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RESEARCH ARTICLE

First Report of *DAX1* Gene Mutations in Patients with Disorders of Sex Development in West Africa

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Abstract

Background: The *NR0B1* gene (*DAX1*) is essential for adrenal and gonadal development. Mutations in *DAX1* are associated with X-linked adrenal hypoplasia congenita and 46,XY disorders of sex development (DSD). However, no studies have explored *DAX1* mutations in West African populations to date.

Methods: We screened the *DAX1* gene in 28 Senegalese patients with DSD using PCR and Sanger sequencing. Identified variants were analyzed using in silico tools and compared to clinical presentations.

Results: Seven distinct variants were identified, including two previously unreported nonsynonymous mutations and two frameshift deletions. Some were found in 46,XY individuals with female phenotypes despite *SRY* positivity.

Conclusion: This is the first report of *DAX1* mutation screening in a West African DSD cohort. Our findings underscore the value of incorporating *DAX1* into molecular diagnostic strategies, particularly in underrepresented populations.

Keywords: *DAX1*, *NR0B1*, Disorders of Sex Development, Senegal, West Africa, Mutation Screening, *SRY*-negative, 46,XY DSD.

1. Introduction

Sex determination is a complex genetic and hormonal process that plays a fundamental role in human development. While most individuals are born with clear sexual characteristics corresponding to their chromosomal sex (46,XX for females and 46,XY for males), some present with atypical development of chromosomal, gonadal, or anatomical sex. These conditions, known as Disorders of Sex Development (DSD), affect approximately 1 in 4,500 births and encompass a wide spectrum of phenotypes, ranging from mild anomalies such as hypospadias to complete sex reversal. In 2006, an international consensus classified DSDs into three main categories based on karyotype: 46,XX DSD, 46,XY DSD, and sex chromosome DSDs, replacing previously used and now obsolete terms such as “hermaphroditism” or “pseudohermaphroditism”[1].

The *SRY* gene, located on Yp11.3, is the principal initiator of testis development in 46,XY embryos.

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In its absence, the bipotential gonads develop into ovaries by default. *SRY* triggers a cascade involving several key genes, including *SOX9, NR5A1, WT1, WNT4, and DAX1* (*NR0B1*)[2,3]. Among these, *DAX1,* located on Xp21.3, encodes an orphan nuclear receptor that plays a regulatory role in adrenal and gonadal development. It is involved in both testicular and ovarian pathways and appears to act as a dosage-sensitive regulator of sex determination. While overexpression or duplication of *DAX1* has been associated with male-to-female sex reversal in 46,XY individuals, loss-of-function mutations lead to X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism, impairing male development[4,5].

The molecular mechanisms of *DAX1* remain incompletely understood. It is hypothesized to inhibit *SF1*-mediated activation of *SOX9* and *AMH*, and to modulate testis-specific enhancers such as TESCO[6]. In animal models, both the absence and overexpression of *DAX1* lead to gonadal dysgenesis, suggesting a tight dosage-dependent balance is required. Moreover, its expression is not restricted to testis development: *DAX1* is also involved in adrenal formation and hypothalamic-pituitary regulation [2,7].

In Sub-Saharan Africa, and particularly in Senegal, genetic studies on DSD remain rare, despite the high birth rate, consanguinity, and limited access to early diagnostic tools. Cultural stigma, lack of awareness, and limited molecular testing contribute to underdiagnosis and late care[8–11]. To address this gap, our study aimed to explore *DAX1* gene variations in a cohort of Senegalese DSD patients, and to assess their molecular and clinical significance, through sequencing, gene expression analysis, and structural modeling. This is, to our knowledge, the first report of *DAX1* mutation screening in a West African DSD population.

2. Materials and Methods

2.1 Study Design and Ethical Considerations

This cross-sectional study was conducted at the Cytogenetics Unit, CHU Aristide Le Dantec, Dakar, Senegal, as part of a doctoral research project at Université Cheikh Anta Diop (UCAD). Ethical approval was obtained from the National Ethics Committee for Health Research (CNERS) in Senegal, under protocol number 053/2021/CER/UCAD. Written informed consent was obtained from all participants or their legal guardians prior to their inclusion in the study.

