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ORIGINAL ARTICLE

Molecular genetics and phenotype/genotype correlation of 5-α reductase deficiency in a highly consanguineous population

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Abstract

Context and objectives 5-α reductase deficiency is a rare 46,XY disorder of sex development. We present detailed phenotypic and genotypic features of a cohort of 24 subjects from a highly consanguineous population of Saudi Arabia

Subjects and Methods We studied the clinical presentation and hormonal profiles of 24 subjects diagnosed with 5-α reductase deficiency and performed genetic testing on DNA isolated from their peripheral blood using polymerase chain reaction and direct sequencing of the *SRD5A2*.

Results All subjects had 46,XY karyotype and presented with atypical appearance of external genitalia ranging from clitoromegaly, micophallus with hypospadias, undescended testes to completely normally looking female genitalia. Thirteen (54%) of them had severe under virilization and were assigned female sex at birth. The other 11 subjects were raised as males. Stimulated Testosterone:Dihydrotestosterone ratio was high in all 16 subjects in whom it was measured. The genetic testing revealed 2 nonsense mutations (p.R103X and p.R227X) in 2 unrelated subjects, 3 missense mutations (p.P181L, p.A228T, p.R246Q) in 11 subjects and a splice site mutation (IVS1-2A > G) in 11 other subjects. There was significant phenotypic variability even in subjects with the same mutation and also within the same family.

Conclusion This is the first and largest report of the clinical and molecular genetics of 5-α reductase deficiency from the Middle East. It shows weak genotype/phenotype correlation and significant phenotypic heterogeneity. IVS1-2A > G mutation is the most common mutation and is likely to be a founder mutation in this part of the world.

Keywords 5-Alpha reductase · Disorders of sex development · Phenotype · Mutation · 46,XY

Introduction

In 46,XY subjects with 5-α reductase type 2 deficiency, the development of external genitalia is usually abnormal [1, 2]. The severity of this condition varies from small phallus and hypospadias to completely female looking genitalia [3]. 5-α reductase type 2 is important for conversion of testosterone (T) to the more active form, dihydrotestosterone (DHT) [4]. DHT is a key hormone for the normal development of external genitalia of 46,XY fetuses in the first few weeks of life in utero. Deficiency of this active hormone leads to undervirilization of 46,XY fetuses with variable degrees of atypical appearance of the external genitalia[4]. There are two isoforms of 5-α reductase, type 1 and type 2 [5]. 5-α reductase type 1 is encoded by *SRD5A1* located on chromosome 5p15 [5]. The enzyme is transiently expressed in the neonatal skin and then repressed until puberty when it is permanently expressed [6]. The other isoenzyme is 5-α reductase type 2 which is encoded by the gene *SRD5A2*

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located on chromosome 2p23 and is expressed in genital skin and prostate [7]. Genetic alterations in *SRD5A2* are the underlying genetic defects in 5-α reductase deficiency [3, 8, 9]. Due to the crucial role of this enzyme in the T:DHT conversion, its deficiency usually leads to variable degrees of undervirilization of 46,XY fetuses [3]. Although the degree of atypical genital appearance varies depending on the residual *SRD5A2* enzymatic activity, 46,XY newborns with this enzymatic deficiency are frequently raised as females [9].

Although series of subjects with this autosomal recessive disorder have been described from different parts of the world, there is sparse data on its phenotypic and genotypic features in our region [10, 11]. Consanguinity rates are high in Saudi Arabia reaching about 54-58% [12, 13] and 5-α reductase deficiency is likely to be common [10, 11]. Therefore, it would be of great interest to report the clinical, biochemical and molecular features of 5-α reductase deficiency from the highly consanguineous population of Saudi Arabia. In this report, we describe the clinical and molecular genetics and the phenotype genotype correlation of 24 subjects with 5-α reductase deficiency evaluated at the largest tertiary care center in the country.

