Genetic analysis of the *FOXL2* gene using quantitative real-time PCR in Chinese patients with blepharophimosis-ptosis-epicanthus inversus syndrome

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**Purpose:** The purpose of this study was to identify the mutation(s) or deletion(s) of the forkhead box protein L2 (*FOXL2*) gene in Chinese patients with blepharophimosis-ptosis-epicanthus inversus syndrome (BPES).

**Methods:** Genomic DNA extracted from peripheral blood was collected from two Chinese families and from one sporadic case. PCR direct sequencing and quantitative real-time PCR-based copy number screening for the whole exon of *FOXL2* were performed.

**Results:** Direct sequencing revealed an indel mutation c.50C→TA in the sporadic case which resulted in a frameshift generating 78 novel amino acids and terminating prematurely at codon 95. Deletions in the *FOXL2* gene were confirmed by quantitative real-time PCR (q-real-time PCR) in two families in which intragenic mutations were excluded by direct sequencing. These changes containing deletions and a de novo mutation were not detected either in the non-carrier relatives or in 100 normal controls.

**Conclusions:** This study identified two deletions and a de novo mutation in the *FOXL2* gene in Chinese BPES patients. This is the first study to report *FOXL2* gene deletions detected by q-real-time PCR in this ethnic group. This technique enriches the diagnostic methods of molecular genetics in BPES patients. The de novo mutation expands the mutation spectrum of *FOXL2*.

Blepharophimosis-ptosis-epicanthus inversus syndrome (BPES, OMIM 110100) is a rare genetic disorder characterized by eyelid malformation and ovarian dysfunction. Based on the presence or absence of premature ovarian failure (POF), two clinical types have been distinguished: type I is associated with POF in affected females, whereas type II is not [1]. BPES is primarily inherited in an autosomal dominant manner, but may also occur sporadically, although the autosomal recessive pattern has also been reported in one consanguineous family [2]. According to cytogenetic rearrangements [3] and linkage analyses [4-6], BPES had been mapped to the human chromosome 3q23 region. Subsequently, the *FOX2* (forkhead box protein L2, OMIM 605597) gene was identified as the pathogenic gene for BPES [7]. Furthermore, non-syndrome POF and granular cell tumors of the ovary may be associated with *FOX2* mutations [8,9].

The FOXL2 protein comprising 376 amino acids is a member of the large family of winged-helix/forkhead transcription factors that play important roles in a variety of developmental processes [10]. FOXL2 contains an unique DNA-binding domain of 100 residues with amino acid positions from 52 to 152, and a polyalanine tract of 14 residues with amino acid positions from 221 to 234 (Figure 1). A comparative analysis shows that the entire open reading frame (ORF) of *FOX2* is highly conserved in several vertebrate species [11]. Expression studies have shown that the FOXL2 protein is expressed in the mesenchyme of developing mouse eyelids and in fetal and adult ovarian granulosa cells, which is consistent with the preconceived role of *FOX2* in early eyelid development and ovarian maintenance [7].

To date, more than 125 mutations have been reported in individuals with BPES type I and II. Among all genetic defects found in BPES, an estimated 72% of cases are due to intragenic *FOX2* mutations [12]; 2% of cases involve cytogenetic rearrangements containing unbalanced translocations and interstitial deletions of 3q23 [13]; about 12% of BPES cases result from deletions involving partial or whole *FOX2* gene deletion and contiguous gene deletion, including *FOX2* and adjacent gene(s) [13]; and about 5% of cases involve regulatory deletions outside the *FOX2* gene.
Using multiplex ligation-dependent probe amplification (MPLA) and quantitative PCR, deletions leading to FOXL2 haploinsufficiency may be detected in individuals with typical BPES in which intragenic mutations were excluded by sequencing of the FOXL2 ORF. The purpose of this study was to identify the mutation(s) or deletion(s) of FOXL2 in two Chinese families and one sporadic case with BPES, using the technique of PCR direct sequencing and quantitative real-time PCR (q-real-time PCR). This is the first report deletion detection in the FOXL2 region using q-real-time PCR in a Chinese ethnic population.

