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Secular trends for age at spermarche among Chinese boys from 11 ethnic minorities, 1995 to 2010: a multiple-cross-sectional study

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Key words: Spermarche; Puberty; Body Mass Index; Ethnic minority; boys

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Abstract

Objectives: We compared the differences in median age at spermarche among 11 ethnic minorities in 2010, estimated the trends regarding age at spermarche in different ethnic minorities from 1995 to 2010, and explored the association of spermarche with BMI.

Methods: We used four cross-sectional Chinese National Surveys on Students' Constitution and Health (CNSSCH, 1995, 2000, 2005, and 2010), and the total sample size was 40 113 children aged 11-18 years. The median age at spermarche of each ethnic minority was determined by using probit analysis. Logistic regression was used to assess the association of spermarche with BMI.

Results: In 2010, the ethnic minorities with earliest age at spermarche were Qiang (12.03 years), Zhuang (12.91 years) and Kirghiz (13.17 years); the three ethnic minorities with latest age at spermarche were Dong (14.73 years), Yao (14.60 years), and Naxi (14.36 years). From 1995 to 2010, age at spermarche showed a decline in almost each minority groups except Yao and Dong. A higher BMI was associated with an increased likelihood of having reached spermarche after adjusting for age, regions or ethnic minorities.

Conclusion: A large variation in age at spermarche was observed among different ethnic minorities and. The age at spermarche showed a downward shift in almost each of the 11 ethnic minorities with different patterns over time, and the children with higher BMI are more likely to enter puberty early.

Key words: Spermarche; Puberty development; Ethnic minority; boys

Strengths and limitations of this study

- Study population was from successive national surveys from 1995 to 2010, and the consistent stratified cluster sampling procedure and methods of measurement contributed to the comparability of the data of different survey year.
- It is the first to describe the trend in decreasing age at spermarche among Chinese ethnic minorities' boys.
- It is the first to assess the relationship between spermarche and BMI among Chinese ethnic minorities' boys and we observed that a higher BMI was associated with an increased likelihood of having reached spermarche.
- Previous findings concerning the age at spermarche in ethnic minorities are limited, and thus, comparative studies across different countries or regions are not available.

Introduction

There are many studies about the trend of age at menarche among girls, but the data for evaluating secular trends in male pubertal development are limited and insufficient. [1] However, some studies have showed that an earlier onset of puberty occurred among boys. [2-3] Some local surveys in China also found the similar trends. [4-5] However, reports of age at spermarche among Chinese boys have been sparse, and many are outdated or focus only on boys of Han ethnicity. [4-6] Data on the timing of spermarche among ethnic minority boys is not available, and the trends in age at spermarche among ethnic minority boys over the past 15 years are unknown.

Previous studies have shown that nutritional status is an important regulator of puberty, i.e. underweight could delay onset of puberty and reduce pubertal growth spurt whereas overweight or obese children are more likely to enter puberty early. [7-10] A study conducted in Chinese Han ethnicity has also found that a higher BMI is associated with an increased likelihood of having reached spermarche. [6] However, the question as to whether an association between spermarche and BMI exists among ethnic minorities remains unanswered.

The majority of the population in China belongs to the Han ethnic group, and the other 55 ethnic groups are referred to as *ethnic minorities*. Although they are in the minority, the absolute number of an ethnic minority in China may be larger than in any other country or region. [11] According to the sixth Chinese national census, conducted in 2010, 113,792,211 (8.49%) of the country's total population belonged to ethnic minorities. [12]

For example, there are now more than 3.5 million Yao people in the world, with approximately 2.8 million living in China, 0.8 million in the Vietnam, and the remainder in other countries, such as Laos, Thailand and the United States. As a multi-ethnic country, China provides a good opportunity to describe the diversity in age at spermarche among different ethnic minorities during the same period.

Previous studies have shown that the estimation of spermarche has been performed successfully in China for many years. [13-14] The age at spermarche in boys can be determined by personal interview, which may be more accurate and convenient than measurement of spermaturia in population-based studies. [13, 15] By using this method, spermarcheal data are collected in the Chinese National Surveys on Students Constitution and Health (CNSSCH), [16-19] which has been conducted every five years using identical methods. As a national sample of school-age children in China, it includes both Han and ethnic minority students and thus provides an opportunity to study the trends regarding age at spermarche among ethnic minorities. The present analysis sought to 1) compare the differences of median age at spermarche among 11 ethnic minorities in 2010; 2) estimate the trends of age at spermarche among different ethnic minorities from 1995 to 2010; and 3) determine any possible association between spermarche and BMI.

Subjects and methods

Subjects

Data were obtained from the 1995, 2000, 2005, and 2010 cycles of the CNSSCH. [16-19] Thus far, it is the largest nationally representative sample of school-aged children in

China (more than 200 000 students aged 7- 22 years in each survey point). The present study included only boys aged 11 to 18- years from the following 11 ethnic minorities: Hui, Zhuang, Yao, Kirghiz, Dong, Hani, Naxi, Dongxiang, Li, Tu and Qiang, who were mainly sampled in the Ethnic Minority Autonomous Region or Ethnic Minority Autonomous Prefecture (Figure 1). The data from the four cycles of CNSSCH were checked for comparability and reliability according to three indicators: (a) all the subjects and their parents were of the same ethnicity origin and had lived in the local areas for at least 1 year; (b) all subjects were uniformly measured in the same year using the same methods and in the same way; (c) all subjects had a thorough medical examination before measurement, and were generally healthy and free from overt disease or physical/mental deformities. The sample sizes of the various ethnic minorities at different survey points ranged from 400 to 2 401 (Table 1); the sample size of each survey ranged from 8 592 to 14734, and the total sample size (all surveys) was 40 113. The project was approved by the Medical Research Ethics Committee of Peking University Health Science Center (IRB00001052-13082).

Measures

Individual spermarcheal data were collected by the *status quo* method. Boys aged ⩾11 years in each CNSSCH were interviewed face to face by male physicians or professionals and were asked whether or not they had experienced a first ejaculation. Almost all school boys of that age group have some knowledge about male pubertal events from their school health education; moreover, the interviewers were well trained to create a harmonious atmosphere and not to let the boys answer under stress. The interviewers

were also prepared to provide sufficient explanation of sperm emission, if necessary, during the interview. Boys were also encouraged to ask questions freely. A dichotomous response (yes/no) was obtained for spermarcheal status. The boys who did not understand sperm emission after explanation, could not remember their ejaculation history, or who refused to answer questions were regarded as invalid cases and excluded from the study. Boys' ages were recorded and calculated as decimal ages (e.g. 11.00–11.99 years, 12.00–12.99 years).

Height (cm) and weight (kg) were all measured using similar instruments at all survey sites. [16-19] Participants were required to wear only light clothing and stand erect, barefoot, and at ease while being measured. Weight was recorded to the nearest 0.1 kg with a standardized scale and height to the nearest 0.1 cm with a portable stadiometer. Both the scales and stadiometers were calibrated before use. BMI was calculated as body weight (kg) divided by height (m) squared (kg/m^2). Measurements at the survey site were conducted by a team of field professionals who had passed a training course in anthropometric measurements. The geographic regions were classified as north China and south China, and north China in present study included Ningxia Hui Autonomous Region, Xinjiang Uyghur Autonomous Region, Gansu and Qinghai, whereas south China included Guangxi Zhuang Autonomous Region, Guizhou, Yunnan, Hainan and Sichuan.

Statistical analyses

The percentages of boys having reached spermarche in each age group were determined. The median age at spermarche and 95% confidence intervals (CI) in subgroups for

different years were calculated using probit analysis. We fitted probit models to the proportion of boys within each age group who had reached spermarche. A cumulative normal curve was fitted to the proportion of boys within each age group who were spermarcheal, and the median age at spermarche was the corresponding age at which 50% of boys in the population were predicted to have reached spermarche. Differences in the percentages of spermarcheal boys among different ethnic minorities in 2010 were compared by using Chi square test, and differences in age at spermarche between 1995 and 2010 survey points were tested by using *Z* test. *P*-value <0.05 was considered as statistically significant. In order to facilitate the classification of ethnic minorities, age at spermarche among the 11ethnic minorities was examined by cluster analyses. Logistic regression was used to assess the association between the log odds of being spermarcheal and BMI, geographic regions age, age and ethnic minorities in 2010 CNSSCH. A two-sided *P* value < 0.05 was considered significant. All analyses were conducted with SPSS 20.0 (SPSS, Chicago, IL).

Results

Age at spermarche among 11 ethnic minorities in 2010

In 2010, only a few boys (6.54%) were spermarcheal in the 11-year-old group. In the 13-year-old group, almost all ethnic minorities were spermarcheal and the percentage of spermarche among Yao was the lowest (13.33%), while that among Kirghiz was the highest (91.06%) ($\chi^2 = 288.80$, $P < 0.01$). By the age of 18, 98.93% ethnic minority boys were spermarcheal. The median ages at spermarche among the three ethnic minorities with the earliest ages at spermarche in 2010 were as follows: Qiang (12.03 years),

Zhuang (12.91 years) and Kirghiz (13.17 years); those with the lastest median ages at spermarche were Dong (14.73 years), Yao (14.60 years), and Naxi (14.36 years) (Table 2). The cluster analysis indicated that the 11 ethnic minorities in 2010 could be classified as two groups: Zhuang, Kirghiz and Qiang were classified as the earlier age group of age at spermarche; and the other 8 ethnic minorities belonged to the later age group of age at spermarche (Figure 2).