Subjects and Methods

Subjects

We reviewed the medical records of persons diagnosed with 5-α reductase deficiency based on their clinical presentation and hormonal profiles. These subjects were referred to King Faisal Specialist Hospital and Research Centre from all over the country as this center is one of the main tertiary care centers dealing with disorders of sex development in Saudi Arabia. A cohort of 24 subjects who were previously diagnosed to have 5-α reductase deficiency was studied. Those subjects were diagnosed to have 5-α reductase deficiency based on clinical and biochemical evaluation. In this study, we prospectively confirmed this diagnosis by genetic testing. The clinical presentation and biochemical and hormonal data and results of genetic testing are summarized in Table 1. They all had 46,XY karyotype and variable degrees of atypical appearance of their external genitalia. The T:DHT ratio was measured 24 h after the last dose of 3 intramuscular injections of human chorionic gonadotrophins (HCG) given at 1500 units per dose every other day.

Molecular testing

After obtaining informed consents and Institutional Review Board approval, genomic DNA was extracted from 3–5 cc blood collected in EDTA-containing tubes using the Gentra Puregene blood kit (Catalog#158389, Qaigen, Valencia, CA, USA) according to the manufacturer's instructions. All the 5 exons and exon-intron boundaries of the *SRD5A2* were amplified by polymerase chain reaction (PCR) using primers that have been previously published [7]. Amplifications were carried out in a total volume of 25 µl containing 100 ng of genomic DNA, 0.2 mM of each deoxynucleotide triphosphates (dNTPs), 1X PCR buffer: 20 mM Tris-HCl (pH 8.4) and 50 mM KCl, 1.5 mM MgCl2, 0.2 µM of forward primer, 0.2 µM of reverse primer, 2 units of Taq DNA Polymerase (Catalog # 10966-018, Platinum® Taq DNA Polymerase, Invitrogen) and the volume was completed to 25 µl by nuclease-free water. Positive and negative controls were used in all reactions. The PCR Thermal Cycler (Catalog# 4375786, Veriti® 96-Well Thermal Cycler, Applied Biosystems) was set to appropriate cycling conditions based on the length of the fragment and the Tm of the primers as previously described [12]. The amplicons were resolved on 2% agarose gel. Successfully amplified fragments were directly sequenced in forward and reverse directions using an ABI PRISM Big-Dye Terminator V3.1 Cycle sequencing Reaction kit (Catalog # 4337455, Applied Biosystems, Foster City, CA 94404, USA) and ABI PRISM® 3730XI Genetic Analyzer (Catalog# 3730 S, Applied Biosystems, Foster City, CA 94404, USA). Sequencing data were analyzed and compared with the *SRD5A2* sequence using the reference sequences in GenBank (Reference sequence Genbank NG_008365.1) and Ensemble.

Results

Clinical and biochemical features

We studied 24 subjects diagnosed clinically and biochemically with 5-α reductase deficiency and their diagnosis was further confirmed in this study by genetic testing. The subjects comprise 14 (58.4%) isolated cases and 10 (41.6%) cases from 4 families with 2–3 affected children per family (Table 1). The most common presentation was small phallus, penoscrotal hypospadias and unilateral or bilateral undescended testes. Due to the severity of genital abnormality, 13 subjects (54%) were assigned female sex at birth. The other 11 children (46%) were raised and named as males. The age at presentation varied from detection of abnormal genitalia by the family or medical team at birth to late presentations during the adolescence age due to under masculinization and lack of secondary sexual characteristics. Biochemically, HCG-stimulated T:DHT ratio was high (> 10) in 16 subjects in whom it was done.

Table 1 Clinical presentations, hormonal data and molecular studies of 24 subjects with 5-α reductase deficiency

