Asian Journal of Andrology (2018) 20, 85–89
www.asiaandro.com; www.ajandrology.com

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ORIGINAL ARTICLE

Male Health

Association between diacylglycerol kinase kappa variants and hypospadias susceptibility in a Han Chinese population

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Previous genome-wide association studies have identified variants in the diacylglycerol kinase kappa (*DGKK*) gene associated with hypospadias in populations of European descent. However, no variants of *DGKK* were confirmed to be associated with hypospadias in a recent Han Chinese study population, likely due to the limited number of single-nucleotide polymorphisms (SNPs) included in the analysis. In this study, we aimed to address the inconsistent results and evaluate the association between *DGKK* and hypospadias in the Han Chinese population through a more comprehensive analysis of *DGKK* variants. We conducted association analyses for 17 SNPs in or downstream of *DGKK* with hypospadias among 322 cases (58 mild, 113 moderate, 128 severe, and 23 unknown) and 1008 controls. Five SNPs (rs2211122, rs4554617, rs7058226, rs7063116, and rs5915254) in *DGKK* were significantly associated with hypospadias ($P < 0.05$), with odds ratios (ORs) of 1.64–1.76. When only mild and moderate cases were compared to controls, 10 SNPs in *DGKK* were significant ($P < 0.05$), with ORs of 1.56–2.13. No significant SNP was observed when only severe cases were compared to controls. This study successfully implicated *DGKK* variants in hypospadias risk among a Han Chinese population, especially for mild/moderate cases. Severe forms of hypospadias are likely due to other genetic factors.
Asian Journal of Andrology (2018) **20**, 85–89; doi: 10.4103/aja.aja_13_17; published online: 9 June 2017

Keywords: association study; diacylglycerol kinase kappa; hypospadias; polymorphisms

INTRODUCTION

Hypospadias, affecting approximately 1 out of every 750 births in Europe,[1] is a common congenital disease characterized by urogenital malformation, in which the position of urethral orifice is abnormal. Hypospadias are caused by incomplete urethral fusion during gestational weeks 8 to 16.[2] The phenotype of hypospadias is divided into mild (glandular), moderate (midpenile), and severe (in scrotum or perineum) depending on the abnormal location of the urethral opening.[2–4] The prevalence of hypospadias has increased in developed nations since the 1960s,[5] and its incidence has plateaued in recent years.[6–8] However, in China, the prevalence of hypospadias has shown an increasing trend, particularly in well-developed areas.[9,10] Although the etiology of hypospadias is largely unknown, genetic factors have been demonstrated to play an important role in the development of hypospadias.[11,12]

Hypospadias is a complex disease affected by both genetic and environmental factors. The first genome-wide association study (GWAS) of hypospadias, among a European population, was reported in 2011, which included 436 Dutch cases and 494 controls.[1] In the study, two single-nucleotide polymorphisms (SNPs) (rs1934179 and rs7063116) were identified in the diacylglycerol kinase kappa (*DGKK*) gene, which is located on the X chromosome and is strongly associated with hypospadias.[1] Subsequently, a fine-mapping study was conducted in an American population to determine whether the association of *DGKK* with hypospadias could be replicated in a more racially diverse population in 2013.[3] Results from this second study confirmed the relationship between hypospadias and the above-mentioned SNPs. Fifteen significant SNPs were also found associated with mild and moderate cases.[3] In 2014, a study of the *DGKK* gene was performed in a Chinese population for the first time.[4] Fourteen tag SNPs in the *DGKK* gene were tested, but all failed to show a statistically significant association with hypospadias.[4] Considering the small number of tag SNPs and limited sample size in the previous Chinese study, we aimed to conduct more comprehensive research of the *DGKK* gene variants with all 17 previously identified SNPs in a larger case–control study. Our hypothesis was that *DGKK* genetic variants would be associated with hypospadias risk in a Han Chinese population.