Secular trends of age at spermarche among 11 ethnic minorities from 1995 to 2010

From 1995 to 2010, the age at spermarche showed a downward shift in almost each of the 11 ethnic minorities, but with different patterns of decline. Some minority groups, such as Hui, Kirghiz, Hani, and Qiang showed a clearly declining trend over time, some minority groups, such as Zhuang, Naxi(Nakhi), Dongxiang, Li, and Tu showed a decreasing trend with upward fluctuations over time, while Yao and Dong showed a relatively flat trend with no statistically significant difference between 1995 and 2010 (Figure 3). As a result, Qiang, Zhuang and Kirghiz showed the largest reductions, with age at spermarche found to be 3.53 ($P < 0.05$), 2.12 ($P < 0.05$) and 1.93 ($P < 0.05$) years earlier, respectively, in 2010 than in 1995. In contrast, Yao and Dong showed the smallest differences, with age at spermarche only 0.02 ($P > 0.05$) and 0.08 ($P > 0.05$) years differences, respectively, between 1995 and 2010 (Table 2).

Association of spermarche with BMI

Table 3 shows that there was an association between age at spermarche and BMI: a higher BMI was associated with an increased likelihood of having reached spermarche

after adjusting for age, regions or ethnic minorities. The ethnic minorities who lived in the south of China were earlier to have reached spermarche than those who lived in the north of China after adjusting for BMI and age (OR=1.84).

Discussion

The present study demonstrated large variations in age at spermarche among different ethnic minorities. Zhuang, Kirghiz and Qiang were classified as the earlier age group of age at spermarche, and the other 8 ethnic minorities belonged to the later age group of age at spermarche in 2010. More than half boys reached spermarche before 13 years among the Zhuang and Qiang boys, whereas less than half peers had spermarche till 14 years among the Hui, Yao, Dong, Naxi (Nakhi) and Tu boys. Because the decreasing age of male sexual maturity runs counter to the delay in the social transition to adulthood that has been documented around the world, the early developers are at an increased risk of negative reproductive health outcomes in both adolescence and adulthood, [21-23] specific health education on the male puberty development is needed to formulate in order to meet the diverse health requirements among different ethnic minorities in China.

To our knowledge, the present study is the first to describe the trend in decreasing age at spermarche among Chinese ethnic minorities' boys. We found that the occurrence of a downward secular trend in age at spermarche was evident in almost all of the 11 ethnic minorities, but to different degrees. Globally, the research on the trend of male puberty development varied greatly across countries and regions. A study in Poland reported an over 3-month decrease between 2000 and 2010 in the age of initial appearance of pubic

hair in boys. [2] In Thailand, the age at testicular enlargement Tanner II declined by 0.15 years/decade (1.8 months/decade) from 1975 to 2012. [3] In the United States, the mean ages of beginning genital and pubic hair growth and early testicular volumes were 6 months to 2 years earlier than in past studies, i.e. HHANES (the Hispanic Health and Examination Survey, 1982-1984) and NHANES III (1988-1992) found attainment of genital stage 2 declined from 12.4 to 10.4 years of age over a 10-year period among Hispanic boys. [24-25] In China, from 1995 to 2010, age at spermarche dropped from 14.57 to 14.03 years with an average decrease of 4.3 months per decade among Han boys. [6] When compared with Chinese Han boys, the 11 ethnic minority groups in the present study could be subdivided into three categories: 1) ethnic minority boys whose differences in age at spermarche from 1995 to 2010 were larger than Han boys, i.e. Hui, Zhuang, kirghiz, Hani, Naxi (Nakhi), Tu, and Qiang; 2) ethnic minority boys whose differences in age at spermarche from 1995 to 2010 were smaller than Han boys, i.e. Dongxiang and Li; and 3) ethnic minority boys whose differences in age at spermarche from 1995 to 2010 were non-significant, such as the Yao and Dong. The results are in agreement with many previous studies in China showing that the age of peak of height velocity (PHV) of student of some ethnic minorities' boys, such as Hui, Qiang, Zhuang, kirghiz, and Hani declined over the past years, [16-19, 26-28] and the morphological development level of the Qiang was better than other ethnic minorities. [28]

The pattern of results thus indicated that some ethnic minorities lived in the same provinces or regions showed similar trends of age at spermarche, i.e. the Hani and Naxi (Nakhi) are both from Yunnan province, and both showed clearly declining trends of age

at spermarche. However, it also pointed that not all ethnic minorities showed a significant decline in age at spermarche during the years in question, suggesting that differences in secular trends among ethnic minorities may well be related to specific features of certain ethnic minority groups. Even among ethnic minorities from the same provinces or regions, large variations in age at spermarche were observed. For example, the Zhuang and Yao are both from Guangxi Zhuang Autonomous Region; however, they showed different patterns concerning age at spermarche trends: Zhuang boys had larger differences regarding average age at spermarche than Han boys, whereas the Yao had no significant differences over the past 15 years.

Considering growth is a product of a continuous and complex interaction of heredity and environment, our findings of difference in age at spermarche of minority boys can be attributed to the effects of both hereditary and environmental factors. Environmental factors can be generally divided into two dimensions: socioeconomic (nutrition, disease, income, occupation, family size, social mobility, urbanization, etc.) and ecological (altitude, season, climate, etc.). [13, 29] In the present study, we observed that higher BMI was associated with the earlier spermarche, which are consistent with previous studies showing that earlier maturing boys were heavier than their coevals, whereas underweight boys developed puberty later, [6, 8, 30] moreover, a cohort study also showed that a significant effect of BMI at 7 years on age at sexual maturation in boys; the heavier at age 7 years the earlier did the children enter puberty. [31] It hints that nutritional strategies and interventions such as the program on improving student nutrition among rural compulsory school students which issued by the State Council in 2011 [32] may have effects on both children's nutritional status and puberty development. Although the ethnic

minorities' children would be benefit from this program to a certain extent, the long-term nutritional strategies targeting on the ethnic minorities should be considered, and the health education focused on the secular trend of male puberty should also be developed correspondingly.

Our study has several limitations. Firstly, it was not a prospective cohort study, because each CNSSCH was a cross-sectional survey conducted with different participants. The average age at spermarche might not reflect the exact situation in the population; this can be clarified only by a longitudinal cohort study. Secondly, information was not obtained about the actual spermarche dates of the boys. However, the *status quo* method — when used in a large study population such as the current sample — is considered to be even more reliable than the recall method for obtaining spermarche dates. Thirdly, the sample size of some ethnic minorities, for example, the Yao, may have been inadequate and larger surveys are needed in the future. In addition, previous findings concerning the age at spermarche in ethnic minorities are limited, and thus, comparative studies across different countries or regions are not available.

Conclusion

Age at spermarche varied widely among different ethnic minorities. From 1995 to 2010, the age at spermarche among Chinese ethnic minority boys declined significantly among 9 of 11 ethnic minorities included in the current study. During that period, seven ethnic minorities showed clearly declining rates, with a decrease of over 4.3 months per decade, which is a larger decrease than that shown among Han boys. Two ethnic minorities

showed decreasing trends and two ethnic minorities showed no significant differences. As Han boys, increasing BMI was associated with an increased likelihood of being spermarcheal when adjusted for age, regions or ethnic minorities. This suggests that nutritional interventions may also have an effect on children's pubertal development.

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References

1. Euling SY, Herman-Giddens ME, Lee PA, Selevan SG, Juul A, Sørensen TI, et al. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics.* 2008; 121(Suppl. 3), S172-91.
2. Kryst Ł, Kowal M, Woronkowicz A, Sobiecki J, Cichocka BA. Secular changes in height, body weight, body mass index and pubertal development in male children and adolescents in Krakow, Poland. *J Biosoc Sci.* 2012; 44(4):495-507.
3. Jaruratanasirikul S, Sriplung H. Secular trends of growth and pubertal maturation of school children in Southern Thailand. *Ann Hum Biol.* 2014; 18:1-8.
4. Sun Y, Tao F, Su PY; China Puberty Research Collaboration. National estimates of pubertal milestones among urban and rural Chinese boys. *Ann Hum Biol.* 2012; 39(6):461-7.
5. Ma HM, Chen SK, Chen RM, Zhu C, Xiong F, Li T, et al. Pubertal development timing in urban Chinese boys. *Int J Androl*. 2011; 34:e435–45.
6. Song Y, Ma J, Wang HJ, Wang Z, Lau PW, Agardh A. Age at spermarche: 15-year trend and its association with Body Mass Index in Chinese school-aged boys. *Pediatric Obesity*. 2015;
7. Wagner IV, Sabin MA, Pfäffle RW, Hiemisch A, Sergeyev E, Körner A, et al. Effects of obesity on human sexual development. *Nat Rev Endocrinol.* 2012; 8(4):246-54.
8. Tomova A, Robeva R, Kumanov P. Influence of the body weight on the onset and progression of puberty in boys. *J Pediatr Endocrinol Metab.* 2015; 28:859-65.
9. Soliman A, De Sanctis V, Elalaily R. Nutrition and pubertal development. *Indian J Endocrinol Metab.* 2014; 18(Suppl 1):S39-47.
10. Marcovecchio ML, Chiarelli F. Obesity and growth during childhood and puberty.

