European Review for Medical and Pharmacological Sciences 2017; 21: 3781-3786

Downregulation of SASH1 correlates with poor prognosis in cervical cancer

J. XIE[1], W. ZHANG[1], J. ZHANG[2], Q.-Y. LV[1], Y.-F. LUAN[1]

[1]Department of Obstetrics and Gynecology, Zhongnan Hospital of Wuhan University, Wuhan, Hubei, China
[2]Department of Obstetrics and Gynecology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China

Abstract. – OBJECTIVE: The aim of this study was to analyze the association of SASH1 expression with clinicopathological features and prognosis in patients suffering cervical cancer.

PATIENTS AND METHODS: The expressions of SASH1 mRNA and protein in cervical cancer tissues and matched normal cervical tissues were detected by Real-time PCR and Immunohistochemistry. Based on the above findings, the association among SASH1 expression and clinicopathological features was analyzed. Overall survival was evaluated using the Kaplan-Meier method. The variables were used in univariate and multivariate analysis by the Cox proportional hazards model.

RESULTS: The results demonstrated that both SASH1 mRNA and proteins were downregulated in cervical cancer tissues compared with those in matched normal tissues (both $p < 0.05$). Also, decreased SASH1 expression in cervical cancer was found to be significantly associated with high FIGO Stage ($p = 0.001$), lymph nodes metastasis ($p = 0.003$) and differentiation ($p = 0.018$). Furthermore, Kaplan-Meier analysis demonstrated that low SASH1 expression level was associated with poorer overall survival ($p < 0.01$). Univariate and multivariate analyses indicated that status of SASH1 was an independent prognostic factor for patients with cervical cancer.

CONCLUSIONS: These findings suggested that SASH1 can be useful as a new prognostic marker and therapeutic target in cervical cancer patients.

Key Words:
SASH1, Cervical cancer, Prognosis.

Introduction

Cervical cancer is the fourth most common cancer after breast, colorectal, and lung cancer in women with about 132,000 new cases in China per year[1,2]. The treatment can achieve a 5-year overall survival rate of 67% with the advancement in therapeutics[3]. However, current therapeutics, including surgery, radiation, and chemotherapy, show limited effectiveness for advanced invasive cervical cancer[4,5]. Predicting prognosis of patients would be of help in the treatment. Clinical factors, such as size, stage, and lymph node metastasis, may be used for prognostic markers, but their relatively low specificity cannot accurately predict survival[6]. Thus, persistent effort is needed to find novel and efficient molecular markers, which can predict tumor progression and guide clinical outcome.

The SAM and SH3 domain containing 1 (SASH1) gene, which includes 22 exons and 21 introns, is located at the chromosomal locus 6q24.3[7]. SASH1 is a member of the SLY-family of signal adapter proteins, encodes a protein containing sterile α motif (SAM) and Src homology domain 3 (SH3), which is required for protein-protein interaction and mediates the formation of signaling complexes[8-10]. Recent studies indicated that SASH1 served as a tumor suppressor gene in various tumors. For instance, He et al[11] reported that forced expression of SASH1 suppressed the metastatic process of hepatocarcinoma cells through suppressing the sonic hedgehog signaling pathway. Ren et al[12] showed that SASH1 overexpression suppressed proliferation and migration of ovarian carcinoma cells, and its low expression was associated with poor prognosis in ovarian carcinoma patients. In line with these studies, a previous study[13] showed a down-regulated expression of SASH1 in cervical cancer, suggesting that SASH1 may play a negative regulator in cervical cancer. However, the prognostic significance of SASH1 in cervical cancer has not been reported.

In the present study, we collected cervical cancer patients from our hospital and detected the expression levels of SASH1 protein and mRNA

Corresponding Author: Wei Zhang, MD; e-mail: zwow03@yeah.net

in cervical cancer tissues. Also, we analyzed the association between SASH1 expression and clinical features and prognosis. To our best knowledge, this is the first study about the prognostic significance of SASH1 in cervical cancer.