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Received: 11 December 2016; Accepted: 14 March 2017

MATERIALS AND METHODS

Study population

This study included 322 unrelated cases and 1008 controls, all of which were Han Chinese. Patients with hypospadias were recruited from the Department of Urology at Shanghai Children's Hospital and were diagnosed by the Department of Urology from January 2013 to January 2014. Only patients with hypospadias without other system abnormalities were included. The severity of hypospadias cases was classified into one of three categories: mild (glandular), moderate (penile), or severe (in the scrotum or perineum) according to the position of the urethral opening. Of the 322 patients ultimately enrolled, there were 58 mild cases, 113 moderate cases, 128 severe cases, and 23 cases with unknown classification. The 1008 healthy controls were recruited from the Chinese Consortium for Prostate Cancer Genetics (ChinaPCa).

Each patient was informed of the purpose of this study, and written consent was obtained from all participants or their parent/legal guardian. Ethical approval was obtained from the Shanghai Children's Hospital in China.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood samples using the Gentra Puregene Blood Kit (Qiagen, Dusseldorf, Germany). Polymerase chain reaction (PCR) and extension reactions were performed according to the manufacturers' protocol. The SNP genotypes were obtained using the MassARRAY iPLEX platform (Sequenom, San Diego, USA) and the genotyping call rates of all SNPs were >97%. Duplicated and water samples were included in each 96-well plate as PCR negative controls.

Selection of tagging SNPs

A total of 17 tagging SNPs were selected based on the Han Chinese population (CHB) in HapMap data and Genome Variation Server (http://gvs.gs.washington.edu/GVS/) with the criteria of $r^2 > 0.8$ and minor allele frequency (MAF) >0.05. The SNP rs5915330 was located in *CCNB3*, and the rest were in *DGKK*. Two SNPs (rs7063116 and rs1934179) were obtained from the first GWAS study of a European population.[1]

Statistical analysis

PLINK software[13] was used to test the association of each SNP with risk of hypospadias. Association analyses were performed on all cases grouped together as well as separate subgroups based on phenotype severity. Mild and moderate cases were grouped together due to their phenotype similarities. The allelic OR and 95% confidence interval (CI) were calculated using logistic regression models to estimate relative risks. We performed clump and haplotype association analyses based on PLINK software. All *P* values were two-sided tests, and $P < 0.05$ was considered statistically significant. $P = 0.003$ (0.05/17) was the significance threshold through the strict Bonferroni correction. The pairwise linkage disequilibrium (LD) structure (r^2) value was calculated using Haploview 4.2 software (https://www.broadinstitute.org/haploview/haploview).[14]

RESULTS

To validate whether the genetic variants of *DGKK* gene were associated with hypospadias risk in a Han Chinese population, we genotyped 17 tagging SNPs. Genotype information is detailed in **Table 1**. Sixteen SNPs are from the *DGKK* gene and one SNP (rs5915330) is located on the *CCNB3* gene, downstream of *DGKK*. With all patients grouped together, 5 of the 16 *DGKK* SNPs (rs2211122, rs4554617, rs7058226, rs7063116, and rs5915254) were significantly associated with hypospadias, which had $P < 0.05$ and were associated with increased risk, with ORs ranging from 1.64 to 1.76. Another 5 SNPs (rs5915330, rs4143304, rs1934188, rs12171755, and rs1934179) in *DGKK* possessed marginal *P* values that were slightly more than 0.05. The other seven SNPs, including the variant in *CCNB3*, did not reach statistical significance with $P < 0.05$. Because of the similar results for mild and moderate cases, we merged them into one group for analysis (data not shown). Among mild/moderate cases, ten SNPs (rs4599945, rs4143304, rs1934188, rs12171755, rs1934179, rs2211122, rs4554617, rs7058226, rs7063116, and rs5915254) had $P < 0.05$ and their ORs were higher compared to the analysis including all cases. For severe cases, no *P* values reached statistical significance and ORs tended to be closer to 1.00.