World Rev Nutr Diet. 2013; 106:135-41.

11. Song Y, Ma J, Agardh A, Lau PW, Hu P, Zhang B. Secular trends in age at menarche among Chinese girls from 24 ethnic minorities, 1985 to 2010. *Glob Health Action.* 2015, 8:26929.
12. National Bureau of Statistics of China. Communiqué of the National Bureau of Statistics of the People's Republic of China on Major Figures of the 2010 Population Census(No.1). [WWW document]. http://www.stats.gov.cn/tjsj/tjgb/rkpcgb/qgrkpcgb/201104/t20110428_30327.html. 2011.
13. Ji CY, Ohsawa S. Onset of the release of spermatozoa (spermarche) in Chinese male youth. *Am J Hum Biol.* 2000; 12(5):577-587.
14. Ji CY. Age at spermarche and comparison of growth and performance of pre- and post-spermarcheal Chinese boys. *Am J Hum Biol.* 2001; 13(1):35-43.
15. Tinggaard J, Mieritz MG, Sørensen K, Mouritsen A, Hagen CP, Aksglaede L, et al. The physiology and timing of male puberty. *Curr Opin Endocrinol Diabetes Obes.* 2012; 19(3):197-203.
16. CNSSCH. Association. Report on the 1995th National Survey on Students' Constitution and Health. Changchun: Jilin Technical and Science Publication; 1997, pp.3-38. [in Chinese].
17. CNSSCH Association. Report on the 2000th National Survey on Students' Constitution and Health. Beijing: People's Educational Publication; 2002, pp.3-46. [in Chinese].
18. CNSSCH Association. Report on the 2005th National Survey on Students'

Constitution and Health. Beijing: China College & University Press; 2007, pp. 3-52. [in Chinese].

19. CNSSCH Association. Report on the 2010th National Survey on Students' Constitution and Health. Beijing: China College & University Press; 2012, pp.4-71. [in Chinese].
20. National Bureau of Statistics of China. The population of each ethnic minority. [WWW document]. http://data.stats.gov.cn/search/keywordlist2?keyword=2010%E5%B9%B4%E4%BA%BA%E5%8F%A3. 2011.
21. Goldstein JR. A secular trend toward earlier male sexual maturity: evidence from shifting ages of male young adult mortality. *PLoS ONE.* 2011; 6(8):e14826.
22. Sabageh AO, Sabageh D, Adeoye OA, Adeomi AA. Pubertal Timing and Demographic Predictors of Adolescents in Southwest Nigeria. *J Clin Diagn Res.* 2015; 9(8):LC11-3.
23. Campbell BC, Prossinger H, Mbzivo M. Timing of pubertal maturation and the onset of sexual behavior among Zimbabwe school boys. *Arch Sex Behav.* 2005; 34(5):505-16.
24. Herman-Giddens ME, Steffes J, Harris D, et al. Secondary sexual characteristics in boys: data from the Pediatric Research in Office Settings Network. *Pediatrics.* 2012; 130: e1058-68.
25. Karpati AM, Rubin CH, Kieszak SM, Marcus M, Troiano RP. Stature and pubertal stage assessment in American boys: the 1988-1994 Third National Health and Nutrition Examination Survey. *J Adolesc Health.* 2002; 30(3):205-12.

26. Zhang TC. Dynamic analysis of the development of students of minority nationalities in plateau areas between 1985 and 2005. J Physical Education. 2008; 15(3):79-83. [in Chinese]

27. Ma J, Li SS, Song Y, Hu PJ, Zhang B. Study on the changing status of morphological development among minority students in China from1985 to 2005. Chin J Epidemiol. 2009; 30(10):1304-08. [in Chinese]

28. Wang YH, Deng ZH, Zhou GX, Yang CL. Dynamic Analysis of Body Shape Variation of Southwest Minority Adolescents from 1995 to 2010 in the View of Social Changes. J Guangzhou Sport University.2014; 34(3):52-8. [in Chinese]

29. Malina RM, Bouchard C. Growth, maturation, and physical activity. Champaign, IL: Human Kinetics. 1991. p 229-249, p 329-352.

30. De Leonibus C, Marcovecchio ML, Chiavaroli, de Giorgis T, Chiarelli F, Mohn A. Timing of Puberty and Physical Growth in Obese Children: A Longitudinal Study in Boys and Girls. *Pediatr Obes*. 2014; 9(4):292-9.

31. Aksglaede L, Juul A, Olsen LW, Sørensen TI. Age at puberty and the emerging obesity epidemic. *PLoS One.* 2009; 4(12):e8450.

32. The central People's Government of the People's Republic of China. The inform of Interim Measures on management of special funds targeted on rural compulsory schools to improve student nutrition program issued by Ministry of Finance and Ministry of Education. (WWW document) http://www.gov.cn/gongbao/content/2012/content_2256575.htm.

Table 1 The distribution and sample size of boys aged 11 to 18 among 11 ethnic minorities from 1995 to 2010

Ethnic minorities	Source province/ region	Number (millions) (20) [a]	Minority population ranking [a]	1995	2000	2005	2010
Hui	Ningxia Hui Autonomous Region	10.59	2	2401	1600	1564	1744
Zhuang	Guangxi Zhuang Autonomous Region	16.93	1	2391	1595	1271	1449
Yao	Guangxi Zhuang Autonomous Region	2.8	12	1200	801	484	408
Kirghiz	Xinjiang Uyghur Autonomous Region	0.19	31	1200	400	-	963
Dong	Guizhou	2.88	10	1200	766	708	741
Hani	Yunnan	1.66	15	1200	-	863	806
Naxi (Nakhi)	Yunnan	0.33	26	1189	-	799	673
Dongxiang	Gansu	0.62	21	930	1136	-	768
Li	Hainan	1.46	16	1200	784	645	596
Tu	Qinghai	0.29	28	599	800	557	827
Qiang	Sichuan	0.31	27	1224	710	-	921
Total				14734	8592	6891	9896

[a] according to the Sixth National Census in 2010

Table 2 Age at spermarche (95%CI) of boys aged 11 to 18 among 11 ethnic minorities from 1995 to 2010

Ethnic minorities	1995	2000	2005	2010	Difference (1995~2010)
Hui	15.93(15.78-16.08)	14.91(14.48-15.31)	14.67(13.45-15.75)	14.31(13.88-14.72)	-1.62*
Zhuang	15.03(14.84-15.21)	15.10(14.96-15.23)	13.34(11.38-14.09)	12.91(12.56-13.22)	-2.12 *
Yao	14.58(14.38-14.79)	15.46(15.03-15.90)	-	14.60(14.29-14.84)	0.02
Kirghiz	15.10(14.86-15.34)	14.85(14.55-15.22)	-	13.17(12.49-13.75)	-1.93*
Dong	14.81(14.66-14.96)	15.45(15.20-15.74)	14.74(14.46-15.01)	14.73(14.02-15.41)	-0.08
Hani	14.37(14.25-14.49)	-	14.20(13.84-14.54)	13.76(13.63-13.89)	-0.61*
Naxi (Nakhi)	14.93(14.72-15.14)	-	13.36(13.19-13.53)	14.36(13.64-14.95)	-0.57*
Dongxiang	14.39(14.12-14.65)	15.98(15.60-16.38)	-	13.96(11.71-15.46)	-0.43*
Li	14.11(13.88-14.34)	14.05(13.00-15.07)	13.50(12.62-14.11)	13.66(13.01-14.14)	-0.45*
Tu	15.37(15.13-15.62)	16.23(15.62-16.88)	15.19(15.02-15.37)	14.35(13.91-14.77)	-1.02*
Qiang	15.56(15.32-15.80)	14.65(14.40-14.92)	-	12.03(11.20-12.55)	-3.53*

* $P < 0.05$ there is a significant difference between 1995 and 2010

Table 3 Logistic regression models predicting spermarcheal status from BMI, geographic regions, age and ethnic minorities in 2010 CNSSCH (OR(95% CI))

Variable	Model 1	Model 2	Model 3	Model 4
BMI	1.49(1.45-1.52)	1.49(1.45-1.52)	1.09(1.06-1.12)	1.13(1.10-1.16)
Regions				
North China		1.00	1.00	
South China		1.68(1.54-1.85)	1.84(1.63-2.08)	NA
Age (years)			2.85(2.73-2.97)	3.40(3.23-3.58)
Ethnic minorities				
Hui				1.00
Zhuang				7.92(6.17-10.17)
Yao				0.64(0.49-0.85)
Kirghiz				0.92(0.64-1.32)
Dong				2.34(1.75-3.12)
Hani				3.40(2.51-4.61)
Naxi (Nakhi)				1.70(1.29-2.52)
Dongxiang				0.90(0.68-1.20)
Li				6.24(4.77-8.17)
Tu				1.00(0.76-1.31)
Qiang				18.62(13.92-24.91)

Legend:

Figure 1 The locations of 11 ethnic minorities sampled in China.