2.2 Study Population and Sample Collection

From a total of 89 patients referred for disorders of sex development (DSD) to the Cytogenetics Unit at CHU Aristide Le Dantec**,** between 2013 and 2020, 28 patients were selected for this study. Inclusion criteria were the presence of a confirmed normal karyotype (46,XX or 46,XY) and the availability of complete clinical and molecular data. All selected patients presented with ambiguous genitalia and/or discordance between phenotypic and chromosomal sex.

Additionally, three healthy fertile controls (two males and one female) were included. Male controls had confirmed fertility (at least one biological child), and the female control had no known reproductive or hormonal disorders.

For all participants, 5 mL of peripheral venous blood was collected in EDTA tubes. These samples were used for DNA and RNA extraction and subsequent molecular analyses. Genomic DNA was extracted using the Quick-DNA Miniprep Kit (Zymo Research), following the manufacturer's protocol. In some cases, DNA was obtained from cell culture pellets.

Clinical data were collected from patient medical records and previous diagnostic reports, including external genitalia phenotype, hormonal profile, *SRY* gene status, and pelvic ultrasound findings(Supplementary Table 1).

Karyotyping had already been performed as part of the initial diagnostic workup, and only patients with normal karyotypes were included in the present study.

2.3 PCR Amplification and Sequencing

Molecular analysis focused on two key genes involved in sex development: *SRY* and *DAX1* (*NR0B1*). Specific primers were used to amplify the coding regions of these genes by polymerase chain reaction (PCR), as previously applied in our earlier studies [10]. The same primers were also used for RT-qPCR to assess *DAX1* gene expression. The sequences and characteristics of all primers used in this study are presented in Table 1.

Table 1. *Primer Sequences Used for PCR and RT-qPCR*

Target Gene	Application	Fragment / Function	Forward Primer (5′→3′)	Reverse Primer (5′→3′)	Product Size
SRY	PCR	Full gene	GGTGTTGAGGGCGGAGAAATGC	GTAGCCATTGTTACCCGATTGTC	779 bp
DAX1	PCR	Exon 1 – F1	CCGCGCCCTTGCCCAGACC	GCCGCCTGCGCTTGATTTGT	786 bp
	PCR	Exon 1 – F2	CGCGCAGAGGCCAGGGGGTAAAG	CCCCGACACTCTCCTGATCACTG	750 bp
	PCR	Exon 2	TTGGGTCTTGTTTAATTGGGATGAA	CCATGAAATTGCTACACTTGTGAAAA	644 bp
DAX1	RT-qPCR	Expression (exon 2)	CGCGCAGAGGCCAGGGGGTAAAG	CCCCGACACTCTCCTGATCACTG	—
GAPDH	RT-qPCR (control)	Endogenous control gene	TGAAGGTCGGAGTCAACGGATTTG	CATGTGGGCCATGAGGTCCACCAC	Not applicable

PCR amplification was followed by agarose gel electrophoresis 2% to verify product size. Amplicons were purified and sequenced using the Sanger method, with forward and reverse primers. Sequence data were analyzed using Mutation Surveyor software, and variants were annotated according to HGVS recommendations.

2.4 In Silico Prediction and ACMG Classification

All identified variants in the *DAX1 (NR0B1)* gene were annotated using HGVS nomenclature. To evaluate the potential impact of these variants, a range of in silico prediction tools was used depending on the variant type. For missense (non-synonymous) variants, the following tools were applied:

PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/): to estimate the potential damaging effect of amino acid substitutions on protein structure and function.

MetaSNP and PhD-SNP (http://genetics.bwh.harvard.edu/pph2/): to predict pathogenicity based on sequence conservation, structural context, and functional impact.

Franklin by Genoox (https://franklin.genoox.com/clinical-db/home): an integrated platform that applies the ACMG/AMP 2015 criteria to classify variants based on population frequency (e.g., gnomAD), clinical databases, predicted functional impact, and mutation type.

For frameshift mutations, synonymous changes, and non-coding variants (e.g., in the 3'UTR), Franklin was used to assess their potential clinical significance.