To explore whether there was a high linkage disequilibrium that existed in these SNPs, we conducted a clump analysis (**Table 2**). The results showed that rs4143304, rs1934188, rs12171755, rs1934179, rs2211122, rs4554617, rs7058226, rs7063116, and rs5915254 were in the same block. All of them were found to be associated with hypospadias when only mild and moderate cases were compared with controls. Four out of these nine SNPs were estimated in the same haplotype block using Haploview 4.2 software (**Figure 1**).

We performed haplotype analysis in a block that included five SNPS (rs2211122, rs4554617, rs7058226, rs7063116, and rs5915254), which were in high linkage disequilibrium and identified three haplotypes (**Table 3**). The haplotype omnibus test revealed overall significant associations between these SNPs and hypospadias ($P = 0.004$). Meanwhile, individual haplotype analyses were consistent with the omnibus test and both ATGGA and GGAAG haplotypes reached significant *P* values ($P = 0.002$ and 0.001, respectively). ATGGA reflected the major allele and was the most common for each SNP, while GGAAG reflected the minor allele and was the next most common for each SNP.

DISCUSSION

In the present study, we found five SNPs (rs2211122, rs4554617, rs7058226, rs7063116, and rs5915254) that were significantly

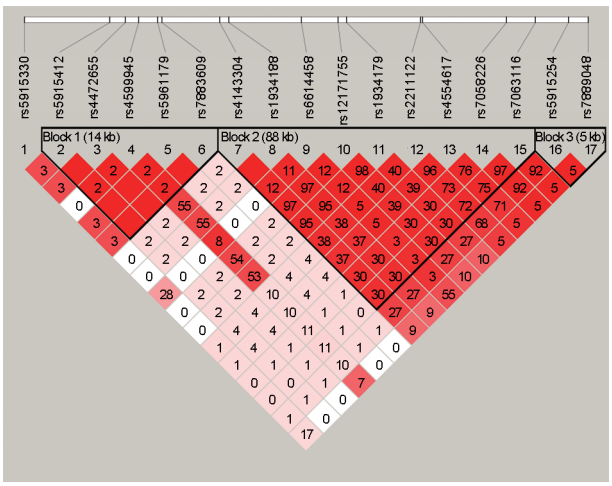

Figure 1: The pairwise linkage disequilibrium (LD) structure of 17 diacylglycerol kinase kappa (*DGKK*) SNPs and estimated haplotype blocks. Bottom, pairwise LD (r^2) values were calculated based on data from the study samples and the color intensity of each SNP corresponded to pairwise D'/log of the odds (LOD). White squares: D' <1 and LOD <2; squares in shades of pink/red: D' <1 and LOD ≥2; bright red squares: D' of 1 and LOD ≥2. SNP: single-nucleotide polymorphism; D': the value of D prime between the two loci.

Table 1: Association of diacylglycerol kinase kappa single-nucleotide polymorphisms with different phenotype severities of hypospadias in a Han Chinese population