Figure 2 The clustering pattern of age at spermache among boys aged 11 to 18 from 11 ethnic minorities.

Figure 3 The secular trends regarding age at spermache of boys aged 11 to 18 among 11 ethnic minorities from 1995 to 2010.

Figure 1 The locations of 11 ethnic minorities sampled in China.

70x48mm (300 x 300 DPI)

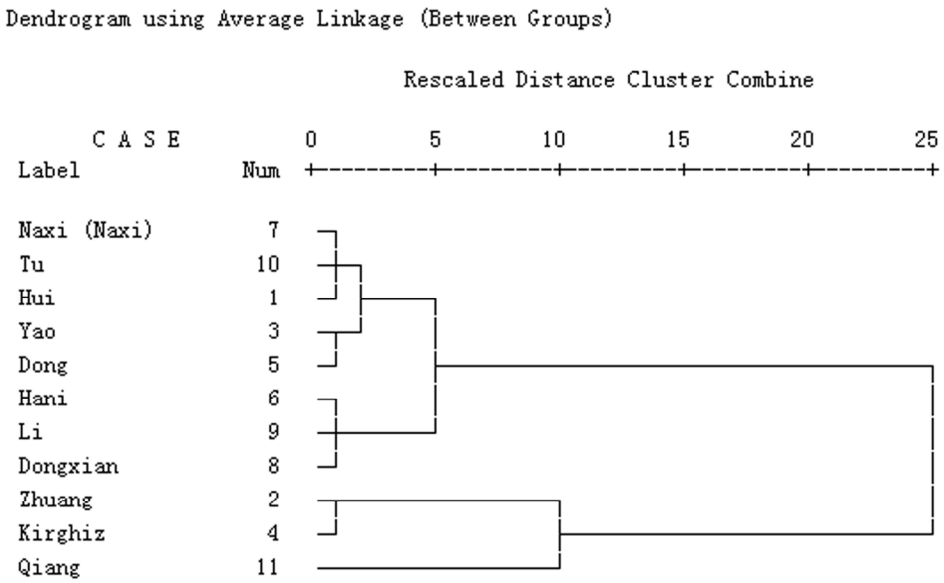

Figure 2 The clustering pattern of age at spermache among boys aged 11 to 18 from 11 ethnic minorities.

73x44mm (600 x 600 DPI)

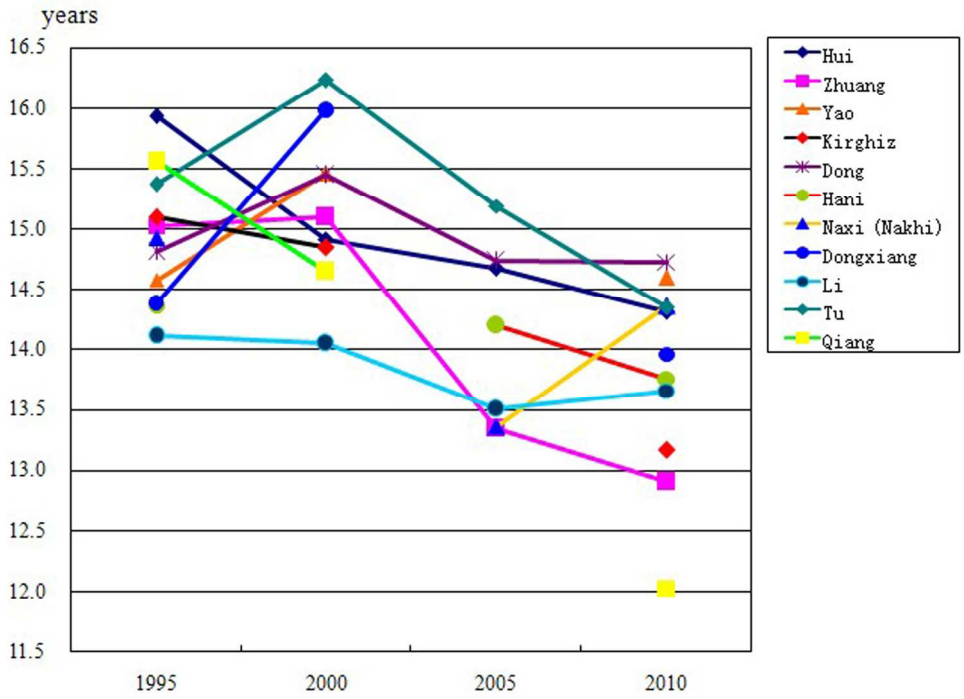

Figure 3 The secular trends regarding age at spermache of boys aged 11 to 18 among 11 ethnic minorities from 1995 to 2010.

89x63mm (600 x 600 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
		Please see page 1, the title and the abstract
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported
		Please see the first two paragraphs of the "Introduction" section.
Objectives	3	State specific objectives, including any prespecified hypotheses
		Please see the last paragraph of the "Introduction" section.
Methods		
Study design	4	Present key elements of study design early in the paper
		Please see the first paragraph of the "Subjects and methods" section.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
		Please see the first paragraph of the "Subjects and methods" section.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		Please see the first paragraph of the "Subjects and methods" section.
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
		Please see the second paragraph of the "Subjects and methods" section.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
		Please see the second and third paragraphs of the "Subjects and methods" section.
Bias	9	Describe any efforts to address potential sources of bias
		Please see the limitation section.
Study size	10	Explain how the study size was arrived at
		Please see the first paragraph of the "Subjects and methods" section.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
		Please see the fourth paragraph of the "Subjects and methods" section.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding

		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses
		Please see the fourth paragraph of the "Subjects and methods" section.
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Please see the first paragraph of the "Subjects and methods" section.
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Please see Table 1.
		(b) Indicate number of participants with missing data for each variable of interest Please see the first paragraph of the "Subjects and methods" section.
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures Please see Table 1.
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Please see Table 2.
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period Please see Table 2 and 3.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Please see Figure 2.
Discussion		
Key results	18	Summarise key results with reference to study objectives Have done in the "Discussion" section.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Please see the limitation section.
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Have done in the "Discussion" section.

Generalisability	21	Discuss the generalisability (external validity) of the study results Have done in the "Discussion" section.
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based We have added the funding information in the "Acknowledgements" section.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Secular trends for age at spermarche among Chinese boys from 11 ethnic minorities, 1995 to 2010: a multiple cross-sectional study

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Secular trends for age at spermarche among Chinese boys from 11 ethnic minorities, 1995 to 2010: a multiple-cross-sectional study

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Short title: Trends of age at spermarche in Chinese minority ethnicities

Key words: Spermarche; Puberty; Body Mass Index; Ethnic minority; boys

Abstract

Objectives: We compared the differences in median age at spermarche among 11 ethnic minorities in 2010, estimated the trends regarding age at spermarche in different ethnic minorities from 1995 to 2010, and explored the association of spermarche with BMI.

Methods: We used four cross-sectional Chinese National Surveys on Students' Constitution and Health (CNSSCH, 1995, 2000, 2005, and 2010), and the total sample size was 40 113 children aged 11-18 years. The median age at spermarche of each ethnic minority was determined by using probit analysis. Logistic regression was used to assess the association of spermarche with BMI.

Results: In 2010, the ethnic minorities with earliest age at spermarche were Qiang (12.03 years), Zhuang (12.91 years) and Kirghiz (13.17 years); the three ethnic minorities with latest age at spermarche were Dong (14.73 years), Yao (14.60 years), and Naxi (14.36 years). From 1995 to 2010, age at spermarche showed a decline in almost each minority groups except Yao and Dong. A higher BMI was associated with an increased likelihood of having reached spermarche after adjusting for age, regions or ethnic minorities.

Conclusion: A large variation in age at spermarche was observed among different ethnic minorities. The age at spermarche showed a downward shift in almost each of the 11 ethnic minorities with different patterns over time, and the children with higher BMI are more likely to enter puberty early.

Key words: Spermarche; Puberty development; Ethnic minority; boys

Strengths and limitations of this study

- Study population was derived from successive national surveys from 1995 to 2010, and the consistent stratified cluster sampling procedure and methods of measurement contributed to the comparability of the data from different survey years.
- This is the first study to describe the trend in decreasing age at spermarche among Chinese ethnic minority boys.
- It is the first to assess the relationship between spermarche and BMI among Chinese ethnic minority boys, showing that a higher BMI was associated with an increased likelihood of having reached spermarche.
- Previous findings concerning the age at spermarche in ethnic minorities are limited, and thus, comparative studies across different countries or regions are not available.