Patients and Methods

Clinical Specimens

Patients who had received operation for cervical cancer at the Zhongnan Hospital of Wuhan University between 2008 and 2011 were recruited with written informed consent. None of the patients had undergone previous treatment including radiation or chemotherapy. Tumor tissues were obtained and stored immediately in liquid nitrogen after surgical resection. The diagnosis of tissue samples was approved by pathologists. The determination of the clinical stage was performed according to the International League of Gynecology and Obstetrics (FIGO). Patients' characteristics are described in Table I. Prior informed consent was obtained from all patients, and the study was approved by the Ethics Committee of Zhongnan Hospital of Wuhan University (Wuhan, Hubei, China).

Quantitative Real-time PCR

Total RNA was isolated from cervical cancer tissue and adjacent normal tissue using the Trizol Total RNA Reagent (Invitrogen, Carlsbad, CA, USA). cDNA was generated from 500 ng of total RNA using PrimeScript™ RT Master Mix Perfect Real-time (TaKaRa, Dalian, China). Quantitative Real-time polymerase chain reactions were performed with 1 μL of cDNA and SYBR Green Real-time PCR Master Mix (TaKaRa, Dalian, China). Reactions were performed in triplicate using $2^{-\Delta\Delta Ct}$ method. GADPH was used as an endogenous control. The primer sequences were as follows: GADPH: 5'-GTCAACGGATTTGGTCTGTATT-3' (forward), 5'-AGTCTTCTGGGTGGCAGTGAT-3' (reverse); SASH1: 5'- CGGGAAAGCGTC AAGTCG GA -3' (forward), 5'-ATCTCCTTTCTTGAG CTTGAG-3' (reverse).

Immunohistochemistry (ICH)

Protein expression was assayed by immunohistochemistry using the paraffin-embedded sections from the patients with cervical cancer. The slides were firstly deparaffinized in xylene and rehydrated in a graded alcohol series and the endogenous peroxidase activity was blocked with 3% H_2O_2. Next, the slides were treated with 1% bovine serum albumin (BSA) for 30 min to block nonspecific reactions. After the cells were washed with phosphate buffered saline (PBS) three times, they were incubated for 1 h in the dark with SASH1-conjugated secondary anti-rabbit antibodies (Invitrogen, Carlsbad, CA, USA). After washing the sections, peroxidase-labeled polymer and substrate-chromogen solutions were used to visualize stained proteins of interest. Negative controls were

Table I. Association of SASH1 expression with different clinicopathological features of cervical cancer patients.

Variable	Number	SASH1 expression		*p*-value
		Low	High	
Age (years)				0.492
< 60	51	23	28	
≥ 60	78	40	38	
Tumor size (cm)				0.515
< 3	72	37	35	
≥ 3	57	26	31	
Histologic type				0.382
Squamous cell	79	41	38	
Adenoma	50	22	28	
Differentiation (grade)				0.036
1/2	97	41	56	
3	32	22	10	
Lymph nodes metastasis				0.003
No	96	34	52	
Yes	43	29	14	
FIGO Stage				0.001
Ib-IIa	82	31	51	
IIb-IIIa	47	32	15	

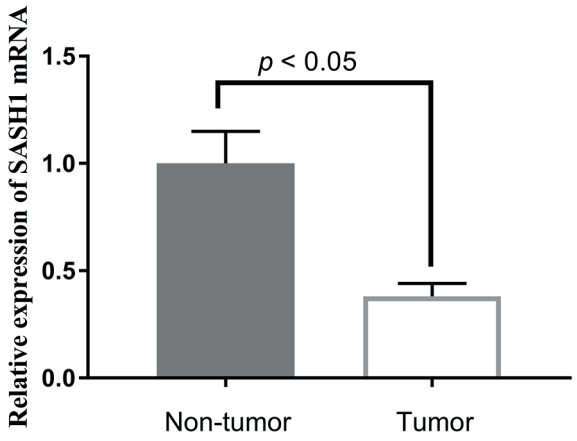

Figure 1. Relative expression of SASH1 mRNA in cervical cancer tissues and matched non-tumor normal tissues were examined by qRT-PCR.

performed by PBS replaced SASH1 antibody during the primary antibody incubation. Slides were examined by two investigators in an independent and random manner. For each tissue sample, the intensity and the proportion of stained tumor cells were recorded. Immunohistochemical grading was performed using the following scoring system, which was described in previous study[14].