Family/ Subject	Age of presentation	Sex assigned at birth	Clinical picture	EMS	Prader score	Stimulated T(nmol/L)/DHT (nmol/L) = ratio	Molecular studies	Current sex	Current age
(1)	20 months	F	HS, UT	6	4	11.8/0.27=42	c.307C>T, p.R103X	M	7 yrs
(2)	8 yrs	M	MP, HS	6	4	3/0.2=14.7	c.542C>T, p.P181L	M	36 yrs[a]
(3)	3 months	F	Female UT	2	2	—	c.542C>T, p.P181L	F	9 yrs
(A4)	8 yrs	F	Female UT	2	2	22.6/0.35=64.6	c.542C>T, p.P181L	M	12 yrs
(A5)	14 yrs	F	Female UT	2	2	—	c.542C>T, p.P181L	M	18 yrs
(6)	6 weeks	M	HS, UT, BS:	5	4	15.7/0.17=91	c.679C>T, p.R227X	M	9 yrs
(7)	Birth	M	HS, UT	6	4	26/0.81=32	c.682G>A, p.A228T	M	5 yrs
(8)	5 months	M	HS, BS	5	4	—	c.682G>A, p.A228T	M	6 yrs
(9)	1 yr	M	BS, HS, UT	6	4	12.61/0.49=25	c.682G>A, p.A228T	M	11 yrs
(10)	5 yrs	M	HS, UT	7	4	—	c.682G>A, p.A228T	M	8 yrs
(B11)	3 months	F	Female UT	2	2	6.8/0.28=24	c.737G>A, p. R246Q	F	30 yrs
(B12)	4 months	F	Female UT	2	2	23/0.29=79	c.737G>A, p. R246Q	F	26 yrs
(B13)	7 months	F	Female UT	2	2	---------	c.737G>A, p. R246Q	F	18 yrs
(14	6 months	F	UT, HS	4	2	17.2/ND	IVS1 -2A>G	M	3 yrs
(15)	14 yrs	F	FG, MP, BS, UT, HS	3	2	14/0.51=27	IVS1 -2A>G	M	17 yrs
(16)	1 month	M	HS, UT	2	2	16.7/1.1=15	IVS1 -2A>G	M	2 yrs
(17)	5 yrs	M	MP, HS, UT	6	4	10.84/0.21=51.6	IVS1 -2A>G	M	17 yrs
(C18)	17yrs	F	UT	2	2	48.8/0.87=55.4	IVS1 -2A>G	M	26 yrs
(C19)	16 yrs	F	FG, LC, UT	3	2	26/1.0=26	IVS1 -2A>G	M	23 yrs
(C20)	13 yrs	M	MP, UT	7	4	—	IVS1 -2A>G	M	16 yrs
(21)	3 yrs	F	MP, HS	6	3	8.8/0.34=25.9	IVS1 -2A>G	M	11 yrs
(22)	14 yrs	F	UT, HS	3	2	8.8/ND	IVS1 -2 A>G	F	15 yrs
(D23)	1 yr	M	MP, BS, Penoscrotal HS	6	4	35.2/0.16=220	IVS1 -2A>G	M	11 yrs
(D24)	1 yr	M	MP, BS, Penoscrotal HS	6	4	4.99/0.036=138.6	IVS1 -2A>G	M	12 yrs

EMS External Masculinization Score, *T* testosterone, *DHT* dihydrotestosterone, *HS* hypospadias, *UT* undescended testes, *MP* microphallus, *BS* bifid scrotum, *FG* female genitalia, *LC* large clitoris

[a]Married but infertile although sperms found on biopsy

Molecular studies

The study revealed 2 non-sense mutations (p.R103X and p.R227X), 3 missense mutations (p.P181L, p.A228T, p.R246Q) and a splice site mutation (c.282-2A > G) (Table 2). The 2 non-sense mutations were present in 2 unrelated subjects (Table 1). The p.P181L missense mutation (Fig. 1) was present in 2 unrelated subjects and 2 siblings (Tables 1 and 2). Although the 2 subjects are unrelated to the 2 siblings, they are from the same region of the country and their remote relationship can't be completely excluded. Alternatively, this mutation might be a founder mutation in this region. p.A228T mutation was detected in 4 unrelated persons from different regions of the country (Table 1). p.R246Q mutation was present in 3 siblings from one family. c.282-2A > G splice site mutation (Fig. 1) was the most common mutation occurring in 11 of 24 subjects (45.8%) suggesting that it might be a founder mutation in this population (Table 1). These subjects were 5 siblings from 2 families and 6 unrelated subjects (Table 1). The mutations were evenly distributed over exon 2–5 and intron 1 of *SRD5A2* gene (Table 1)