Introduction

Since the timing of puberty onset in childhood is associated with type 2 diabetes, cardiovascular disease, and other adverse outcomes in adulthood, [1] it thus appears to have a profound impact on later health. A better understanding of trends in puberty timing may improve the planning of health services, and provide the potential target for early preventive interventions of those chronic diseases. Although there are many studies about the trend of age at menarche among girls, the data for evaluating secular trends in male pubertal development are limited and insufficient. [2] However, some studies have showed that an earlier onset of puberty occurred among boys. [3-4] Some local surveys in China have also found similar trends. [5-6] However, reports of age at spermarche among Chinese boys have been sparse, and many are outdated or focus only on boys of Han ethnicity. [5-7] Data on the timing of spermarche among ethnic minority boys is not available, and the trends in age at spermarche among ethnic minority boys over the past 15 years are unknown.

Previous studies have shown that nutritional status is an important regulator of puberty, i.e. underweight could delay onset of puberty and reduce pubertal growth spurt whereas overweight or obese children are more likely to enter puberty early. [8-11] A study conducted in Chinese Han ethnicity has also found that a higher BMI is associated with an increased likelihood of having reached spermarche. [7] However, the question as to whether an association between spermarche and BMI exists among ethnic minorities remains unanswered.

The majority of the population in China belongs to the Han ethnic group, and the other 55 ethnic groups are referred to as *ethnic minorities*. Although they are in the minority, the absolute number of an ethnic minority in China may be larger than in any other country or region. [12] According to the sixth Chinese national census, conducted in 2010, 113,792,211 (8.49%) of the country's total population belonged to ethnic minorities. [13] For example, there are now more than 3.5 million Yao people in the world, with approximately 2.8 million living in China, 0.8 million in the Vietnam, and the remainder in other countries, such as Laos, Thailand and the United States. As a multi-ethnic country, China provides a good opportunity to describe the diversity in age at spermarche among different ethnic minorities during the same period.

Previous studies have shown that the estimation of spermarche has been performed successfully in China for many years. [14-15] The age at spermarche in boys can be determined by personal interview, which may be more accurate and convenient than measurement of spermaturia in population-based studies. [14, 16] By using this method, spermarcheal data are collected in the Chinese National Surveys on Students Constitution and Health (CNSSCH), [17-20] which has been conducted every five years using identical methods. As a national sample of school-age children in China, it includes both Han and ethnic minority students and thus provides an opportunity to study the trends regarding age at spermarche among ethnic minorities. The present analysis sought to 1) compare the differences of median age at spermarche among 11 ethnic minorities in 2010; 2) estimate the trends of age at spermarche among different ethnic minorities from 1995 to 2010; and 3) determine any possible association between spermarche and BMI.

Subjects and methods

Subjects

Data were obtained from the 1995, 2000, 2005, and 2010 cycles of the CNSSCH. [17-20] Thus far, it is the largest nationally representative sample of school-aged children in China (more than 200 000 students aged 7- 22 years in each survey point). Twenty of the 55 ethnic minorities were investigated in 1995, but only 11 ethnic minorities with spermarcheal data were available in at least three rounds of survey year. Thus, the present study included only boys aged 11 to 18 years from the following 11 ethnic minorities: Hui, Zhuang, Yao, Kirghiz, Dong, Hani, Naxi, Dongxiang, Li, Tu and Qiang, who were mainly sampled in the Ethnic Minority Autonomous Region or Ethnic Minority Autonomous Prefecture (Figure 1). Because some ethnic minorities did not participate in the CNSSCH in 2000 or in 2005, information concerning the Hani and Naxi was missing in the 2000 CNSSCH, and information concerning the Yao, Kirghiz, Dongxiang and Qiang was missing in the 2005 CNSSCH. The data from the four cycles of CNSSCH were checked for comparability and reliability according to three indicators: (a) all the subjects and their parents were of the same ethnicity origin and had lived in the local areas for at least 1 year; (b) all subjects were uniformly measured in the same year using the same methods and in the same way; (c) all subjects had a thorough medical examination before measurement, and were generally healthy and free from overt disease or physical/mental deformities. The sample sizes of the various ethnic minorities at different survey points ranged from 400 to 2 401 (Table 1); the sample size of each survey ranged from 8 592 to 14734, and the total sample size (all surveys) was 40 113.

The project was approved by the Medical Research Ethics Committee of Peking University Health Science Center (IRB00001052-13082).

Measures

Individual spermarcheal data were collected by the *status quo* method. Boys aged ⩾11 years in each CNSSCH were interviewed face to face by male physicians or professionals and were asked whether or not they had experienced a first ejaculation. Almost all school boys of that age group have some knowledge about male pubertal events from their school health education; moreover, the interviewers were well trained to create a harmonious atmosphere and not to let the boys answer under stress. The interviewers were also prepared to provide sufficient explanation of sperm emission, if necessary, during the interview. Boys were also encouraged to ask questions freely. A dichotomous response (yes/no) was obtained for spermarcheal status. The boys who did not understand sperm emission after explanation, who could not remember their ejaculation history, or who refused to answer questions were regarded as invalid cases and excluded from the study. Boys' ages were recorded and calculated as decimal ages (e.g. 11.00–11.99 years, 12.00–12.99 years).

Height (cm) and weight (kg) were all measured using similar instruments at all survey sites. [17-20] Participants were required to wear only light clothing and stand erect, barefoot, and at ease while being measured. Weight was recorded to the nearest 0.1 kg with a standardized scale and height to the nearest 0.1 cm with a portable stadiometer. Both the scales and stadiometers were calibrated before use. BMI was calculated as body weight

(kg) divided by height (m) squared (kg/m^2). Measurements at the survey site were conducted by a team of field professionals who had passed a training course in anthropometric measurements. The geographic regions were classified as north China and south China, and north China in the present study included Ningxia Hui Autonomous Region, Xinjiang Uyghur Autonomous Region, Gansu and Qinghai, whereas south China included Guangxi Zhuang Autonomous Region, Guizhou, Yunnan, Hainan, and Sichuan.

Statistical analyses

The percentages of boys having reached spermarche in each age group were determined. The median age at spermarche and 95% confidence intervals (CI) in subgroups for different years were calculated using probit analysis. We fitted probit models to the proportion of boys within each age group who had reached spermarche. A cumulative normal curve was fitted to the proportion of boys within each age group who were spermarcheal, and the median age at spermarche was the corresponding age at which 50% of boys in the population were predicted to have reached spermarche. Differences in the percentages of spermarcheal boys among different ethnic minorities in 2010 were compared by using Chi square test, and differences in age at spermarche between 1995 and 2010 survey points were tested by using *Z* test. In order to facilitate the classification of ethnic minorities, age at spermarche among the 11ethnic minorities was examined by cluster analyses. Stepwise logistic regression was used to assess the association between the log odds of being spermarcheal and BMI, geographic regions, age and ethnic minorities in 2010 CNSSCH. A two-sided *P* value < 0.05 was considered significant. All analyses were conducted with SPSS 20.0 (SPSS, Chicago, IL).

Results

Age at spermarche among 11 ethnic minorities in 2010

In 2010, only a few boys (6.54%) were spermarcheal in the 11-year-old group. In the 13-year-old group, almost all ethnic minorities were spermarcheal and the percentage of spermarche among Yao was the lowest (13.33%), while that among Kirghiz was the highest (91.06%) ($\chi^2 = 288.80$, $P = 0.00$). By the age of 18, 98.93% ethnic minority boys were spermarcheal. The median ages at spermarche among the three ethnic minorities with the earliest ages at spermarche in 2010 were as follows: Qiang (12.03 years), Zhuang (12.91 years), and Kirghiz (13.17 years); those with the highest median ages at spermarche were Dong (14.73 years), Yao (14.60 years), and Naxi (14.36 years) (Table 2). The cluster analysis indicated that the 11 ethnic minorities in 2010 could be classified as two groups: Zhuang, Kirghiz and Qiang were classified as the earlier age group of age at spermarche, and the other 8 ethnic minorities belonged to the later age group of age at spermarche (Figure 2).

Secular trends of age at spermarche among 11 ethnic minorities from 1995 to 2010

From 1995 to 2010, the age at spermarche showed a downward shift in almost each of the 11 ethnic minorities, but with different patterns of decline. Some minority groups, such as Hui, Kirghiz, Hani, and Qiang showed a clearly declining trend over time, some minority groups, such as Zhuang, Naxi(Nakhi), Dongxiang, Li, and Tu showed a decreasing trend with upward fluctuations over time, while Yao and Dong showed a relatively flat trend with no statistically significant difference between 1995 and 2010 (Figure 3). As a result, Qiang, Zhuang and Kirghiz showed the largest reductions, with age at spermarche found

to be 3.53 (P = 0.00), 2.12 (P = 0.00) and 1.93 (P = 0.00) years earlier, respectively, in 2010 than in 1995. In contrast, Yao and Dong showed the smallest differences, with age at spermarche only 0.02 (P = 0.74) and 0.08 (P = 0.08) years differences, respectively, between 1995 and 2010 (Table 2).