Statistical Analysis

The results were analyzed using SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). The statistical significance of differences between two groups was calculated using the unpaired Student's *t*-test. Fisher's exact probability test was used to assess the correlation between SASH1 expression levels and clinicopathological characteristics. Both Kaplan-Meier method and a log-rank test were implemented to determine the significant difference in overall survival of patients. Cox multivariate proportional hazards model was applied to analyze the survival variables. All *p*-values were 2-sided and statistical significance was determined at $p < 0.05$.

Results

Protein Expression of SASH1 mRNA and Protein in Human Cervical Cancer Tissues

The expression of SASH1 mRNA in cervical cancer tissues and adjacent tissues were analyzed by qRT-PCR. The results were shown in Figure 1; we found that SASH1 mRNA expression in cervical cancer tissues was significantly downregulated compared with that in adjacent tissues ($p < 0.05$). Then, we analyzed cervical cancer tissues and normal cervical tissues by ICH. Representative photomicrographs of SASH1 immunostaining are shown in Figure 2. It was observed that 48.8% (63/129) of the tumor tissue samples showed low SASH1 expression and 51.2% (66/129) showed high expression. In contrast, all of the normal cervical tissue showed strong SASH1 expression.

Relationship Between SASH1 Expression and Clinicopathological Characteristics

Next, we analyzed the association between the SASH1 expression and various clinicopatholo-

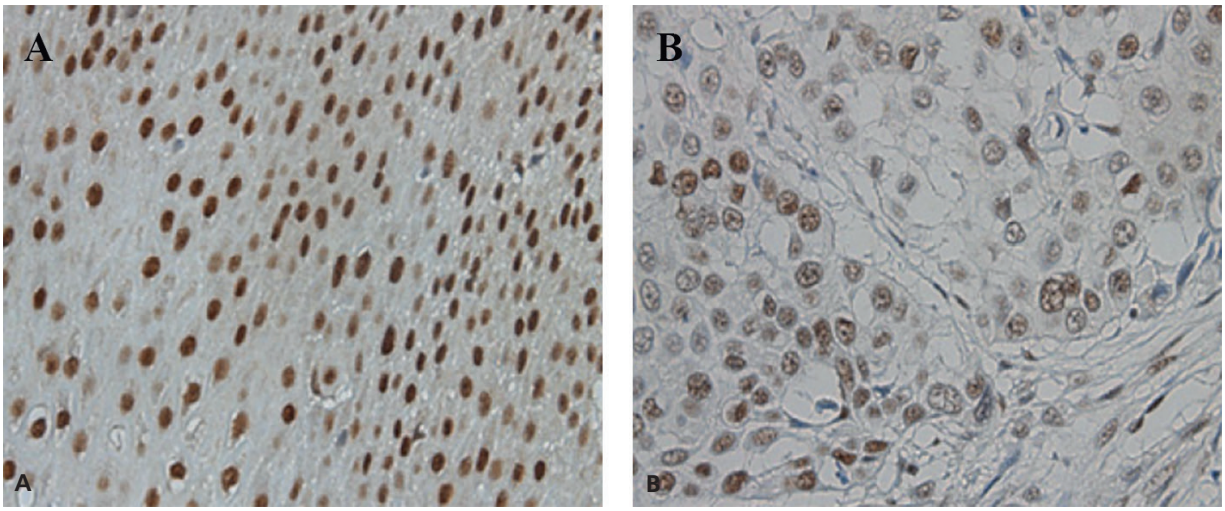

Figure 2. IHC staining for SASH1 protein expression in cervical cancer tissues and matched non-tumor normal tissues. ***A,*** High SASH1 expression in normal tissues. ***B,*** Low SASH1 expression in cervical cancer tissues.