SpliceAI (https://spliceailookup.broadinstitute.org/) was additionally used for variants near canonical splice sites or located in untranslated regions, although no splicing impact was predicted. Variant classification followed the ACMG-AMP guidelines, with each variant assigned to one of the five recognized categories: benign, likely benign, variant of uncertain significance (VUS), likely pathogenic, or pathogenic.

2.5 Gene Expression Analysis by qPCR

Expression of the *DAX1 (NR0B1)* gene was evaluated by quantitative real-time PCR (qPCR) in 14 patients and three fertile controls, targeting exon 1. The GAPDH gene was used as an internal control for normalization. qPCR reactions were performed in a final volume of 20 µL, using SYBR Green Master Mix and specific primers. Thermal cycling conditions followed previously established protocols (Gueye et al., 2023 for *DAX1*; (Saxena et al., 2020 for *GAPDH)*.

Relative quantification (RQ) of *DAX1* expression was calculated using the 2^(-ΔΔCt) method, with controls as calibrators.

The list of individuals included in the qPCR analysis, along with their karyotype and *SRY* gene status, is provided in Supplementary Table 2.

2.6 Structural Modeling of Missense Variants

Three protein sequences were analyzed using the INTFOLD7 server[13] (https://www.reading.ac.uk/bioinf/IntFOLD/), including the wild-type *DAX1* sequence (UniProt P51843) and two mutated versions containing the substitutions *p.Ala2Glu* and *p.Ala25Glu*, respectively. INTFOLD7 provides structural predictions using integrated modules

- ModFOLD for per-residue confidence scoring,
- DomFOLD for domain identification,
- FunFOLD2 for ligand-binding site prediction[14],
- DISOclust for intrinsic disorder prediction.

All outputs were visualized directly using JSmol, without further processing. The generated models were compared descriptively to the wild-type structure

to identify any structural differences or changes in predicted functional regions.

3. Results

3.1 Clinical and Cytogenetic Characteristics

Out of 89 patients referred for suspected disorders of sex development (DSD), 28 individuals with a confirmed normal karyotype (46,XX or 46,XY) and an ambiguous sexual phenotype were included in the study.

The *SRY* gene was present in 17 patients and absent in 11.

Clinical features included micropenis, clitoral hypertrophy, hypospadias, empty scrotum, peno-scrotal ambiguity, and discordance between external phenotype and genetic sex.

Full clinical descriptions are provided in Supplementary Table 1.

3.2 *DAX1* Gene Variants

Sequencing of the *DAX1 (NR0B1)* gene revealed 7 distinct variants in 13 patients. These variants were distributed as follows

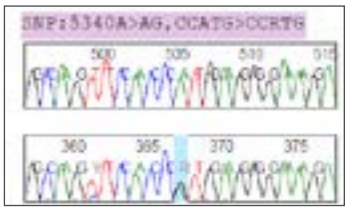
- Exon 1: 6 variants
  - 2 missense: *c.5C>A (p.Ala2Glu)*, *c.74C>A (p.Ala25Glu)*
  - 2 synonymous: *c.498G>A (p.Arg166Arg)*, *c.1029G>A (p.Leu343Leu)*
  - 2 frameshift deletions: *c.615delC*, *c.616delC*
- Exon 2: 1 variant
  - 1 non-coding 3′UTR variant: *c.40A>G*

Some individuals (e.g., P52, P55, P80, P87) carried more than one variant. All variants, along with clinical and molecular information, are detailed in Table 2, and representative Sanger sequencing chromatograms are shown in Figure 1.