Association of spermarche with BMI

Table 3 shows that there was an association between age at spermarche and BMI: a higher BMI was associated with an increased likelihood of having reached spermarche after adjusting for age, regions or ethnic minorities. The ethnic minorities who lived in the south of China reached spermarche earlier than those who lived in the north of China after adjusting for BMI and age (OR=1.84).

Discussion

The present study demonstrated large variations in age at spermarche among different ethnic minorities. Zhuang, Kirghiz and Qiang were classified as the earlier age group of age at spermarche, and the other 8 ethnic minorities belonged to the later age group of age at spermarche in 2010. More than half reached spermarche before 13 years among the Zhuang and Qiang boys, whereas less than half had spermarche till 14 years among the Hui, Yao, Dong, Naxi (Nakhi) and Tu boys. Currently, the decreasing age of male sexual maturity runs counter to the delay in the social transition to adulthood that has been documented around the world [22], and one of the consequences is that the early developers are at an increased risk of negative reproductive health outcomes in both adolescence and adulthood. [23-24] Therefore, specific health education on the male puberty

development needs to be developed in order to meet the diverse health requirements among different ethnic minorities in China.

To our knowledge, the present study is the first to describe the trend in decreasing age at spermarche among Chinese ethnic minority boys. We found that the occurrence of a downward secular trend in age at spermarche was evident in almost all of the 11 ethnic minorities, but to different degrees. Globally, research on the trend of male puberty development has varied greatly across countries and regions due to different assessment methods. Some studies used pubarche and testicular enlargement to assess the timing and tempo of puberty. [3-4, 25-26] For example, a study in Poland reported an over 3-month decrease between 2000 and 2010 in the age of initial appearance of pubic hair in boys. [3] In Thailand, the age at testicular enlargement Tanner II declined by 0.15 years/decade (1.8 months/decade) from 1975 to 2012. [4] In the United States, the mean ages of beginning genital and pubic hair growth and early testicular volumes were 6 months to 2 years earlier than in past studies, i.e. HHANES (the Hispanic Health and Examination Survey, 1982-1984) and NHANES III (1988-1992) found attainment of genital stage 2 declined from 12.4 to 10.4 years of age over a 10-year period among Hispanic boys. [25-26] Some studies used age at spermarche as an indicator. [6-7] In China, from 1995 to 2010, age at spermarche dropped from 14.57 to 14.03 years with an average decrease of 4.3 months per decade among Han boys. [7] On the population level, secular trends in the timing of puberty could influence the health of both genders. Several epidemiologic studies in boys showed that the current secular trends in puberty timing seem to be associated with adverse health implications, such as increased risk for testicular cancer and a greater

incidence of conduct and behavioral disorders. [24, 27] However, the mechanisms that determine puberty timing and the reasons for its alterations are not clear. Thus, periodic surveillances of pubertal development, not only age at spermarche but also indicators of pubarche and testicular enlargement and related health consequences or longitudinal tracking between pubertal development and boys' future health are needed.

When compared with Chinese Han boys, the 11 ethnic minority groups in the present study could be subdivided into three categories: 1) ethnic minority boys whose differences in age at spermarche from 1995 to 2010 were larger than Han boys, i.e. Hui, Zhuang, Kirghiz, Hani, Naxi (Nakhi), Tu, and Qiang; 2) ethnic minority boys whose differences in age at spermarche from 1995 to 2010 were smaller than Han boys, i.e. Dongxiang and Li; and 3) ethnic minority boys whose differences in age at spermarche from 1995 to 2010 were non-significant, such as the Yao and Dong. The results are in alignment with many previous studies in China showing that the age of peak of height velocity (PHV) among some ethnic minority boys, such as Hui, Qiang, Zhuang, Kirghiz, and Hani declined over the past years, [16-19, 28-30] and the morphological development level of the Qiang was better than other ethnic minorities. [29]

The pattern of results thus indicated that some ethnic minorities who lived in the same provinces or regions showed similar trends of age at spermarche; e.g. the Hani and Naxi (Nakhi) are both from Yunnan province, and both showed clearly declining trends of age at spermarche. However, not all ethnic minorities showed a significant decline in age at spermarche during the years in question, suggesting that differences in secular trends

among ethnic minorities may well be related to specific features of certain ethnic minority groups. Even among ethnic minorities from the same provinces or regions, large variations in age at spermarche were observed. For example, the Zhuang and Yao are both from Guangxi Zhuang Autonomous Region; however, they showed different patterns concerning age at spermarche trends: Zhuang boys had larger differences regarding average age at spermarche than Han boys, whereas the Yao had no significant differences over the past 15 years.

Considering that growth is a product of a continuous and complex interaction between heredity and the environment, our findings of difference in age at spermarche of minority boys can be attributed to the effects of both hereditary and environmental factors. Environmental factors can be generally divided into two dimensions: socioeconomic (nutrition, disease, income, occupation, family size, social mobility, urbanization, etc.) and ecological (altitude, season, climate, etc.). [14, 31] In the present study, we observed that higher BMI was associated with an earlier spermarche, which is consistent with previous studies showing that earlier maturing boys were heavier than their peers, whereas underweight boys developed puberty later. [7, 9, 32] Moreover, a cohort study also showed that a significant effect of BMI at 7 years on age at sexual maturation in boys; the heavier at age 7 years, the earlier was puberty. [33] This suggests that nutritional strategies and interventions such as the program on improving student nutrition among rural compulsory school students, initiated by the State Council in 2011, [34] may have effects on both children's nutritional status and puberty development. Although ethnic minority children would benefit from this program to a certain extent, long-term nutritional strategies targeting the ethnic minorities should be considered, and health education

focused on the secular trend of male puberty should also be developed correspondingly.

The primary limitation of this study was that only association but not causuality can be inferred, given the cross-sectional design of the CNSSCH. Since the enlargement of testicular volume to 4 ml is considered as the best clinical marker for detecting the onset of puberty in boys, the average age at spermarche might not reflect the exact timing of puberty onset in this sample. . In addition, the actual spermarche dates of boys are unavailable. The *status quo* method — when used in a large study population such as the current sample — is considered to be even more reliable than the recall method for obtaining spermarche dates. Moreover, the same method was used to evaluate the puberty timing across the survey years and the different ethnicities in this study, and these limitations are not likely to affect our conclusions concerning the trends of puberty timing. Further, information concerning environmental factors was not collected in these surveys, and adjustment for such factors may potentially modify our results. The sample size of some ethnic minorities in the present study, such as Yao, may not have been adequate for obtaining reliable estimates. Surveys with large samples and data on important environmental factors are needed to confirm our findings.

Conclusion

Age at spermarche varied widely among different ethnic minorities. From 1995 to 2010, the age at spermarche among Chinese ethnic minority boys declined significantly among 9 of 11 ethnic minorities included in the current study. During that period, seven ethnic minorities showed clearly declining rates, with a decrease of over 4.3 months per decade,

which is a larger decrease than that shown among Han boys. Two ethnic minorities showed decreasing trends and two ethnic minorities showed no significant differences. Among Han boys, increasing BMI was associated with an increased likelihood of being spermarcheal when adjusted for age, regions or ethnic minorities. This suggests that nutritional interventions may also have an effect on children's pubertal development.

Acknowledgements

YS conceived and designed the study, carried out the initial analyses and prepared the first draft of the manuscript. JM, LBL, BD and ZW critically reviewed and revised the manuscript. YS and JM conducted the research and collected the data. AA, ZW and BD interpreted data, developed materials analysis tools and revised the manuscript. All authors read and approved the final manuscript. None of the authors have any conflicts of interest. We thank WK Liao, WH Xing, and X Zhang for their permission concerning accessing the 1995, 2000, 2005 and 2010 Chinese National Survey on Student's Constitution and Health data. We also thank the students who participated in the surveys for their cooperation.

Contributorship Statement: YS conceived and designed the study, carried out the initial analyses and prepared the first draft of the manuscript. JM, LBL, BD and ZW critically reviewed and revised the manuscript. YS and JM conducted the research and collected the data. AA, ZW and BD interpreted data, developed materials analysis tools and revised the manuscript. All authors read and approved the final manuscript.

Competing interests: The authors declared no competing interests.

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Data sharing statement: No additional data are available.

References

1. Day FR, Elks CE, Murray A, Ong KK, Perry JR. Puberty timing associated with diabetes, cardiovascular disease and also diverse health outcomes in men and women: the UK Biobank study. Sci Rep. 2015; 5:11208.
2. Euling SY, Herman-Giddens ME, Lee PA, Selevan SG, Juul A, Sørensen TI, et al. Examination of US puberty-timing data from 1940 to 1994 for secular trends: panel findings. *Pediatrics.* 2008; 121(Suppl. 3), S172-91.
3. Kryst Ł, Kowal M, Woronkowicz A, Sobiecki J, Cichocka BA. Secular changes in height, body weight, body mass index and pubertal development in male children and adolescents in Krakow, Poland. *J Biosoc Sci.* 2012; 44(4):495-507.
4. Jaruratanasirikul S, Sriplung H. Secular trends of growth and pubertal maturation of school children in Southern Thailand. *Ann Hum Biol.* 2014; 18:1-8.
5. Sun Y, Tao F, Su PY; China Puberty Research Collaboration. National estimates of pubertal milestones among urban and rural Chinese boys. *Ann Hum Biol.* 2012; 39(6):461-7.