**METHODS**

Patients: Five BPES patients and four of their relatives were recruited from Heilongjiang province in the northeast of China, as well as two unrelated families (F1, F2) and a sporadic case (S1). Informed consent was obtained from their parents or guardians. One hundred healthy normal controls were also involved in this study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Heilongjiang Institutional Review Board (Harbin, China).

Clinical information about the patients was obtained from an ophthalmologist, an endocrinologist, and a gynecologist using the standardized diagnostic criteria. Where possible, facial photographs were obtained with patient approval.

Mutation detection: Blood samples from BPES patients and their healthy relatives were collected and stored at −20 °C. Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany).

The whole exon of the FOXL2 gene containing coding and flanking regions with BPES was amplified by the polymerase chain reaction (PCR) method using the primers listed in Table 1. The PCR reaction mixture (50 µl) contained 12.5 µl 2× GC Buffer II, 200 µmol/l dNTP mix, 1 unit of LA Taq polymerase (TakaRa Biotechnology Co. Ltd., Dalian, China), 10 pmol primer pairs and 100 ng genomic DNA. PCR cycling conditions were as follows: pre-degeneration at 95 °C for 3 min, then 38 cycles of denaturation, annealing and extension, followed by a final extension at 72 °C for 10 min. The PCR products were directly sequenced on an automated sequencer (ABI 3730XL Genetic Analyzer; Applied Biosystems, Foster City, CA) to perform mutation analysis.

**TABLE 1. List of the primers and annealing temperatures used for the amplification of the whole exon of the FOXL2 gene containing coding and flanking regions with BPES.**

| Primer | Sequence (5'–3') | Product length (bp) | Annealing temperature (°C) |
|--------|------------------|---------------------|---------------------------|
| AF     | GTGGAGCCCATACGAATCAG | 610               | 62                        |
| AR     | GTACGAGTACGGGGGCTTCT | 545               | 64                        |
| BF     | CAGCAGCTGGAGGCGAGAG | 517               | 66                        |
| BR     | CTTGCCGGGCTGGAATGTC | 500               | 68                        |
| CF     | GACCGGCGCTGGAAGACA  | 467               | 51                        |
| CR     | GCACCGGTGCAGATGTC   | 518               | 55                        |
| DF     | CGCGGCCGCTGTGACTCAAG| 542               | 55                        |
| DR     | GTTGCCGCGGCGCGTCTGC| 467               | 51                        |
| EF     | CCTCTTTGTCCTCCCTCTGCTTA| 500              | 68                        |
| ER     | CGGTGTAACCCGAGTACAGG| 518               | 55                        |
| FF     | AGAAAGGACGCGCAATGAC| 518               | 55                        |
| FR     | CAGATAGAGGAGGAGGAGG | 542               | 55                        |
| GF     | GAAATATGTCGGCCCTGGAGT| 610              | 62                        |
| GR     | ATTTATCTGGGAAATCGACAAG | 610              | 62                        |

Figure 1. Diagram of the FOXL2 gene and positions of the amplification segments by the q-real-time PCR primers in FOXL2. Shaded areas represent the DNA-binding domain and the polyalanine tract in the coding region of FOXL2, respectively. Black boxes indicate the location of the amplification segments of two pairs of primers with respect to the nucleotide (top) numbering.
Quantitative real-time PCR analysis: The real-time primers, as listed in Table 2, were designed and provided by TakaRa (TakaRa Biotechnology Co. Ltd.). The amplification segments of the two pairs of primers were located in the 5′ and 3′ ends of the FOXL2 gene, respectively (Figure 1). The gDNA was used as template in q-real-time PCR reactions with SYBR® Green PCR Master Mix (TakaRa Biotechnology Co. Ltd.) and performed using a 7000 real-time PCR system (Applied Biosystems). The quantification of the target sequences was normalized to an assay of chromosome 21, C2, and the relative copy number (RCN) was determined on the basis of the comparative ΔΔcycle threshold (Ct) method with a normal control DNA as the calibration standard [16]. The experiments were repeated three times. A ≈0.5-fold change in RCN was used as the benchmark for deletion.