Table 2. *DAX1 (NR0B1) gene variants identified in 13 patients with DSD/ (GRCh37)*

Patient ID	Variant (DNA)	cDNA NM_000475.5	Protein Change	Type	Exon	Karyotype	*SRY* Status	Phenotype	dbSNP ID
P26	1013G>A	c.498	p.Arg166Arg	Synonymous	Exon 1	46,XX	+	DSD testicular	rs753580144
P49	1013G>A	c.498	p.Arg166Arg	Synonymous	Exon 1	46,XX	+	DSD testicular	rs753580144
P52	1013G>A	c.498	p.Arg166Arg	Synonymous	Exon 1	46,XX	+	DSD testicular	rs753580144
P52	1131delC	c.616delC	—	Frameshift	Exon 1	46,XX	+	DSD testicular	—
P55	1013G>A	c.498	p.Arg166Arg	Synonymous	Exon 1	46,XY	–	DGP	rs753580144
P55	5340A>G	c.*40	—	3′UTR variant	Exon 2	46,XY	–	DGP	rs181865731
P61	1013G>A	c.498	p.Arg166Arg	Synonymous	Exon 1	46,XX	+	Partial AIS	rs753580144
P64	589C>A	c.74	p.Ala25Glu	Missense	Exon 1	46,XY	+	DGP	—
P66	1131delC	c.616delC	—	Frameshift	Exon 1	46,XY	+	DGP	—
P73	1544G>A	c.1029	p.Leu343Leu	Synonymous	Exon 1	46,XY	+	DGP	rs112775648
P80	1013G>A	c.498	p.Arg166Arg	Synonymous	Exon 1	46,XX	–	DSD testicular	rs753580144
P80	1130delC	c.615delC	—	Frameshift	Exon 1	46,XX	–	DSD testicular	—
P87	1013G>A	c.498	p.Arg166Arg	Synonymous	Exon 1	46,XX	+	DSD testicular	rs753580144
P87	520C>A	c.5	p.Ala2Glu	Missense	Exon 1	46,XX	+	DSD testicular	—
P88	1013G>A	c.498	p.Arg166Arg	Synonymous	Exon 1	46,XY	+	DSD testicular	rs753580144
P89	1013G>A	c.498	p.Arg166Arg	Synonymous	Exon 1	46,XX	+	MRKH	rs753580144
P13	589C>A	c.74	p.Ala25Glu	Missense	Exon 1	46,XY	+	Ovotesticular DSD	—
P2	1131delC	c.616delC	—	Frameshift	Exon 1	46,XY	+	DGP	—

Variants exon 1 DAX1



Variant exon 2 DAX1

Figure 1. *Electropherograms showing the identified DAX1 (NR0B1) variants in patients with DSD.*

3.3 In Silico Prediction and ACMG Classification

The seven variants identified in the *DAX1* gene were analyzed using prediction tools and classified following ACMG guidelines.

- Missense variants (*p.Ala2Glu*, *p.Ala25Glu*) were predicted to be benign or neutral by PolyPhen-2, Meta-SNP, and PhD-SNP.
- Synonymous variants (*p.Arg166Arg*, *p.Leu343Leu*) showed no predicted impact on splicing (SpliceAI score = 0) and were classified as benign.
- Frameshift deletions (*c.615delC*, *c.616delC*) were classified as likely pathogenic based on loss-of-function predictions and ACMG criteria (PVS1, PM2).
- The 3′UTR variant (*c.*40A>G*) was found in public databases with high frequency and predicted to be benign.

All classifications and prediction scores are summarized in Table 3.

Table 3. *In silico predictions and ACMG classification of DAX1 gene variants*

Variant (HGVS)	Variant Type	PolyPhen-2	MetaSNP	PhD-SNP	Franklin ACMG	ACMG Criteria	Final Interpretation
c.616delC	Frameshift	—	—	—	Likely Pathogenic	PVS1, PM2	Likely pathogenic
c.615delC	Frameshift	—	—	—	Likely Pathogenic	PVS1	Likely pathogenic
c.498G>A (p.Arg166Arg)	Synonymous	—	—	—	Benign	BA1	Benign
c.1029G>A (p.Leu343Leu)	Synonymous	—	—	—	Benign	BA1	Benign
*c.40A>G	Non-coding (3'UTR)	—	—	—	Benign	BA1	Benign
c.74C>A (p.Ala25Glu)	Missense (non synonymous)	0.211 – Benign	0.136 – Neutral	0.430 – Neutral	VUS	PM2	Variant of Uncertain Significance
c.5C>A (p.Ala2Glu)	Missense (non synonymous)	0.227 – Benign	0.136 – Neutral	0.185 – Neutral	VUS	PM2	Variant of Uncertain Significance

