illustration of mean values for (A) semen variables and (B) sperm function assay in control and infertile group with $DAZ3/4 + CDY1a$ SNV pattern. Error bars indicate mean ± SE.
Figure 4.7: Histogram shows the mean scores for (A) semen variables and (B) sperm function assay in control and infertile group with $DAZ3/4 + CDY1b$ SNV pattern. Error bars indicate mean ± SE.

Figure 4.8: Distribution of different types of AZFc partial deletions in normozoospermic control and infertile subjects with $DAZ3/4 + CDY1a$ SNV pattern.
Table 4.1: Deletion frequency for different types of DAZ and CDY1 SNV deletion combinations among Siddi tribe, Normozoospermic controls and infertile groups

<table>
<thead>
<tr>
<th>Deletion type</th>
<th>Study group</th>
<th>Total number of subjects with SNVs</th>
<th>Number of subjects with specific SNV pattern</th>
<th>Deletion in percentage</th>
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<tbody>
<tr>
<td>$DAZ1/2$ + $CDY1a$</td>
<td>Siddi tribe</td>
<td>10</td>
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<td>Nil</td>
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<tr>
<td></td>
<td>Normozoospermic</td>
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<td>7.69%</td>
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<td></td>
<td>Infertile</td>
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<td>3</td>
<td>11.5%</td>
</tr>
<tr>
<td>$DAZ1/2$ + $CDY1b$</td>
<td>Siddi tribe</td>
<td>10</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Normozoospermic</td>
<td>13</td>
<td>1</td>
<td>7.69%</td>
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<tr>
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<td></td>
<td>Infertile</td>
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</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>Infertile</td>
<td>26</td>
<td>6</td>
<td>23%</td>
</tr>
</tbody>
</table>

Table 4.2: Independent t-test analysis for different $DAZ$ + $CDY1$ deletion patterns and their comparison with different semen parameters and sperm function test variables among normozoospermic controls and infertile individuals

<table>
<thead>
<tr>
<th>Semen parameter and function tests</th>
<th>Mean ± SD</th>
<th>95% Confidence interval of the difference</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Infertile</td>
<td>Lower</td>
</tr>
<tr>
<td>1. $DAZ1/2$ + $CDY1a$ deletion pattern</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morphology</td>
<td>7.00 ± 0</td>
<td>7.75 ± 1.76</td>
<td>26.7</td>
</tr>
<tr>
<td>Vitality</td>
<td>62.0 ± 0</td>
<td>37.5 ± 3.53</td>
<td>30.5</td>
</tr>
<tr>
<td>Count</td>
<td>75.0 ± 0</td>
<td>5.25 ± 4.17</td>
<td>4.80</td>
</tr>
<tr>
<td>Motility</td>
<td>50.0 ± 0</td>
<td>55.0 ± 7.07</td>
<td>105.0</td>
</tr>
<tr>
<td>NCD</td>
<td>65.0 ± 0</td>
<td>50.0 ± 6.84</td>
<td>95.0</td>
</tr>
<tr>
<td>HOS</td>
<td>62.5 ± 0</td>
<td>40.0 ± 17.7</td>
<td>362.6</td>
</tr>
<tr>
<td>AIT</td>
<td>58.5 ± 0</td>
<td>30.0 ± 2.94</td>
<td>48.5</td>
</tr>
</tbody>
</table>
**2. DAZ3/4 + CDY1a deletion pattern**

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Total with deletions</th>
<th>OR Exp(B)</th>
<th>95% CI for Exp (B)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normozoospermic</td>
<td>Infertile</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DAZ3/4 + CDY1a</strong></td>
<td>1</td>
<td>3</td>
<td>0.63</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>DAZ3/4 + CDY1b</strong></td>
<td>3</td>
<td>6</td>
<td>1.30</td>
<td>1.02</td>
</tr>
</tbody>
</table>

**3. DAZ3/4 + CDY1b deletion pattern**

<table>
<thead>
<tr>
<th>Morphology</th>
<th>Total with deletions</th>
<th>OR Exp(B)</th>
<th>95% CI for Exp (B)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normozoospermic</td>
<td>Infertile</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>DAZ3/4 + CDY1a</strong></td>
<td>1</td>
<td>3</td>
<td>0.63</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>DAZ3/4 + CDY1b</strong></td>
<td>3</td>
<td>6</td>
<td>1.30</td>
<td>1.02</td>
</tr>
</tbody>
</table>

* indicate p value < 0.05 as determined by binary logistic regression method

**Table 4.3:** Different types of DAZ + CDY1 SNV deletion patterns. Binary logistic regression analysis with odds ratio between normozoospermic and infertile males shows risk factor for male infertility by deletion of specific SNV pattern.

* * *
Table 4.4: Different types of AZFc partial deletions in $DAZ3/4 + CDY1a$ SNV patterns. Binary logistic regression analysis with odds ratio between normozoospermic and infertile males shows risk factor for male infertility by deletion of specific AZFc deletion in combination with $DAZ3/4 + CDY1a$ SNV pattern.

<table>
<thead>
<tr>
<th>AZFc deletion type in $DAZ3/4 + CDY1a$</th>
<th>Total number subjects with deletions</th>
<th>OR Exp(B)</th>
<th>95% CI for Exp (B)</th>
<th>$p$ value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normozoospermic</td>
<td>Infertile</td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>gr/gr</td>
<td>8</td>
<td>12</td>
<td>1.86</td>
<td>0.48 7.25</td>
</tr>
<tr>
<td>b1/b3</td>
<td>1</td>
<td>3</td>
<td>0.63</td>
<td>0.06 6.82</td>
</tr>
<tr>
<td>b2/b3</td>
<td>3</td>
<td>14</td>
<td>1.65</td>
<td>0.31 8.79</td>
</tr>
</tbody>
</table>

* indicate $p$ value < 0.05 as determined by binary logistic regression method

** OR - odds ratio; CI - confidence interval