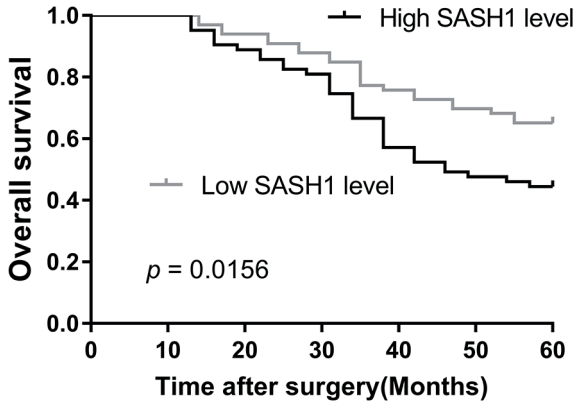

Figure 3. Overall survival curve of cervical cancer patients with different SASH1 expression. The patients with a lower expression of SASH1 had a lower survival time compared with those with a higher expression of SASH1 ($p = 0.0156$).

gical factors of the cervical cancer patients. As shown in Table I, our results indicated that the decreased expression of SASH1 in cervical cancer was positively associated with high FIGO Stage ($p = 0.001$), lymph nodes metastasis ($p = 0.003$) and differentiation ($p = 0.018$). However, there was no association between SASH1 expression and other clinicopathologic characteristics (all $p > 0.05$). These data suggested that SASH1 might play an important role in the progression of cervical cancer.

Prognostic Values of SASH1 Expression in Cervical Cancer

The Kaplan-Meier analysis was performed to show the relationship between patient survival and the expression of SASH1. As shown in Figure 3, we observed that SASH1 expression was significantly associated with overall survival of cervical cancer patients ($p = 0.0156$). Next, we performed univariate and multivariate analysis to identify the predictors of overall survival. The results were shown in Table II, in univariate analysis, differentiation ($p = 0.0018$), lymph nodes metastasis ($p = 0.008$), FIGO Stage ($p = 0.005$) and low SASH1 expression ($p = 0.006$) were significant predictive factors for poor outcome. Furthermore, in multivariate analysis, SASH1 expression level was proved to be independent prognosis factors for cervical cancer ($p = 0.009$).

Discussion

Cervical cancer remains a major public health problem, specifically in advanced cases[15]. In clinical practice, favorable prognosis and increased long-term survival time have been proven to be correlated with early diagnosis and precise therapy in cervical cancer[16]. Recent studies[17] have demonstrated that some abnormal molecular biology changes may predict the prognosis of tumor patients. For instance, Kim et al[18] reported that high OCT4 protein expression showed worse overall survival rates when compared to the low-expression group. Wang et al[19] found that high FABP5 expression was significantly correlated with lymph node metastasis, lymphovascular space invasion and poor overall survival time in cervical cancer. In the present study, we focused on SASH1.

As a member of the SH3-domain containing expressed in lymphocytes (SLY1) gene family, which is involved in various cellular signal pathway, SASH1 plays an important role in development and progression of several tumors[20]. For example, Joshua et al[21] reported that down-regulation of SASH1 was observed in breast cancer tissues and cell line, and high SASH1 expression is an independent marker of favorable prognosis in breast cancer. Sun et al[22] found that forced SASH1 expression suppressed proliferation and EMT of

Table II. Univariate and multivariate Cox regression analyses for overall survival.

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p	HR (95% CI)	p
Age (< 60 *vs.* ≥ 60)	1.341 (0.522-1.892)	0.371	-	-
Tumor size (< 3 *vs.* ≥ 3)	1.763 (0.894-2.239)	0.129	-	-
Histologic type (squamous cell *vs.* adenoma)	1.523 (0.781-1.994)	0.291	-	-
Differentiation (grade) (1/2 *vs.* 3)	2.521 (1.223-4.239)	0.018	2.198 (1.033-3.781)	0.021
Lymph nodes metastasis (no *vs.* yes)	3.132 (1.871-5.441)	0.008	2.761 (1.483-4.356)	0.011
FIGO Stage (Ib-IIa *vs.* IIb-IIIa)	3.872 (1.772-6.821)	0.005	3.213 (1.482-5.661)	0.007
SASH1 expression (low *vs.* high)	2.562 (1.569-4.239)	0.006	2.139 (1.228-3.519)	0.009

thyroid cancer cells through PI3K/Akt signaling pathway. For cervical cancer, a previous study by Chen et al[13] indicated that overexpression of SASH1 suppressed cervical cancer cell proliferation and invasion by suppressing the FAK pathway. However, there are no reports on prognosis value of SASH1 in cervical cancer.