SNP	*Location*	*Nearby gene*	*BP*	*Minor allele*	*Major allele*	*MAF of cases*	*MAF of controls*	*Mild + moderate cases*		*Severe cases*		*All cases*	
								OR (95% CI)	*P*	*OR (95% CI)*	*P*	*OR (95% CI)*	*P*
rs5915330	Intron	*CCNB3*	50091920	A	G	0.364	0.310	1.26 (0.90–1.76)	0.185	1.36 (0.92–2.0)	0.117	1.27 (0.98–1.66)	0.073
rs5915412	Intron	*DGKK*	50115970	A	G	0.084	0.093	0.99 (0.57–1.72)	0.962	0.66 (0.31–1.39)	0.268	0.90 (0.57–1.40)	0.627
rs4472655	Intron	*DGKK*	50120200	G	A	0.084	0.094	0.98 (0.56–1.70)	0.933	0.65 (0.31–1.37)	0.256	0.89 (0.57–1.39)	0.594
rs4599945	Intron	*DGKK*	50123966	A	C	0.244	0.205	1.56 (1.08–2.24)	0.016	1.04 (0.66–1.64)	0.876	1.25 (0.93–1.69)	0.138
rs5961179	Coding	*DGKK*	50129369	C	T	0.084	0.094	0.97 (0.56–1.70)	0.927	0.65 (0.31–1.37)	0.254	0.88 (0.57–1.38)	0.588
rs7883609	Intron	*DGKK*	50130544	T	C	0.084	0.094	0.98 (0.56–1.71)	0.947	0.65 (0.31–1.38)	0.262	0.89 (0.57–1.39)	0.610
rs4143304	Coding	*DGKK*	50146570	A	G	0.309	0.258	1.66 (1.18–2.33)	0.003	0.90 (0.59–1.40)	0.655	1.29 (0.98–1.69)	0.074
rs1934188	Intron	*DGKK*	50149245	T	C	0.309	0.257	1.67 (1.19–2.34)	0.003	0.91 (0.59–1.41)	0.676	1.29 (0.98–1.71)	0.067
rs6614458	Intron	*DGKK*	50169456	G	A	0.244	0.254	0.91 (0.62–1.33)	0.631	0.92 (0.60–1.42)	0.705	0.94 (0.71–1.26)	0.700
rs12171755	Intron	*DGKK*	50179749	A	G	0.309	0.261	1.64 (1.17–2.30)	0.004	0.90 (0.58–1.38)	0.618	1.27 (0.96–1.67)	0.089
rs1934179[a]	Intron	*DGKK*	50182184	T	C	0.309	0.258	1.66 (1.18–2.33)	0.003	0.91 (0.59–1.40)	0.657	1.29 (0.98–1.70)	0.074
rs2211122	Intron	*DGKK*	50202750	G	A	0.188	0.122	2.08 (1.39–3.10)	0.000	1.04 (0.60–1.81)	0.893	1.67 (1.19–2.34)	0.003
rs4554617	Intron	*DGKK*	50203402	G	T	0.184	0.121	2.10 (1.41–3.15)	0.000	1.05 (0.60–1.84)	0.855	1.64 (1.17–2.30)	0.004
rs7058226	Intergenic	*DGKK*	50226867	A	G	0.156	0.097	2.08 (1.35–3.21)	0.001	1.15 (0.63–2.08)	0.649	1.72 (1.19–2.48)	0.004
rs7063116[a]	Intergenic	*DGKK*	50235002	A	G	0.156	0.095	2.13 (1.38–3.30)	0.000	1.18 (0.66–2.13)	0.592	1.76 (1.22–2.55)	0.002
rs5915254	Intergenic	*DGKK*	50244281	G	A	0.162	0.103	1.93 (1.25–2.97)	0.002	1.15 (0.65–2.04)	0.631	1.68 (1.17–2.41)	0.004
rs7889048	Intergenic	*DGKK*	50249685	G	A	0.310	0.300	1.01 (0.71–1.43)	0.965	1.07 (0.71–1.60)	0.751	1.05 (0.80–1.38)	0.716

[a]Two SNPs selected from van der Zanden *et al*.[1] MAF: minor allele frequency; OR: odds ratio; CI: confidence interval; SNPs: single-nucleotide polymorphisms; *DGKK*: diacylglycerol kinase kappa; BP: base pair

Table 2: Clump analysis based on linkage disequilibrium between diacylglycerol kinase kappa single-nucleotide polymorphisms