RESULTS

Clinical findings: All three probands indicated typical features of BPES, including small palpebral fissure, ptosis of the eyelids, epicanthus inversus, and telecanthus. In addition to eyelid malformations, other eye abnormalities were detected in some of these patients. In family 1 (Figure 2A), the proband (F1-III:4) was diagnosed with bilateral amblyopia and strabismus. His mother (F1-II:6), who had undergone eyelid surgeries in childhood, did not suffer from POF at the time of this study, and was diagnosed BPES type II. In family 2 (Figure 2B), the BPES type of the female child (F2-III:1) could not be determined. Her father (F2-II:1) was a 27-year-old BPES patient who presented some abnormalities such as bilateral amblyopia, strabismus, and ophthalmoplegia, besides ocular abnormalities characteristic of BPES. In addition to the typical ocular manifestations of BPES, the 3-year-old girl in the sporadic case (S1) had no other developmental abnormalities. Neither of her parents had clinical evidence of this disorder. Clinical data for the patients in this study are summarized in Table 3.

Mutations in FOXL2: In the sporadic case (S1), bidirectional sequencing of the whole exon of FOXL2 revealed an indel mutation, C→TA at nucleotide 50 (c.50 delCinsTA, Figure 3A). This was a frameshift mutation expected to cause...
miscoding of 78 amino acids from codon 17, and eventually a premature stop codon at codon 95. This change was not detected in either of proband’s parents or in the 100 normal controls (Figure 3B).

Quantitative real-time PCR analysis: Since intragenic mutations were excluded by direct sequencing of FOXL2 in the two BPSE families, we then considered whether FOXL2 gene deletion existed in the patients. This was subsequently confirmed by the q-real-time PCR technique. The study found that the relative copy numbers (RCNs) of the patients were about half that of the healthy individuals (Figure 4). Copy number variations (CNVs) measured by q-real-time PCR signified that the deletion of FOXL2 led to haploinsufficiency. To confirm the CNVs found in the present study, the q-real-time PCRs were performed at least three times to eliminate handling error. As expected, no deletion was detected either in non-carrier relatives or in BPES-free controls.

DISCUSSION
In two families (F1, F2) with BPES, two deletions in FOXL2 were detected and characterized by an efficient technique using q-real-time PCR. Compared to fluorescent in situ hybridization (FISH) and multiplex ligation-dependent probe amplification (MLPA) analysis, q-real-time PCR appeared to be more convenient [15]. In the present study, the copy numbers of the amplification segments located at the 5′ and 3′ ends of FOXL2 were approximately 50% that of healthy individuals (Figure 4), which suggested that FOXL2 was deleted in the region encompassing these amplification segments. Since FOXL2 deletions are found in at least 12% of BPSE cases [13], deletion screening is now routinely used for molecular diagnosis of BPES. Based on the predictions of Beysen et al. [14], deletions encompassing FOXL2 have no reliable genotype-phenotype correlations with regard to POF. However, according to a study by D’haene et al. [15], FOXL2 deletions may be associated with varying degrees of ovarian dysfunction. Therefore, we attempted to assess the BPES type in the two families. In family 1, the absence of female infertility or POF in II:6 suggested that this gene deletion did not affect the ovarian expression of FOXL2, and thus led to BPES type II. However, in family 2, the BPES type could not be determined owing to the prepubertal developmental stage of the female child. FOXL2 deletions causing haploinsufficiency of this gene may affect ovarian function, leading to POF with a variable age of onset [15]. Therefore, apart from the ophthalmological follow-up, young

| Individual | Type of BPES | Clinical data |
|------------|--------------|---------------|
| F1*        | II:6         | II 32-year-old female with refractive error and normal levels of sex hormones. |
|            | III:4        | 7-year-old boy with bilateral amblyopia and strabismus, no clinical data about mental retardation. |
| F2*        | II:1         | Undetermined 27-year-old male with bilateral amblyopia and strabismus and ophthalmoplegia. |
|            | III:1        | 1-year-old girl with the normal growth development and the undetermined BPES type. |
| S*         | S1           | 3-year-old girl with the risk of having POF, no clinical data about other abnormal development. |

*F1=family 1; F2=family 2; S=sporadic case.
female patients of undetermined phenotype require a close endocrinological and gynecological follow-up. Importantly, this study is the first to report a FOXL2 gene deletion in a Chinese ethnic population detected by quantitative real-time PCR. Consequently, we have identified q-real-time PCR as a relatively reliable, convenient and inexpensive molecular diagnostic tool for deletion screening of FOXL2, which will facilitate genetic counseling for BPES patients and help identify those female patients who require an extended clinical follow-up for POF.