In the present study, we found that SASH1 protein and mRNA were down-regulated in cervical cancer tissues compared with these in matched normal tissues. This data was in line with a previous study. Also, we found that decreased SASH1 expression in cervical cancer tissues was significantly correlated with aggressive clinicopathological features. Furthermore, the Kaplan-Meier analysis and log-rank tests showed that patients with lower SASH1 expression levels had dramatically shorter overall survival than that observed in those with higher levels. Further, multivariate Cox analysis confirmed that low SASH1 expression was an independent poor prognostic factor for long-term outcome in cervical cancer patients.

Conclusions

Our study suggested that SASH1 may be a powerful marker to predict the prognosis of cervical cancer patients. However, further studies are required to elucidate underlying mechanisms.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Sartor F, Anderson J, McCaig C, Miedzybrodzka Z, Müller B. Mutation of genes controlling mRNA metabolism and protein synthesis predisposes to neurodevelopmental disorders. Biochem Soc Trans 2015; 43: 1259-1265.
2) Testa A, Giannuzzi R, Sollazzo F, Petrongolo L, Bernardini L, Daini S. Psychiatric emergencies (part I): psychiatric disorders causing organic symptoms. Eur Rev Med Pharmacol Sci 2013; 1: 55-64.
3) Yeargin-Allsopp M, Boyle C. Overview: the epidemiology of neurodevelopmental disorders. Ment Retard Dev Disabil Res Rev 2002; 8: 113-116.
4) Mullin AP, Gokhale A, Moreno-De-Luca A, Sanyal S, Waddington JL, Faundez V. Neurodevelopmental disorders: mechanisms and boundary definitions from genomes, interactomes and proteomes. Transl Psychiatry 2013; 3: e329.
5) Picker JD, Walsh CA. New innovations: therapeutic opportunities for intellectual disabilities. Ann Neurol 2013; 74: 382-390.
6) Kendler KS. A history of the DSM-5 scientific review committee. Psychol Med 2013; 43: 1793-1800.
7) Moeschler JB. Genetic evaluation of intellectual disabilities. Semin Pediatr Neurol 2008; 15: 2-9.
8) Moeschler JB. Medical genetics diagnostic evaluation of the child with global developmental delay or intellectual disability. Curr Opin Neurol 2008; 21: 117-122.
9) Salvador-Carulla L, Reed GM, Vaez-Azizi LM, Cooper SA, Martinez-Leal R, Bertelli M, Adnams C, Cooray S, Deb S, Akoury-Dirani L, Girimaji SC, Katz G, Kwok H, Luckasson R, Simeonsson R, Walsh C, Munir K, Saxena S. Intellectual developmental disorders: towards a new name, definition and framework for "mental retardation/intellectual disability" in ICD-11. World Psychiatry 2011; 10: 175-180.
10) Silva PA, Williams S, McGee R. A longitudinal study of children with developmental language delay at age three: later intelligence, reading and behaviour problems. Dev Med Child Neurol 1987; 29: 630-640.
11) Shetty P. Speech and language delay in children: a review and the role of a pediatric dentist. J Indian Soc Pedod Prev Dent 2012; 30: 103-108.
12) Coplan J, Gleason JR, Ryan R, Burke MG, Williams ML. Validation of an early language milestone scale in a high-risk population. Pediatrics 1982; 70, 677-683.
13) Graham SA, Fisher SE. Decoding the genetics of speech and language. Curr Opin Neurobiol 2013; 23: 43-51.
14) Bitsika V, Sharpley CF, Andronicos NM, Agnew LL. Prevalence, structure and correlates of anxiety-depression in boys with an autism spectrum disorder. Res Dev Disabil 2016; 49-50: 302-311.
15) Ramocki MB, Zoghbi HY. Failure of neuronal homeostasis results in common neuropsychiatric phenotypes. Nature 2008; 455: 912-918.
16) Scouarnec LS, Gribble SM. Characterising chromosome rearrangements: recent technical advances in molecular cytogenetics. Heredity (Edinb) 2012; 108: 75-85.
17) Shaffer LG; American College of Medical Genetics Professional Practice and Guidelines Committee. American College of Medical Genetics guideline on the cytogenetic evaluation of the individual with developmental delay or mental retardation. Genet Med 2005; 7: 650-654.
18) Flint J, Knight S. The use of telomere probes to investigate submicroscopic rearrangements associated with mental retardation. Curr Opin Genet Dev 2003; 13: 310-316.
19) Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Sche-

rer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 2010; 86: 749-764.

20) Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, Devon RS, St Clair DM, Muir WJ, Blackwood DH, Porteous DJ. Disruption of two novel genes by a translocation co- segregating with schizophrenia. Hum Mol Genet 2000; 9: 1415-1423.

21) Chelly J, Tümer Z, Tønnesen T, Petterson A, Ishikawa-Brush Y, Tommerup N, Horn N, Monaco AP. Isolation of a candidate gene for Menkes disease that encodes a potential heavy metal binding protein. Nat Genet 1993; 3: 14-19.

22) Cordoba M, Andriole S, Evans SM, Britt D, Chu Lam M, Evans MI. Integrating microarrays into routine prenatal diagnosis: determinants of decision making. Fetal Diagn Ther 2016; 40: 135-140.

23) Cooper GM, Coe BP, Girirajan S, Rosenfeld JA, Vu TH, Baker C, Williams C, Stalker H, Hamid R, Hannig V, Abdel-Hamid H, Bader P, McCracken E, Niyazov D, Leppig K, Thiese H, Hummel M, Alexander N, Gorski J, Kussmann J, Shashi V, Johnson K, Rehder C, Ballif BC, Shaffer LG, Eichler EE. A copy number variation morbidity map of developmental delay. Nat Genet 2011; 43: 838-846.

24) Hochstenbach R, van Binsbergen E, Engelen J, Nieuwint A, Polstra A, Poddighe P, Ruivenkamp C, Sikkema-Raddatz B, Smeets D, Poot M. Array analysis and karyotyping: workflow consequences based on a retrospective study of 36,325 patients with idiopathic developmental delay in the Netherlands. Eur J Med Genet 2009; 52: 161-169.

25) Vissers LE, de Vries BB, Veltman JA. Genomic microarrays in mental retardation: from copy number variation to gene, from research to diagnosis. J Med Genet 2010; 47: 289-297.

26) Yao F, Ariyaratne PN, Hillmer AM, Lee WH, Li G, Teo AS, Woo XY, Zhang Z, Chen JP, Poh WT, Zawack KF, Chan CS, Leong ST, Neo SC, Choi PS, Gao S, Nagarajan N, Thoreau H, Shahab A, Ruan X, Cacheux-Rataboul V, Wei CL, Bourque G, Sung WK, Liu ET, Ruan Y. Long span DNA paired-end-tag (DNA-PET) sequencing strategy for the interrogation of genomic structural mutations and fusion-point-guided reconstruction of amplicons. PLoS One 2012; 7: e46152.

27) Fullwood MJ, Wei CL, Liu ET, Ruan Y. Next-generation DNA sequencing of paired-end tags (PET) for transcriptome and genome analyses. Genome Res 2009; 19: 521-532.

28) Korbel JO, Urban AE, Affourtit JP, Godwin B, Grubert F, Simons JF, Kim PM, Palejev D, Carriero NJ, Du L, Taillon BE, Chen Z, Tanzer A, Saunders AC, Chi J, Yang F, Carter NP, Hurles ME, Weissman SM, Harkins TT, Gerstein MB, Egholm M, Snyder M. Paired-end mapping reveals extensive structural variation in the human genome. Science 2007; 318: 420-426.

29) Kidd JM, Cooper GM, Donahue WF, Hayden HS, Sampas N, Graves T, Hansen N, Teague B, Alkan C, Antonacci F, Haugen E, Zerr T, Yamada NA, Tsang P, Newman TL, Tüzün E, Cheng Z, Ebling HM, Tusneem N, David R, Gillett W, Phelps KA, Weaver M, Saranga D, Brand A, Tao W, Gustafson E, McKernan K, Chen L, Malig M, Smith JD, Korn JM, McCarroll SA, Altshuler DA, Peiffer DA, Dorschner M, Stamatoyannopoulos J, Schwartz D, Nickerson DA, Mullikin JC, Wilson RK, Bruhn L, Olson MV, Kaul R, Smith DR, Eichler EE. Mapping and sequencing of structural variation from eight human genomes. Nature 2008; 453: 56-64.