Genotype/phenotype correlation

In the subjects carrying the two non-sense mutations p.R103X and p.R227X, T:DHT ratio was significantly high indicating severe enzymatic deficiency that would be expected to cause severe under virilization. However, these subjects manifested only with hypospadias and micophallus and their external masculinization score (EMS) was 6 and 5 (subjects 1 and 6, Table 1). The missense mutation p.P181L was present in 4 subjects (2 siblings and 2 unrelated subjects) and showed significant differences in their degree of under virilization with EMS of 6 in 1 subject (subject 2, Table 1) and EMS of 2 in the other 3 subjects (subjects 3, A4, A5, Table 1). The other missense mutation (c.682 G > A, p.A228T) also was present in 4 unrelated subjects but their EMS was similar (Subjects 7, 8, 9, 10, Table 1). These 4 subjects were assigned male sex since birth. The missense mutation p.R246Q occurred in one family with 3 affected siblings who showed severe feminization with EMS of 2 in all of them (subjects B11, B12, B13, Table 1). They were all raised as females.

The splice site mutation (IVS1 -2A) occurred in 11 subjects. Their EMS varies between 2–6 and their T:DHT ratios were quite variable ranging between 15-220. Due to the variability of their external genitalia appearance, 5 of these subjects were raised as males while 6 were assigned a female sex at birth (Table 1). In one family (subjects C18. C19, C20, Table 1) with IVS1 -2A > G mutation, 2 siblings were severely undervirilized (EMS 2 and 3) and were assigned a female sex at birth while the third sibling was less affected (EMS 6) and was assigned a male sex.

To further study the heterogeneity of clinical phenotype, we reviewed all studies which reported the mutations we found in our cohort and compared the degree of genital abnormalities in subjects described in these studies with our subjects (Table 2). Since not all studies provided clear description of genital appearance, we used the sex assignment at birth as indicative of the degree of genital abnormalities when no detailed description of the clinical phenotype was provided. Subjects with 46,XY karyotype with severe genital abnormalities are likely to be severely undervirilized and raised as females while those with lesser degree of genital abnormalities are likely to look like males and raised as males (Table 2).

Sex of rearing

Thirteen subjects (54%) had severe under virilization that they were raised and named as females (Table 1). Eight (61.5%) of these subjects underwent corrective surgeries and were converted to male sex later (Table 1). The other five persons (38.5%) continued as females. None of the subjects who were assigned male sex at birth changed to female.

Discussion

In this report, the molecular genetics of a large series of subjects with 5-α reductase deficiency from the highly inbred population of Saudi Arabia are presented. This is to our knowledge, the first and largest study that describes molecular genetics of this rare disorder from this region. In addition, the clinical and biochemical profiles, sex of rearing and management of these subjects are described.

Of 24 subjects described in this report, 13 (54%) had severe abnormalities of their external genitalia appearance to the extent that they were assigned a female sex at birth and raised as females. This is lower than a previously reported rate of 72% in a multiethnic study of 55 subjects [3]. This may reflect a less severe form of enzymatic deficiencies in some of the mutations that we found in our subjects. The clinical phenotypes ranged from micro phallus with variable degree of hypospadias with or without undescended testes to severe degree of feminization with low EMS score (Table 1). We found a significant heterogeneity of the clinical presentation not only between individual subjects but also in subjects with the same mutations and within the same family. For example, 2 unrelated subjects (subjects 2 and 3, Tables 1) and 2 siblings (subjects A3, A4, Table 1) had the same mutation (c.542 C > T, p.P181L). Three of these subjects were severely

Table 2 Summary of subjects with the 6 mutations described in the current and previous studies