BPES features typically include epicanthus inversus (fold curving in the mediolateral direction, inferior to the inner canthus), low nasal bridge, and ptosis of the eyelids leading to both vertical and horizontal narrowing of the palpebral fissures. Thus, subjects with BPES have smaller than normal eyelid openings. The ptosis is usually bilateral and symmetric. Additional dysmorphic features of the eye include nystagmus, microphthalmos, microcornea, and stenosis of the lateral canaliculi [17]. The F1 and F2 families shared similar features to those described above that characterize BPES, including ptosis of the eyelids, epicanthus inversus and telecanthus. Other ocular abnormalities in the F1 and F2 families included bilateral amblyopia, strabismus, and ophthalmoplegia, which may not be common features of BPES. Apart from the eye abnormalities, patients carrying deletions in FOXL2 presented more frequently associated clinical findings. D’haene et al. [15] commented that psychomotor retardation was noted in some patients with a haploinsufficiency of the FOXL2 gene. Microcephaly was reported in some cases with large deletions of FOXL2 involving the neighboring ataxia telangiectasia and Rad3 related (ATR) gene [18]. As reported here, however, III:4 in F1 carrying a deletion in the FOXL2 region presented normal psychomotor and mental developments at age 7 years, which suggested that there was not an exact genotype-phenotype correlations attributed to deletions of the FOXL2 region. Owing to the infancy of III:1 in F2, associated clinical findings such as psychomotor delay and microcephaly could not be determined. Therefore, it may be important for providing a prognosis regarding associated clinical findings in new borns with BPES carrying a FOXL2 deletion.

In a 3-year-old girl with sporadic BPES (S1), we found a de novo mutation which had a C deletion associated with a TA dinucleotide insert at position 50, resulting in a frameshift generating 78 novel amino acids and terminating prematurely at codon 95. The mutation led to a truncated protein in which the entire forkhead DNA-binding domain was erased; this was not found in her parents who lacked clinical evidence of the disorder. Predictions from De Baere et al. [19] suggested that intragenic mutations that resulted in proteins truncated before the polyalanine tract probably led to BPES type I. Therefore, the female child will need regular evaluation by an endocrinologist or gynecologist to explore the possibility of sterility or to anticipate POF [20].

The deletions of FOXL2 reported in this study which caused the haploins sufficiency leading to the presence of a null allele may result in a disable transcript undergoing nonsense-mediated decay [21] as a causative mechanism for BPES. It has been reported that the truncated protein formed as a result of intragenic mutations is strongly aggregated in the nucleus [11]. Aggregation of the protein seriously impairs its DNA-binding function, and then influences interactions with the other proteins. Since the entire polyalanine tract of the COOH-terminus of FOXL2 is important for transcriptional repression of the steroidogenic acute regulatory (StAR) gene [22], either the FOXL2 deletion or mutation-dependent protein truncation before the polyalanine tract might increase StAR expression, thereby resulting in the development of POF. The clinical findings and genetic analysis in the sporadic case, together with findings that intragenic mutations can seriously impair the function of the FOXL2 protein [11] suggest that a single mutation in FOXL2 can cause complete inactivation of the gene product. As a result, the genetic mutation in the sporadic case can, in effect, produce the same disruption in FOXL2.
gene function as gene deletions, leading to development of the BPES and POF phenotypes.

In conclusion, this study provides the first report of FOXL2 gene deletions in a Chinese ethnic population detected by quantitative real-time PCR. It supports the application of quantitative real-time PCR techniques as a relatively reliable, convenient and inexpensive method for detecting genetics abnormalities in BPES patients. Meanwhile, the de novo mutation in the sporadic case broadens the mutation spectrum of FOXL2. The new information concerning mutations in FOXL2 and the more widespread use of q-real-time PCR for deletion screening is likely to facilitate the clinical genetic diagnosis of BPES and lead to improved genetic counseling for a larger number of BPES patients.