SNPs[a]	*BP*	*P*	*Total*	*SP2*[b]
rs7063116	50235002	0.002	8	rs4143304, rs1934188, rs12171755, rs1934179, rs2211122, rs4554617, rs7058226, rs5915254
rs5915330	50091920	0.073	1	rs6614458
rs4599945	50123966	0.138	0	None
rs5961179	50129369	0.589	3	rs5915412, rs4472655, rs7883609
rs7889048	50249685	0.716	0	None

[a]These as index SNPs; [b]list of other SNP names in clump; SNPs: single-nucleotide polymorphisms; BP: base pair

associated with hypospadias in *DGKK* ($P < 0.05$). When only mild and moderate cases were compared with controls, ten SNPs in *DGKK* were significantly associated with hypospadias ($P < 0.05$). No risk SNP was found to be associated with severe cases. Heritability of hypospadias is approximately 65%–75%, and the risk was estimated to be increased 12- to 20-fold among first-degree relatives.[15–17] A previous study also demonstrated that genetic factors have a more important role in causing familial hypospadias than intrauterine environmental factors.[17] GWAS analysis is a useful method in elucidating the genetic contributions of common variants. The first GWAS of hypospadias was conducted in a European population and identified two SNPs (rs1934179 and rs7063116) of *DGKK*, which had compelling evidence for association with hypospadias.[1] These two susceptibility genetic loci and multiple other genetic loci of *DGKK* were further confirmed in moderate and mild cases in an independent American study population.[3] However, the first study in a Han Chinese population indicated that the role of *DGKK* genetic variants was not likely to have major influences on hypospadias for the failure of identifying significant susceptibility genetic variants in *DGKK*.[4] In our study, we confirmed the previous findings in the European population[1,3] and identified several novel variants in *DGKK* in a Han Chinese population, supporting the evidence that *DGKK* variants do contribute to urethral development as a major susceptibility gene for hypospadias.

According to previous studies, the severity of hypospadias might affect the association with *DGKK* SNPs;[3] thus, we divided the 322 cases into mild, moderate, and severe subgroups. For the two previously reported SNPs,[1,3] SNP rs7063116 is outside of the *DGKK* coding sequence, located upstream of the coding region, and rs1934179 is located in an intron. We validated that SNP rs7063116 increased risk of hypospadias, except in severe cases, while SNP rs1934179 possessed only a marginal *P* value (0.05) in all cases, and did not show a significant difference after the strict Bonferroni correction. We also identified additional, neighboring SNPs (rs2211122, rs4554617, rs7058226, and rs5915254) that significantly increased risk for hypospadias. Two SNPs (rs4143304 and rs1934188) previously reported in an American population[3] also showed significant association with hypospadias in our Han Chinese population. SNP rs12171755 was a novel genetic risk variant, which was significantly associated with mild to moderate hypospadias among the Han Chinese population in our study. Despite that none of these SNPs were found to have a relationship with hypospadias in severe cases in a Han Chinese population, our results indicated that genetic variants of *DGKK* play a major role in moderate and mild cases.

The *DGKK* gene, located on chromosome Xp11.22, encodes diacylglycerol kinase, which plays an important role in signal transduction by modulating the balance between diacylglycerol and phosphatidic acid.[18] *DGKK* mRNA is found to be most abundant in the testis and second in the placenta.[18] In addition, real-time quantitative PCR analyses showed that *DGKK* was expressed in the preputial skin of 10 healthy boys and 14 hypospadias cases.[6] However, little is

Table 3: Association of diacylglycerol kinase kappa haplotypes with hypospadias among all cases