Legend

- **—** = not applicable or not tested
- **BA1**: high allele frequency in population databases → benign
- **PVS1**: null variant in a gene where loss of function is a known mechanism
- **PM2**: extremely low frequency in population databases → potential significance
- **VUS**: Variant of Uncertain Significance (pending functional or familial evidence)

3.4 *DAX1* Gene Expression Analysis

Expression levels of the ***DAX1*** gene (exon 1) were evaluated by quantitative PCR (qPCR) in 14 DSD patients carrying ***DAX1*** variants and in four fertile controls (TF, TH1, TH2, TN). The housekeeping gene GAPDH was used as an internal reference. Relative quantification (RQ) was calculated using the 2^(-ΔΔCt) method. As shown in Figure 2, the majority of patients exhibited reduced *DAX1* expression compared to fertile controls. Notably

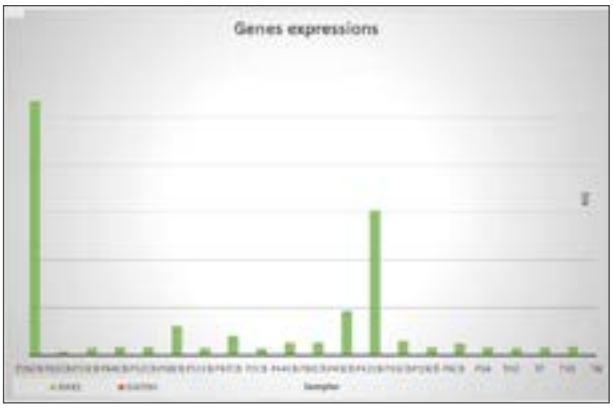


Figure 2. *Relative expression levels of DAX1 in DSD patients and fertile controls (TF, TH1, TH2, TN). Expression values were normalized to GAPDH and calculated using the 2^(-ΔΔCt) method. Most patients show reduced DAX1 expression compared to controls, with variability between individuals.*

- P26 and P42 showed markedly elevated expression relative to other patients.
- Most other patients, including those with frameshift deletions (e.g., P55 and P80), displayed very low expression.
- Some, such as P52, had moderately reduced levels.

These results indicate variable *DAX1* expression among patients, possibly related to the type or position of the variant. The complete list of analyzed individuals is provided in Supplementary Table 2.

3.5 Structural Modeling of *DAX1* Missense Variants

The 3D models generated for variants *p.Ala2Glu* and *p.Ala25Glu* showed similar overall structure compared to the wild-type *DAX1* protein. According to ModFOLD outputs, per-residue confidence was highest in the central core regions of the models and lower at the terminal ends. Domain predictions (DomFOLD) showed no changes in domain organization between the wild-type and mutant models. Ligand-binding residues, predicted by FunFOLD2, were located in similar regions across both variants, though minor differences in the number and identity of residues were observed between models. Disorder predictions (DISOclust) revealed no significant intrinsic disorder at or near the mutated positions in either variant, indicating consistent local structural order. Illustrations of the predicted structures are shown in Figure 3.

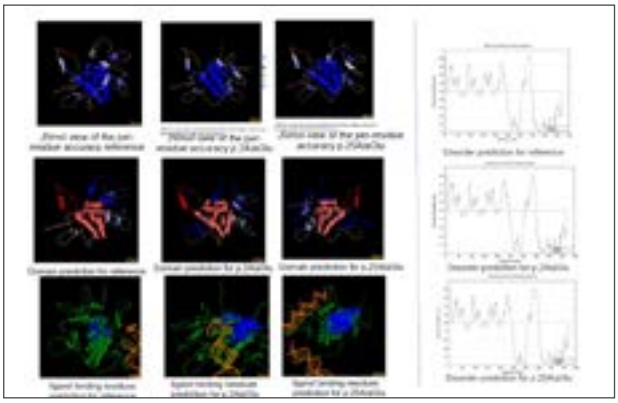


Figure 3. *Predicted 3D structures and computational analysis of DAX1 protein (UniProtP51843)for variants p.Ala2Glu and p.Ala25Glu.*

Legend: The reference structure (top left) is compared to the p.Ala2Glu and p.Ala25Glu variants using models generated by INTFOLD7. Top-center images show per-residue accuracy coloring (JSmol), ranging from blue (high confidence) to red (low confidence). Middle panels display domain predictions (DomFOLD), and bottom panels show predicted ligand-binding residues (FunFOLD2). Line graphs on the right represent disorder prediction profiles (DISOclust) for each variant. Residues involved in predicted ligand interaction are localized to similar regions in both variants, with minor differences in residue count and identity.