ACKNOWLEDGMENTS

We are grateful to all patients, their families, and the healthy volunteers for agreeing to participate in the study, as well as all the people who helped us successfully complete the research. This study was supported by the National Science & Technology Pillar Program of China (No. 2008BAH24B05), the Science and Technology Department of Heilongjiang Province Projects of International Cooperation (WB09C104) and the Natural Science Foundation of China (Grant No. 30801274). Professors Xu Ma (genetics@263.net.cn) and Yanhua Qi contributed equally to the research project and can be considered as equal co-corresponding authors.

REFERENCES

1. Zlotogora J, Sagi M, Cohen T. The blepharophimosis, ptosis, and epicanthus inversus syndrome: delineation of two types. Am J Hum Genet 1983; 35:1020-7. [PMID: 6613996]
2. Nallathambi J, Moumne L, De Baere E, Beyesen D, Usha K, Sundaresan P, Veitia RA. A novel polyalanine expansion in FOXL2: the first evidence for a recessive form of the blepharophimosis syndrome (BPES) associated with ovarian dysfunction. Hum Genet 2007; 121:107-12. [PMID: 17089161]
3. Fryns JP, Stromme P, van den Berghe H. Further evidence for the location of the blepharophimosis syndrome (BPES) at 3q22.3-q23. Clin Genet 1993; 44:149-51. [PMID: 8275574]
4. Amati P, Chomel JC, Nivelon-Chavallier A, Gilgenkrantz S, Kitzis A, Kaplan J, Bonneau D. A gene for blepharophimosis-ptosis-epicanthus inversus syndrome maps to chromosome 3q23. Hum Genet 1995; 96:213-5. [PMID: 7635472]
5. Harrar HS, Jeffery S, Patton MA. Linkage analysis in blepharophimosis-ptosis syndrome confirms localisation to 3q21–24. J Med Genet 1995; 32:774-7. [PMID: 8558553]
6. Amati P, Gasparini P, Zlotogora J, Zelante L, Chomel JC, Kitzis A, Kaplan J, Bonneau D. A gene for premature ovarian failure associated with eyelid malformation maps to chromosome 3q22-q23. Am J Hum Genet 1996; 58:1089-92. [PMID: 8651270]
7. Crisponi L, Deiana M, Loi A, Chiappe F, Uda M, Amati P, Bisceglia L, Zelante L, Nagaraja R, Porcu S, Ristaldi MS, Marzella R, Rocchi M, Nicolinoc M, Lienhardt-Roussie A, Nivelon A, Verloes A, Schlessinger D, Gasparini P, Bonneau D, Cao A, Pilia G. The putative forkhead transcription factor FOXL2 is mutated in blepharophimosis/ptosis/epicanthus inversus syndrome. Nat Genet 2001; 27:159-66. [PMID: 11175783]
8. Laisseau P, Lakhal B, Benayoun BA, Dipietromaria A, Brahnam R, Elghezal H, Philibert P, Saad A, Sultan C, Fellous M, Veitia RA. Functional evidence implicating FOXL2 in non-syndromic premature ovarian failure and in the regulation of the transcription factor OSR2. J Med Genet 2009; 46:455-7. [PMID: 19429596]
9. Jamieson S, Butzow R, Andersson N, Alexiades M, Unkila-Kallio L, Heikinheimo M, Fuller PJ, Anttonen M. The FOXL2 C134W mutation is characteristic of adult granulosa cell tumors of the ovary. Mod Pathol 2010; 23:1477-85. [PMID: 20693978]
10. Kaufmann E, Knöchel W. Five years on the wings of fork head. Mech Dev 1996; 57:3-20. [PMID: 8817449]
11. Cocquet J, Painlhoux F, Jaubert F, Servel N, Xia X, Pannetier M, De Baere E, Messiaen L, Cotinot C, Fellous M, Veitia RA. Evolution and expression of FOXL2. J Med Genet 2002; 39:916-21. [PMID: 12471206]
12. Beyesen D, De Jaegere S, Amor D, Bouchard P, Christin-Maître S, Fellous M, Touraine P, Grix AW, Hennekam R, Meire F, Oyen N, Wilson LC, Barel D, Clayton-Smith J, de Ravel T, Decock C, Delbeke P, Ensenuaire R, Ebinger F, Gillessen-Kaestbach G, Hendriks Y, Kimonis V, Laframboise R, Laisseau P, Leppig K, Leroy BP, Miller DT, Mowat D, Neumann L, Plomp A, Van Regemorter N, Wieczorek D, Veitia RA, De Paepe A, De Baere E. Identification of 34 novel and 56 known FOXL2 mutations in patients with Blepharophimosis syndrome. Hum Mutat 2008; 29:E205-19. [PMID: 18642388]
13. Beyesen D, De Paepe A, De Baere E. FOXL2 mutations and genomic rearrangements in BPES. Hum Mutat 2009; 30:158-69. [PMID: 18726931]
14. Beyesen D, Raes J, Leroy BP, Lucassen A, Yates JR, Clayton-Smith J, Ilyina H, Brooks SS, Christin-Maître S, Fellous M, Fryns JP, Kim JR, Lapunzina P, Lemyre E, Meire F, Messiaen LM, Oley C, Splitt M, Thomson J, Van de Peer Y, Veitia RA, De Paepe A, De Baere E. Deletions involving long-range conserved nongenic sequences upstream and downstream of FOXL2 as a novel disease-causing mechanism in blepharophimosis syndrome. Am J Hum Genet 2005; 77:205-18. [PMID: 15962237]
15. Dhaene B, Nevado J, Pugeat M, Pierquin G, Lowry RB, Reardon W, Delicado A, García-Miñaur S, Palomares M, Courtens W, Stefanova M, Wallace S, Watkins W, Shelling AN, Wieczorek D, Veitia RA, De Paepe A, De Baere E. FOXL2 copy number changes in the molecular pathogenesis of BPES: unique cohort of 17 deletions. Hum Mutat 2010; 31:E1332-47. [PMID: 20232352]
16. Sun M, Ma F, Zeng X, Liu Q, Zhao XL, Wu FX, Wu GP, Zhang ZF, Gu B, Zhao YF, Tian SH, Lin B, Kong XY, Zhang XL, Yang W, Lo WH, Zhang X. Triphalangeal thumb-polydactyly syndrome and syndactyly type IV are caused by genomic duplications involving the long range, limb-specific SHH enhancer. J Med Genet 2008; 45:589-95. [PMID: 18417549]
17. Cai T, Tagle DA, Xia X, Yu P, He XX, Li LY, Xia JH. A novel case of unilateral blepharophimosis syndrome and mental
retardation associated with de novo trisomy for chromosome 3q. J Med Genet 1997; 34:772-6. [PMID: 9321768]