Mutation	c.307C>T, p. R103X	c.542C>T, p.P181L	c.682G>A, p.A228T	c.679C>T, P.R227X	c.737G>A, p.R246Q	IVS1 -2A>G
Type of mutation	Nonsense	Missense	Missense	Nonsense	Missense	Splice site
Current study	Subject # 1: Female (Table 1)	1 male (subject #2) and 3 females (subjects # 3, A4, A5) (Table 1)	4 subjects all assigned male sex (Subjects # 7, 8, 9, 10) (Table 1)	Male (subject # 7)	Three sisters with female looking external genitalia (Subjects B11, B12, B13) (Table 1)	5 Males and 6 females (Subjects # 14-20) (Table 1)
Report 1	Female: Prader 3 [18]	1 Female: HS, UT 1 Female with Heterozygous mutation (P181L) only: female external genitalia with testes palpable in the labia majora [8]	Male with MP, HS [14]	1 Female: masculine appearance and and clitromegly [9, 22]	Two Pakistani subjects: 1 Male with microphallus, penoscortal hypospidus and UT 1 Male, MP, HS and UT	1 Female genitalia with undescended testes [16]
Report 2	Female: Normal external female genitalia [19]	The gender and genital appearance of the subject were not described [21]			1 Female with heterozygous for p.R246Q [31]	5 Cypriot subjects raised as females. Three of them had Prader 1–2 and had homozygous mutation and 2 had compound heterozygous with other mutations and presented with Clitoromegaly, HS and UT [30]
Report 3	Male: EMS 8 [32]	1 Female with clitoromegaly and UT. Compound heterozygous (IVS-2A>G /P181L)			2 males with UT and HS [24]	
Report 4					1 boy with MP [26]	
Report 5					A young man with infertility and oligospermia only [27]	

MP microphallus, *HS* hypospadias, *UT* undescended testes

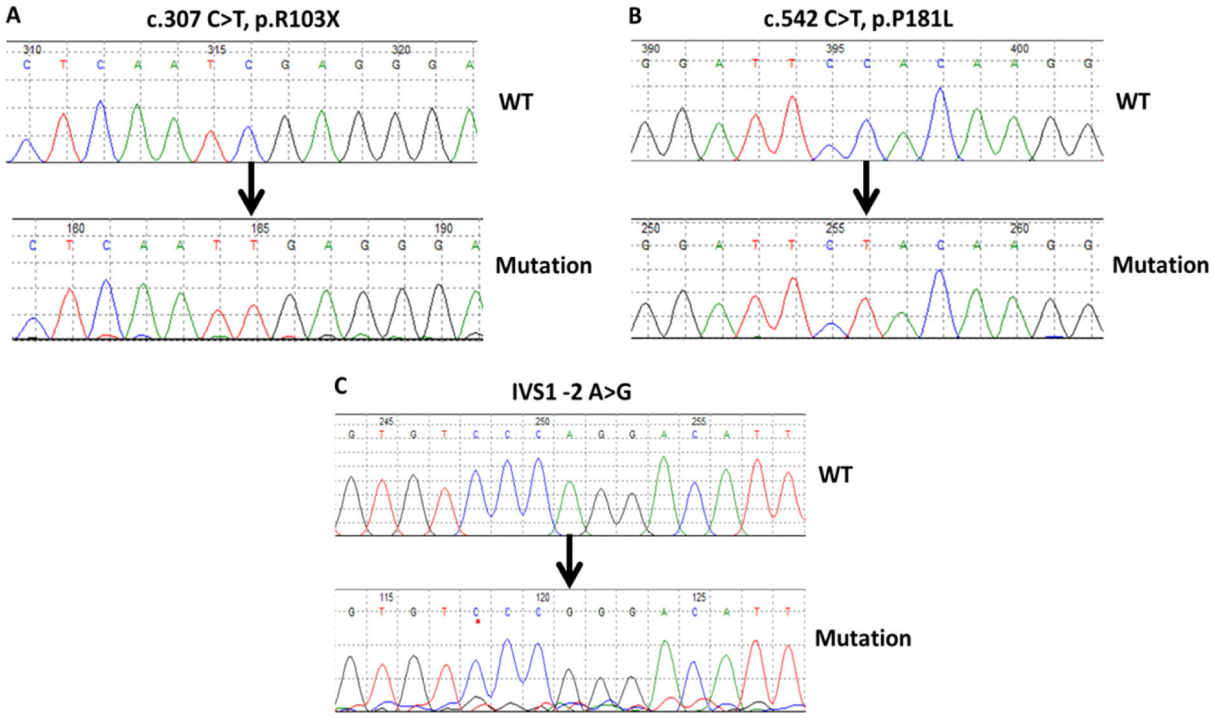

Fig. 1 Sequence chromatographs showing examples of *SRD5A2* mutations found in subjects described in this study. **a** shows a nonsense mutation (c.307 C > T, p.R103X) in subject #1, **b** shows a missense mutation (c.542 C > T, p.P181L) in subjects # 2, 3, A4, A5 and **c** shows a splice site mutation (IVS1 -2A > G) found in subjects# 14-24