Locus	*Haplotype*	*Frequency in cases*	*Frequency in controls*	*P*	*SNPs*
WIN1	GG	0.185	0.120	0.003	rs2211122|rs4554617
WIN1	AT	0.815	0.880	0.003	rs2211122|rs4554617
WIN2	Omnibus[a]	NA	NA	0.008	rs4554617|rs7058226
WIN2	GA	0.156	0.095	0.002	rs4554617|rs7058226
WIN2	GG	0.028	0.026	0.831	rs4554617|rs7058226
WIN2	TG	0.816	0.879	0.004	rs4554617|rs7058226
WIN3	AA	0.156	0.093	0.002	rs7058226|rs7063116
WIN3	GG	0.844	0.907	0.002	rs7058226|rs7063116
WIN4	AG	0.156	0.095	0.002	rs7063116|rs5915254
WIN4	GA	0.844	0.905	0.002	rs7063116|rs5915254
WIN5	Omnibus[a]	NA	NA	0.006	rs2211122|rs4554617|rs7058226
WIN5	GGA	0.156	0.094	0.002	rs2211122|rs4554617|rs7058226
WIN5	GGG	0.028	0.026	0.844	rs2211122|rs4554617|rs7058226
WIN5	ATG	0.816	0.880	0.003	rs2211122|rs4554617|rs7058226
WIN6	Omnibus[a]	NA	NA	0.007	rs4554617|rs7058226|rs7063116
WIN6	GAA	0.157	0.094	0.002	rs4554617|rs7058226|rs7063116
WIN6	GGG	0.028	0.025	0.755	rs4554617|rs7058226|rs7063116
WIN6	TGG	0.816	0.881	0.003	rs4554617|rs7058226|rs7063116
WIN7	AAG	0.157	0.096	0.002	rs7058226|rs7063116|rs5915254
WIN7	GGA	0.843	0.904	0.002	rs7058226|rs7063116|rs5915254
WIN8	Omnibus[a]	NA	NA	0.005	rs2211122|rs4554617|rs7058226|rs7063116
WIN8	GGAA	0.157	0.092	0.001	rs2211122|rs4554617|rs7058226|rs7063116
WIN8	GGGG	0.028	0.025	0.754	rs2211122|rs4554617|rs7058226|rs7063116
WIN8	ATGG	0.815	0.883	0.002	rs2211122|rs4554617|rs7058226|rs7063116
WIN9	Omnibus[a]	NA	NA	0.006	rs4554617|rs7058226|rs7063116|rs5915254
WIN9	GAAG	0.157	0.093	0.001	rs4554617|rs7058226|rs7063116|rs5915254
WIN9	GGGA	0.028	0.025	0.759	rs4554617|rs7058226|rs7063116|rs5915254
WIN9	TGGA	0.814	0.881	0.002	rs4554617|rs7058226|rs7063116|rs5915254
WIN10	Omnibus[a]	NA	NA	0.004	rs2211122|rs4554617|rs7058226|rs7063116|rs5915254
WIN10	GGAAG	0.158	0.092	0.001	rs2211122|rs4554617|rs7058226|rs7063116|rs5915254
WIN10	GGGGA	0.028	0.025	0.758	rs2211122|rs4554617|rs7058226|rs7063116|rs5915254
WIN10	ATGGA	0.814	0.883	0.002	rs2211122|rs4554617|rs7058226|rs7063116|rs5915254

[a]Omnibus test is a H-1 degree of freedom test for H haplotypes (of each *vs* all others); SNPs: single-nucleotide polymorphisms; NA: not available

known about the functions of *DGKK,* and the biological mechanisms underlying the association between *DGKK* genetic variants and hypospadias risk remain elusive.

There are still several limitations in our study. First, the limited sample size may not have enough power to validate the genetic variants that exert effects on hypospadias in a Han Chinese population. Furthermore, the recruitment methods of cases and controls were different, which may have caused selection bias; thus, a larger sample size is warranted to confirm the association in a Han Chinese population. Second, little is known about the biological mechanisms of *DGKK*, and functional studies are needed in future projects.

CONCLUSIONS

In summary, we validated the finding of the first GWAS study of hypospadias and identified several novel SNPs in a Han Chinese population. These results from our fine-mapping study indicated that genetic variants of *DGKK* were associated with hypospadias in mild and moderate Han Chinese cases. Our findings support that further investigations and more comprehensive studies are warranted to address the functions and biological mechanisms of *DGKK*.