6. Ma HM, Chen SK, Chen RM, Zhu C, Xiong F, Li T, et al. Pubertal development timing in urban Chinese boys. *Int J Androl*. 2011; 34:e435–45.
7. Song Y, Ma J, Wang HJ, Wang Z, Lau PW, Agardh A. Age at spermarche: 15-year trend and its association with Body Mass Index in Chinese school-aged boys. *Pediatric Obesity*. 2015;
8. Wagner IV, Sabin MA, Pfäffle RW, Hiemisch A, Sergeyev E, Körner A, et al. Effects of obesity on human sexual development. *Nat Rev Endocrinol.* 2012; 8(4):246-54.
9. Tomova A, Robeva R, Kumanov P. Influence of the body weight on the onset and progression of puberty in boys. *J Pediatr Endocrinol Metab.* 2015; 28:859-65.
10. Soliman A, De Sanctis V, Elalaily R. Nutrition and pubertal development. *Indian J Endocrinol Metab.* 2014; 18(Suppl 1):S39-47.
11. Marcovecchio ML, Chiarelli F. Obesity and growth during childhood and puberty. *World Rev Nutr Diet.* 2013; 106:135-41.
12. Song Y, Ma J, Agardh A, Lau PW, Hu P, Zhang B. Secular trends in age at menarche among Chinese girls from 24 ethnic minorities, 1985 to 2010. *Glob Health Action.* 2015, 8:26929.
13. National Bureau of Statistics of China. Communiqué of the National Bureau of Statistics of the People's Republic of China on Major Figures of the 2010 Population Census(No.1). [WWW document]. http://www.stats.gov.cn/tjsj/tjgb/rkpcgb/qgrkpcgb/201104/t20110428_30327.html. 2011.
14. Ji CY, Ohsawa S. Onset of the release of spermatozoa (spermarche) in Chinese male youth. *Am J Hum Biol.* 2000; 12(5):577-587.

15. Ji CY. Age at spermarche and comparison of growth and performance of pre- and post-spermarcheal Chinese boys. *Am J Hum Biol.* 2001; 13(1):35-43.

16. Tinggaard J, Mieritz MG, Sørensen K, Mouritsen A, Hagen CP, Aksglaede L, et al. The physiology and timing of male puberty. *Curr Opin Endocrinol Diabetes Obes.* 2012; 19(3):197-203.

17. CNSSCH. Association. Report on the 1995th National Survey on Students' Constitution and Health. Changchun: Jilin Technical and Science Publication; 1997, pp.3-38. [in Chinese].

18. CNSSCH Association. Report on the 2000th National Survey on Students' Constitution and Health. Beijing: People's Educational Publication; 2002, pp.3-46. [in Chinese].

19. CNSSCH Association. Report on the 2005th National Survey on Students' Constitution and Health. Beijing: China College & University Press; 2007, pp. 3-52. [in Chinese].

20. CNSSCH Association. Report on the 2010th National Survey on Students' Constitution and Health. Beijing: China College & University Press; 2012, pp.4-71. [in Chinese].

21. National Bureau of Statistics of China. The population of each ethnic minority. [WWW document]. http://data.stats.gov.cn/search/keywordlist2?keyword=2010%E5%B9%B4%E4%BA%BA%E5%8F%A3. 2011.

22. Goldstein JR. A secular trend toward earlier male sexual maturity: evidence from shifting ages of male young adult mortality. *PLoS ONE.* 2011; 6(8):e14826.

23. Sabageh AO, Sabageh D, Adeoye OA, Adeomi AA. Pubertal Timing and Demographic Predictors of Adolescents in Southwest Nigeria. *J Clin Diagn Res.* 2015; 9(8):LC11-3.

24. Campbell BC, Prossinger H, Mbzivo M. Timing of pubertal maturation and the onset of sexual behavior among Zimbabwe school boys. *Arch Sex Behav.* 2005; 34(5):505-16.

25. Herman-Giddens ME, Steffes J, Harris D, et al. Secondary sexual characteristics in boys: data from the Pediatric Research in Office Settings Network. *Pediatrics.* 2012; 130: e1058-68.

26. Karpati AM, Rubin CH, Kieszak SM, Marcus M, Troiano RP. Stature and pubertal stage assessment in American boys: the 1988-1994 Third National Health and Nutrition Examination Survey. *J Adolesc Health.* 2002; 30(3):205-12.

27. Golub MS, Collman GW, Foster PM, Kimmel CA, Rajpert-De Meyts E, Reiter EO, et al. Public health implications of altered puberty timing. *Pediatrics.* 2008; 121 Suppl 3:S218-30.

28. Zhang TC. Dynamic analysis of the development of students of minority nationalities in plateau areas between 1985 and 2005. J Physical Education. 2008; 15(3):79-83. [in Chinese]

29. Ma J, Li SS, Song Y, Hu PJ, Zhang B. Study on the changing status of morphological development among minority students in China from1985 to 2005. Chin J Epidemiol. 2009; 30(10):1304-08. [in Chinese]

30. Wang YH, Deng ZH, Zhou GX, Yang CL. Dynamic Analysis of Body Shape Variation of Southwest Minority Adolescents from 1995 to 2010 in the View of

Social Changes. J Guangzhou Sport University.2014; 34(3):52-8. [in Chinese]

31. Malina RM, Bouchard C. Growth, maturation, and physical activity. Champaign, IL: Human Kinetics. 1991. p 229-249, p 329-352.
32. De Leonibus C, Marcovecchio ML, Chiavaroli, de Giorgis T, Chiarelli F, Mohn A. Timing of Puberty and Physical Growth in Obese Children: A Longitudinal Study in Boys and Girls. *Pediatr Obes*. 2014; 9(4):292-9.
33. Aksglaede L, Juul A, Olsen LW, Sørensen TI. Age at puberty and the emerging obesity epidemic. *PLoS One.* 2009; 4(12):e8450.
34. The central People's Government of the People's Republic of China. The inform of Interim Measures on management of special funds targeted on rural compulsory schools to improve student nutrition program issued by Ministry of Finance and Ministry of Education. (WWW document) http://www.gov.cn/gongbao/content/2012/content_2256575.htm.

Table 1 The distribution and sample size of boys aged 11 to 18 among 11 ethnic minorities from 1995 to 2010

Ethnic minorities	Source province/ region	Number (millions) [21 a]	Minority population ranking [a]	1995	2000	2005	2010
Hui	Ningxia Hui Autonomous Region	10.59	2	2401	1600	1564	1744
Zhuang	Guangxi Zhuang Autonomous Region	16.93	1	2391	1595	1271	1449
Yao	Guangxi Zhuang Autonomous Region	2.8	12	1200	801	484	408
Kirghiz	Xinjiang Uyghur Autonomous Region	0.19	31	1200	400	-	963
Dong	Guizhou	2.88	10	1200	766	708	741
Hani	Yunnan	1.66	15	1200	-	863	806
Naxi (Nakhi)	Yunnan	0.33	26	1189	-	799	673
Dongxiang	Gansu	0.62	21	930	1136	-	768
Li	Hainan	1.46	16	1200	784	645	596
Tu	Qinghai	0.29	28	599	800	557	827
Qiang	Sichuan	0.31	27	1224	710	-	921
Total				14734	8592	6891	9896

[a] according to the Sixth National Census in 2010

Table 2 Age at spermarche (95% CI) of boys aged 11 to 18 among 11 ethnic minorities from 1995 to 2010 and compared with Han boys

Ethnic minorities	1995	2000	2005	2010	Difference (1995~2010)
Hui	15.93(15.78-16.08)	14.91(14.48-15.31)	14.67(13.45-15.75)	14.31(13.88-14.72)	-1.62**
Zhuang	15.03(14.84-15.21)	15.10(14.96-15.23)	13.34(11.38-14.09)	12.91(12.56-13.22)	-2.12 **
Yao	14.58(14.38-14.79)	15.46(15.03-15.90)	-	14.60(14.29-14.84)	0.02
Kirghiz	15.10(14.86-15.34)	14.85(14.55-15.22)	-	13.17(12.49-13.75)	-1.93**
Dong	14.81(14.66-14.96)	15.45(15.20-15.74)	14.74(14.46-15.01)	14.73(14.02-15.41)	-0.08
Hani	14.37(14.25-14.49)	-	14.20(13.84-14.54)	13.76(13.63-13.89)	-0.61**
Naxi (Nakhi)	14.93(14.72-15.14)	-	13.36(13.19-13.53)	14.36(13.64-14.95)	-0.57**
Dongxiang	14.39(14.12-14.65)	15.98(15.60-16.38)	-	13.96(11.71-15.46)	-0.43**
Li	14.11(13.88-14.34)	14.05(13.00-15.07)	13.50(12.62-14.11)	13.66(13.01-14.14)	-0.45**
Tu	15.37(15.13-15.62)	16.23(15.62-16.88)	15.19(15.02-15.37)	14.35(13.91-14.77)	-1.02**
Qiang	15.56(15.32-15.80)	14.65(14.40-14.92)	-	12.03(11.20-12.55)	-3.53**
Han [7]	14.57(14.35-14.78)	14.33(14.07-14.58)	14.10(13.79-14.39)	14.03(13.72-14.32)	-0.54*