undervirilized and were assigned a female sex at birth while the fourth subject (subject 2, Table 1) was mildly undervirilized and was raised as a male. Another example is a family with 3 affected 46,XY members (C18, C19, C20, Table 1) who had the IVS1 -2A > G splice site mutation. Two of these siblings had a similar severely affected phenotype (EMS 2 and 3) and were raised as females. The third sibling who carries the same mutation had much milder degree of feminization (EMS 7) and was raised as a boy (Table 1). This situation where subjects with the same mutation have significant phenotypic variability has only been rarely reported [3, 9, 14]. It is been suggested that other factors such as the androgen receptor sensitivity, the circulating and local testosterone concentration in utero and other environmental factors might mediate some of this variability beyond the primary defect of decreased 5-α reductase enzymatic activity [3, 9, 14].

The most common mutation in our cohort was the splice site mutation IVS1 -2A > G occurring in 11 subjects with 5 subjects from 2 families and 6 unrelated persons (Table 1). This suggests that this mutation is a founder mutation in Saudi population. This mutation has also been reported to be a common founder mutation in other Middle Eastern populations including the Turks and Cypriots [15–17]. The severity of the genital abnormalities in subjects with this mutation varied from an EMS of 2 to 6 (Table 1). This has resulted in assigning 6 of these children a female sex at birth and 5 subjects a male sex (Table 1). Interestingly, All the female-assigned subjects with IVS1 -2A > G mutation, except one who is still 15 month old, were changed to male sex and underwent corrective surgeries (Table 1).

p.R103X was found in one of our subjects and described at least 3 times in the literature (Table 2). Our subject had an EMS of 6 and was named and raised as female and so did the two other previously described subjects [18, 19]. The third child was not described in details but he was raised as a male and was given an EMS of 8 [20].

In our cohort, p.P181L mutation was found in 2 unrelated subjects and 2 siblings from one family (Table 1). This mutation was also previously reported separately in 3 unrelated subjects (Table 2) [8, 15, 21]. In all of them, the subjects were raised as females indicating severe impact of this mutation on enzymatic activity resulting in severe feminization.

The p.A228T mutation was found in 4 unrelated subjects in our study (Table 1). All of them were raised as males due to less degree of abnormalities of their external genitalia. Another male subject with hypospadias carrying this mutation was reported [14]. This suggested that this mutation has mild effects on the 5-α reductase enzymatic activity and sexual differentiation of 46,XY subjects.

The p.R227X nonsense mutation was found in one of our child who had an EMS of 5 and was raised as a male. A previously reported subject with the same mutation was raised as a female (Table 2). She presented with clitoromegaly and significant genital abnormalities at birth and with masculine appearance and lack of female secondary sexual characteristics at pubertal age [9, 22]

In our study, the p.R246Q mutation was found in a family with 3 affected siblings (Table 1). All of them had severe genital abnormalities with an EMS of 2 and were named and raised as females. This mutation was previously frequently reported in persons with 5-α reductase deficiency who had variable phenotypes (Table 2) [9, 21, 23–29].

Similarly, the splice site mutation c.282-2 A > G was the most common mutation occurring in 11 of our subjects and manifested with variable degrees of abnormal genital appearance. This mutation was previously reported in a number of subjects [15–17]. In all of these studies, the phenotype was more like females and the children were raised as females indicating significant effect of this mutation on the enzymatic activity and the clinical phenotype.

In summary, we have for the first time presented a cohort of children and young persons with 5-α reductase deficiency from a highly inbred population. More than half of these subjects had significantly atypical genital appearance to the extent that they were assigned female sex at birth. The study also shows a significant phenotypic heterogeneity not only between individual unrelated subjects but even within the same family indicating weak phenotype genotype correlation. The IVS1 -2A > G splice site mutation was the most common variant suggesting that this mutation is a founder mutation consistent with other reports from the Middle Eastern region [15, 16, 30].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study was approved by the Institutional Review Board of the King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.

Informed consent Informed consents were obtained from all subjects or their parents.

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