4. Discussion

This study reports the first molecular screening of the *DAX1 (NR0B1)* gene in a West African cohort of patients with Disorders of Sex Development (DSD). Among 28 patients with normal karyotypes and ambiguous sexual phenotypes, seven distinct variants were identified in 13 individuals, including two novel missense mutations and two frameshift deletions. These findings contribute new data on the genetic basis of DSD and underscore the clinical significance of *DAX1* in gonadal differentiation.

The synonymous variant *c.498G>A (p.Arg166Arg)* was the most frequent in our cohort, identified in nine patients. Although considered benign, its high prevalence in our study population may reflect ethnic-specific variation or unrecognized regulatory effects. The variant *c.1029G>A (p.Leu343Leu)* and the 3′UTR variant **c.40A>G* were also detected; both are present in public databases but are not associated with functional impact. However, the latter *(rs181865731)* is not currently annotated in ClinVar.

Two missense variants, *p.Ala2Glu* and *p.Ala25Glu,* were previously unreported in public databases. While in silico tools predicted them to be benign, their potential impact cannot be excluded, particularly given the reduced *DAX1* expression observed in some carriers. This highlights the value of integrating transcriptomic data into variant interpretation. Of particular interest, the frameshift deletions *c.615delC* and *c.616delC*, found in four patients (P2, P52, P66, P80), were classified as likely pathogenic according to ACMG guidelines. Among the variants, the c.616delC variant warranted particular attention due to its novelty and its potential functional impact. The *c.616delC* variant was identified in three patients (P2, P52, and P66). This frameshift deletion, which leads to a premature stop codon (p.His206ThrfsTer58), is not listed in any public variant databases, including dbSNP, gnomAD, ClinVar, or 1000 Genomes. It was validated using VariantValidator (GRCh37: g.30326866del), and its classification as likely pathogenic is based on ACMG criteria PVS1 and PM2. In Table 2, the dbSNP ID was intentionally left blank to reflect this novelty. The absence of an rsID might have led to confusion during peer review. We therefore confirm that c.616delC is a novel variant, reported here for the first time, and supported by both bioinformatics prediction tools and experimental evidence (electropherograms in Figure 1, and VariantValidator output in Supplementary Material). These variants are expected to truncate the *DAX1* protein, potentially leading to impaired transcriptional repression and disrupted gonadal or adrenal development.

From a mechanistic standpoint, *DAX1* is expressed early in the gonadal ridge, preceding the *SRY* peak by several days, and persists throughout Sertoli cell differentiation[2]. In Dosage-Sensitive Sex Reversal (DSS), overexpression of *DAX1* can antagonize the action of *SRY* by inhibiting SF1-dependent activation of *SOX9,* a key gene in testis determination[3,6]. This antagonistic interplay may explain the phenotype observed in P43, who presented partial gonadal dysgenesis despite a 46,XY karyotype and *SRY* positivity. Similar mechanisms have been described in other studies where *DAX1* overexpression disrupts testis formation and follicle maintenance[15,16].

These observations reinforce the model in which *DAX1* antagonizes *SOX9* via suppression of *SF1-dependent transcription*, particularly at the *TESCO enhancer* [6,17]. While the classical view presents *DAX1* as an “anti-testis” or “pro-ovary” gene, more recent data suggest it also has a supportive role in testicular development, with its effect being tightly dosage-dependent[7,10,18].

Additionally, three polymorphic variants observed in our study (*rs2269345, rs112775648, and rs181865731*) have been previously associated with X-linked adrenal hypoplasia congenita (AHC) and sex reversal phenotypes[19,20]. These cases underline the pleiotropic role of *DAX1* in both adrenal and gonadal development. The AHC phenotype is frequently associated with testicular dysfunction and delayed puberty in 46,XY individuals, sometimes with preserved gonadal structures but disrupted function[21,22].