AUTHOR CONTRIBUTIONS

HX, XLL, JX, and FC participated in conceiving and designing the study. HX, XLL, and SZ, participated in drafting the manuscript; XLL, SZ, and HTC participated in statistical analyses; HX, LY, XXL, YCH, and YQL participated in administrative, technical, and material support. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

ACKNOWLEDGMENTS

This study was sponsored by the Shanghai Municipal Commission of Health and Family Planning (No. 20134283) and the Shanghai Municipal Commission of Science and Technology (No. 14411950403).

REFERENCES

1 van der Zanden LF, van Rooij IA, Feitz WF, Knight J, Donders AR, *et al.* Common variants in DGKK are strongly associated with risk of hypospadias. *Nat Genet* 2011; 43: 48–50.
2 Fredell L, Kockum I, Hansson E, Holmner S, Lundquist L, *et al.* Heredity of hypospadias and the significance of low birth weight. *J Urol* 2002; 167: 1423–7.
3 Carmichael SL, Mohammed N, Ma C, Iovannisci D, Choudhry S, *et al.* Diacylglycerol kinase K variants impact hypospadias in a California study population. *J Urol* 2013; 189: 305–11.
4 Ma Q, Tang Y, Lin H, Xu M, Xu G, *et al.* Diacylglycerol kinase kappa (DGKK) variants and hypospadias in Han Chinese: association and meta-analysis. *BJU Int* 2015; 116: 634–40.
5 Paulozzi LJ. International trends in rates of hypospadias and cryptorchidism. *Environ Health Perspect* 1999; 107: 297–302.
6 Bergman JE, Loane M, Vrijheid M, Pierini A, Nijman RJ, *et al.* Epidemiology of

hypospadias in Europe: a registry-based study. *World J Urol* 2015; 33: 2159–67.
7 Caione P. Prevalence of hypospadias in European countries: is it increasing? *Eur Urol* 2009; 55: 1027–9.
8 Fisch H, Lambert SM, Hensle TW, Hyun G. Hypospadias rates in new york state are not increasing. *J Urol* 2009; 181: 2291–4.
9 Li Y, Mao M, Dai L, Li K, Li X, *et al*. Time trends and geographic variations in the prevalence of hypospadias in China. *Birth Defects Res A Clin Mol Teratol* 2012; 94: 36–41.
10 Sun G, Tang D, Liang J, Wu M. Increasing prevalence of hypospadias associated with various perinatal risk factors in Chinese newborns. *Urology* 2009; 73: 1241–5.
11 George M, Schneuer FJ, Jamieson SE, Holland AJ. Genetic and environmental factors in the aetiology of hypospadias. *Pediatr Surg Int* 2015; 31: 519–27.
12 Soderhall C, Korberg IB, Thai HT, Cao J, Chen Y, *et al*. Fine mapping analysis confirms and strengthens linkage of four chromosomal regions in familial hypospadias. *Eur J Hum Genet* 2015; 23: 516–22.
13 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81: 559–75.
14 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005; 21: 263–5.
15 Carmichael SL, Shaw GM, Lammer EJ. Environmental and genetic contributors to hypospadias: a review of the epidemiologic evidence. *Birth Defects Res A Clin Mol Teratol* 2012; 94: 499–510.
16 Harris EL. Genetic epidemiology of hypospadias. *Epidemiol Rev* 1990; 12: 29–40.
17 Schnack TH, Zdravkovic S, Myrup C, Westergaard T, Christensen K, *et al*. Familial aggregation of hypospadias: a cohort study. *Am J Epidemiol* 2008; 167: 251–6.
18 Imai S, Kai M, Yasuda S, Kanoh H, Sakane F. Identification and characterization of a novel human type II diacylglycerol kinase, DGK kappa. *J Biol Chem* 2005; 280: 39870–81.