** $P < 0.01$ there is a significant difference between 1995 and 2010

Table 3 Logistic regression models predicting spermarcheal status from BMI, geographic regions, age and ethnic minorities in 2010 CNSSCH (OR(95% CI))

Variable	Model 1	Model 2	Model 3	Model 4
BMI	1.49(1.45-1.52)	1.49(1.45-1.52)	1.09(1.06-1.12)	1.13(1.10-1.16)
Regions				NA
North China		1.00	1.00	
South China		1.68(1.54-1.85)	1.84(1.63-2.08)	
Age (years)			2.85(2.73-2.97)	3.40(3.23-3.58)
Ethnic minorities				
Hui				1.00
Zhuang				7.92(6.17-10.17)
Yao				0.64(0.49-0.85)
Kirghiz				0.92(0.64-1.32)
Dong				2.34(1.75-3.12)
Hani				3.40(2.51-4.61)
Naxi (Nakhi)				1.70(1.29-2.52)
Dongxiang				0.90(0.68-1.20)
Li				6.24(4.77-8.17)
Tu				1.00(0.76-1.31)
Qiang				18.62(13.92-24.91)

Note: Stepwise logistic regression analyses to determine the association between spermarcheal status and BMI in the first step (Model 1); regions in the second step (Model 2); age in the third step (Model 3); and the association between spermarcheal status and BMI, age and Ethnic minorities in the Model 4.

Legend:

Figure 1 The locations of 11 ethnic minorities sampled in China.

Figure 2 The clustering pattern of age at spermache among boys aged 11 to 18 from 11 ethnic minorities.

Figure 3 The secular trends regarding age at spermache of boys aged 11 to 18 among 11 ethnic minorities from 1995 to 2010.

Figure 1 The locations of 11 ethnic minorities sampled in China.

70x48mm (300 x 300 DPI)

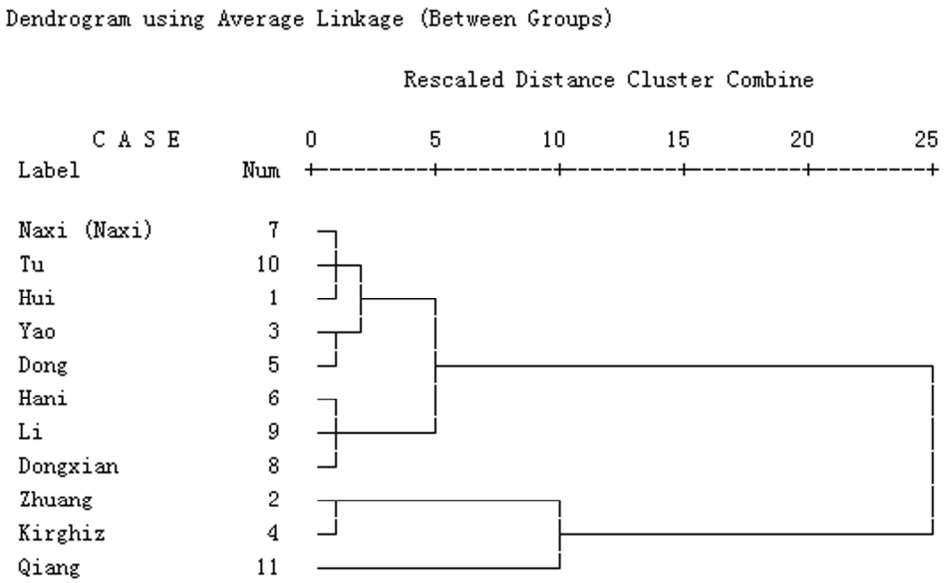

Figure 2 The clustering pattern of age at spermache among boys aged 11 to 18 from 11 ethnic minorities.

73x44mm (600 x 600 DPI)

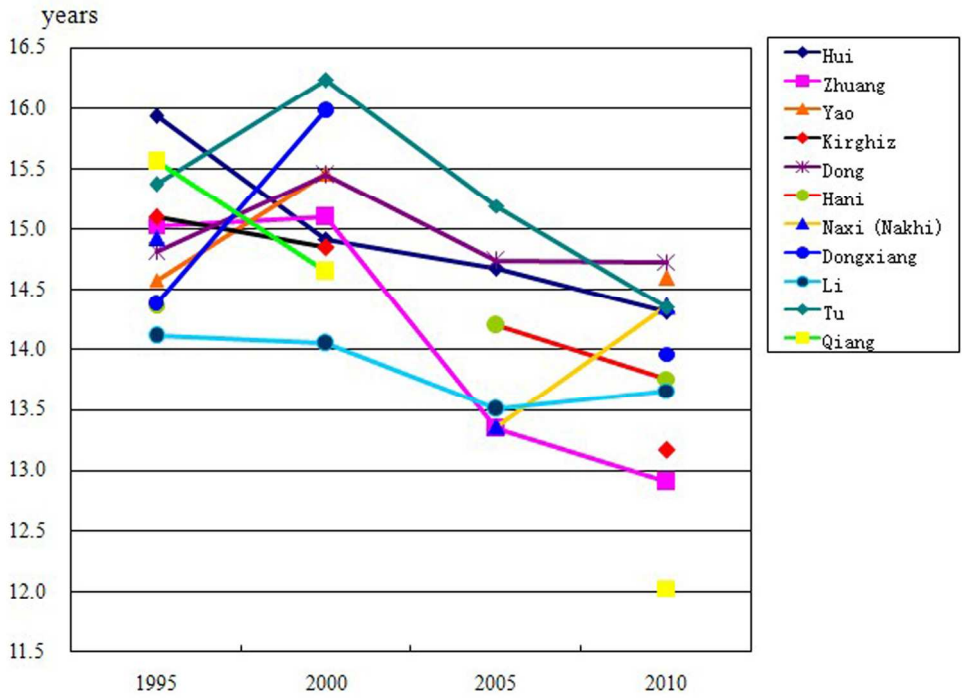

Figure 3 The secular trends regarding age at spermache of boys aged 11 to 18 among 11 ethnic minorities from 1995 to 2010.

89x63mm (600 x 600 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found Please see page 1, the title and the abstract
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported Please see page 5, the first two paragraphs of the "Introduction" section.
Objectives	3	State specific objectives, including any prespecified hypotheses Please see page 6, the last paragraph of the "Introduction" section.
Methods		
Study design	4	Present key elements of study design early in the paper Please see page 7, the first paragraph of the "Subjects and methods" section.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection Please see page 7, the first paragraph of the "Subjects and methods" section.
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up N/A *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls N/A *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants Please see page 7, the first paragraph of the "Subjects and methods" section.
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed N/A *Case-control study*—For matched studies, give matching criteria and the number of controls per case N/A.
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable Please see page 8, the second paragraph of the "Subjects and methods" section.
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group Please see page 8-9, the second and third paragraphs of the "Subjects and methods" section.
Bias	9	Describe any efforts to address potential sources of bias Please see page 15, limitation section in the Discussion.
Study size	10	Explain how the study size was arrived at Please see page 7, the first paragraph of the "Subjects and methods" section.

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why Please see page 9, the fourth paragraph of the "Subjects and methods" section.
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding Please see page 9 the fourth paragraph of the "Subjects and methods" section.
		(*b*) Describe any methods used to examine subgroups and interactions Please see page 9, the fourth paragraph of the "Subjects and methods" section.
		(*c*) Explain how missing data were addressed N/A.
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy N/A.
		(*e*) Describe any sensitivity analyses N/A.
Results		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed Please see page 7, the first paragraph of the "Subjects and methods" section.
		(b) Give reasons for non-participation at each stage N/A.
		(c) Consider use of a flow diagram N/A.
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders Please see page 22, Table 1.
		(b) Indicate number of participants with missing data for each variable of interest N/A.
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) N/A.
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time N/A.
		Case-control study—Report numbers in each exposure category, or summary measures of exposure N/A.
		Cross-sectional study—Report numbers of outcome events or summary measures Please see page 22, Table 1.
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included Please see page 23, Table 2.
		(*b*) Report category boundaries when continuous variables were categorized N/A.
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful

		time period N/A.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses Please see page 27, Figure 2.
Discussion		
Key results	18	Summarise key results with reference to study objectives Please see page 15-16, ~~Have done in~~conclusion section in the "Discussion" ~~section~~.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias Please see page 15, ~~the~~ limitation section in the Discussion~~.~~
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence Please see page 11-16, ~~Have done~~ in the "Discussion" section.
Generalisability	21	Discuss the generalisability (external validity) of the study results N/A.~~Have done in the "Discussion" section.~~
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based Please see page 16, ~~-~~we have added the funding information in the "Acknowledgements" section.

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.