From a regional perspective, this study highlights the lack of molecular data on DSD in West Africa,

despite a high prevalence of genetic conditions due to consanguinity and high birth rates [8,10,11,23,24]. Cultural stigma and lack of resources often delay diagnosis and care[25,26], and contribute to the social marginalization of affected individuals.

As of 2025, no recent studies have specifically focused on *DAX1* mutation screening in DSD, either globally or in large public databases such as gnomAD or ClinVar. This reinforces the novelty of our findings and underscores the need for population-specific research and diagnostics in underrepresented regions.

5. Conclusion

This study presents the first report of *DAX1 (NR0B1)* mutation screening in a West African cohort of patients with Disorders of Sex Development (DSD). Through the identification of seven distinct variants, including two novel missense mutations and two frameshift deletions, our results confirm the involvement of *DAX1* in 46,XY DSD phenotypes and underscore the complexity of its dosage-sensitive role in sex determination[2,3]. The integration of genetic, transcriptomic, and in silico data reinforces the importance of multi-level molecular analysis in understanding DSD pathogenesis[27]. In resource-limited settings, where DSD diagnosis is often delayed due to limited infrastructure and sociocultural barriers, such approaches offer a valuable framework to improve patient care and reduce diagnostic uncertainty African-specific variants. This study thus represents a timely and novel contribution, and calls for the inclusion of *DAX1* in diagnostic panels for DSD, especially in underrepresented populations such as those in sub-Saharan Africa.

5.1 Data Availability Statement

Data generated or analyzed in this study is available upon reasonable request from the corresponding author.

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CREDIT statement

Fatou Diop Gueye: Conceptualization, patient recruitment, molecular analysis, bioinformatics interpretation, data analysis, manuscript drafting. Arame Ndiaye: Sequencing analysis, data interpretation, manuscript review. Adji Dieynaba Diallo: PCR and qPCR optimization, sample processing. Mame Venus Gueye: Clinical data collection, patient evaluation, coordination of clinical records, and interpretation of clinical findings. Ndiaga Diop: Critical revision and final editing. Rokhaya Ndiaye and Mama Sy Diallo: Scientific supervision, project administration, final validation of the manuscript. All authors read and approved the final manuscript.

Ethics Statement

This study was conducted in accordance with the ethical standards of the institutional and national research committee. Ethical approval was obtained from the National Ethics Committee for Health Research (CNERS) in Senegal. Written informed consent was obtained from all participants or their legal guardians.

Conflict of Interest Statement

The authors declare no conflict of interest.

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Genomic (g.)	Validation status	Notes		
Transcript (RefSeq)	Variant (c.)	Protein (p.)	Genomic (g.) (GRCh37)	Variant Type
NM_000475.5	c.5C>A	p.(Ala2Glu)	NC_000023.10:g.30327476G>T	Missense
NM_000475.5	c.74C>A	p.(Ala25Glu)	NC_000023.10:g.30327407G>T	Missense
NM_000475.5	c.498G>A	p.(Arg166Arg)	NC_000023.10:g.30314670G>A	Synonymous
NM_000475.5	c.615delC	p.(His206Thrfs*58)	NC_000023.10:g.30326864del	Frameshift
NM_000475.5	c.616delC	p.(His206Thrfs*58)	NC_000023.10:g.30326866del	Frameshift
NM_000475.5	c.1029G>A	p.(Leu343=)	NC_000023.10:g.30326452C>T	Synonymous
NM_000475.5	c.*40A>G	—	NC_000023.10:g.30327715T>C	3′UTR

Validation	Notes
☑ OK	Validated
☑ OK	Validated
☑ OK	Validated
☑ OK	Validated, frameshift
☑ OK	Validated, frameshift
☑ OK	Validated
☑ OK	Non-coding UTR variant

Separate Supplementary files

Supplementary Table 1: Clinical and paraclinical picture of the 28 patients

Supplementary Table 2: List of patients included in the RT-qPCR expression analysis of DAX1