18. de Ru MH, Gille JJ, Nieuwint AW, Bijlsma JB, van der Blij JF, van Hagen JW. Interstitial deletion in 3q in a patient with blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) and microcephaly, mild mental retardation and growth delay: clinical report and review of the literature. Am J Med Genet A 2005; 137:81-7. [PMID: 16015581]

19. De Baere E, Deysen D, Oley C, Cocquet J, De Sutter P, Devriendt K, Dixon M, Fellous M, Fryns JP, Garza A, Jonsrud C, Koivisto PA, Krause A, Leroy BP, Meire F, Plomp A, Van Maldergem L, De Paepe A, Veitia R, Messiaen L. FOXL2 and BPES: mutational hotspots, phenotypic variability, and revision of the genotype-phenotype correlation. Am J Hum Genet 2003; 72:478-87. [PMID: 12529855]

20. De Baere E. Blepharophimosis, Ptosis, and Epicanthus Inversus. GeneReviews. 1993–2004 [PMID: 11468277]

21. De Baere E, Dixon MJ, Small KW, Jabs EW, Leroy BP, Devriendt K, Gilliot Y, Mortier G, Meire F, Van Maldergem L, Courtens W, Hjalgrim H, Huang S, Liebaers I, Van Regemorter N, Touraine F, Praphanphoj V, Verloes A, Udar N, Yellore V, Chalukya M, Yelchits S, De Paepe A, Kutten F, Fellous M, Veitia R, Messiaen L. Spectrum of FOXL2 gene mutations in blepharophimosis-ptosis-epicanthus inversus (BPES) families demonstrates a genotype–phenotype correlation. Hum Mol Genet 2001; 10:1591-600. [PMID: 11468277]

22. Pisarska MD, Bae J, Klein C, Hsueh AJ. Forkhead I2 is expressed in the ovary and represses the promoter activity of the steroidogenic acute regulatory gene. Endocrinology 2004; 145:3424-33. [PMID: 15059956]