

## Sexy Small Copy Numbers in Hereditary Gastric Carcinogenesis

Hidetaka Yamada<sup>1</sup>, Hiromi Sakamoto<sup>2</sup> and Haruhiko Sugimura<sup>1\*</sup>

<sup>1</sup>Hamamatsu University School of Medicine, Shizuoka, Japan

<sup>2</sup>National Cancer Center Research Institutes, Tokyo, Japan

\*Corresponding author: Haruhiko Sugimura, Hamamatsu University School of Medicine, Shizuoka, Japan, Tel: +81-53-435-2220; Fax: +81-53-435-2225; E-mail: [hsugimur@hama-med.ac.jp](mailto:hsugimur@hama-med.ac.jp)

Received date: June 16, 2014, Accepted date: July 17, 2014, Published date: July 25, 2014

Copyright: © 2014 Yamada H, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Commentary

Occurrence of gastric cancer in a family has been known for many years, and since the discovery of germline mutations of CDH1 in Maori families [1], the entity of hereditary diffuse gastric cancer (HDGC) (OMIM #137215) has been almost established. The guidelines of diagnosis and management have been regularly updated [2-5]. In many countries including Japan, where gastric cancer is endemic, the search and surveillance of the family has been continued. Reviewing the publications for the past two decades, the progress is not rapid, but considerably steady. New mutations are coming out from many countries every few months. The cases do not always fulfill the criteria of age and family histories; some occurred in the older patients [6], and the others occur in the patients without any family history [7-9]. The reports on the related diseases, lobular carcinoma of the breast [10], prostate cancer [11], colorectal cancer [12], and cleft lips [13,14] are also among the literatures.

I would like to draw the readers' attention to four reports recently published in the journals related to gastroenterology. They are on the germline alterations of CDH1 copy numbers in diffuse hereditary gastric cancer. With an introduction of Multiplex ligation-dependent probe amplification (MLPA) [15] method to CDH1 germline test, Oliveira et al. [16] identified exon-size CDH1 deletions in 6 probands. The sizes of the deletions encompassed 5'UTR-exon1 (1 family), exon1 to exon2 (3 families), exon14 to exon16 (1 family), and exon16 (1 family) of the CDH1. Next, we reported a case of exon3 deletion in Japanese family [17]. Japan has been notorious for being endemic with gastric cancer and a pursuit for HDGC was difficult because the application of the consensus guidelines to recommend genetic test pick up too many cases. The pessimism has been rampant on the prevalence of CDH1 germline changes in Japanese familial gastric cancer for the last 15 years especially in the community of clinicians [18]. The improved detection methods, easier sequencing (no more single-strand conformation polymorphism) covering whole exons and MLPA, re-motivated the attending clinicians such as endoscopists, surgeons, and attending pathologists to refer to genetic test labs when they fell any peculiarities in cases. The case referred to us 2 years after the first publication of a Japanese copy number deletion case was prominent. The multiple signet ring cell carcinoma occurred in a 30's without any family history of gastric cancer. Since the attending pathologists noticed that the features of the case resembled to the ones of HDGC [19], they asked us on testing for them. The sequencing of the whole exons of CDH1 and MLPA analysis was performed. The exon 11 of CDH1 was deleted in the germline of this proband and in his son. De novo occurrence of this deletion was confirmed by showing absence of it in his parents. Importantly his son succeeded it from his father [20] and surveillance program was considered. Just after this report, another very impressive case was reported from

National Cancer Center Hospital, Tokyo appeared [21]. They applied array-comparable genomic hybridization (CGH) technology (Agilent Technologies, Santa Clara, CA) to the cases (the proband and his mother, a 56 years old post operative recurrence case) and discovered 275 kb deletion encompassing exon 7 to 16 of CDH1, a largest deletion in HDGC so far demonstrated (Table 1).

Authors	Ethnic origin	Cancer type	Copy number changes Ordered by the exons of CDH1
Oliviera et al. (16)	Southern European	HDGC	Deletion of 5'-UTR-exon 1
Oliviera et al. (16)	Northern European	HDGC	Deletion of exon 1-2
Oliviera et al. (16)	Canadian	HDGC	Deletion of exon 1-2
Oliviera et al. (16)	Eastern European	HDGC	Deletion of exon 1-2
Yamada H et al. (17)	Japanese	HDGC	Deletion exon 3
Benusiglio et al. (10)	French	LBC	Deletion exon 3
Yamada M et al. (21)	Japanese	HDGC	Deletion of exon 7-16
Sugimoto et al. (20)	Japanese	Early-onset DGC	Deletion of exon 11
Benusiglio et al. (10)	French	HDGC	Deletion of exon 11
Oliviera et al. (16)	Central European	HDGC	Deletion of exon 14-16
Oliviera et al. (16)	Central European	HDGC	Deletion of exon 16

**Table 1:** Summary of the cases with germline copy number change in CDH1 gene; HDGC: hereditary diffuse gastric cancer; DGC: diffuse gastric cancer; LBC: lobular breast carcinoma

The lesson we experienced is obvious. Our methods are still insufficient to recover all the deleterious genetic alterations from the subjects who daily visit the clinic; limitation of the access, lack of sharing the knowledge, and insufficient resource in genetics including history takers and counselors are hurdles in practical settings, even in Japan, where complete national health insurance coverage is famous. Insufficiency in our methods will be continued in front of us as to

identification of the genes besides CDH1, which are responsible for familial gastric cancer, more than 50% of them.

Usages of next generation sequencing (NGS) would be an obvious option for the institutes where the instruments and fund are available. In the recent American Association Cancer Research Annual Meeting in San Diego, two posters presented the first results of exome analyses. Identification of novel susceptibility genes in familial gastric cancer using next generation sequencing and identity-by-descent mapping; Genetic basis of hereditary gastric cancer: Beyond the CDH1 locus). Both and another Japanese group adopted whole exome analysis approaches, and we are now watching their validation and replication processes. Whole exome sequence (WES) will provide other gene mutations. Actually CTNNA1 mutation was identified using exome analysis [22] in a hereditary gastric cancer family. CTNNA1, a candidate gene associated with CDH1 in signal transduction, was not changed in another cohort of hereditary gastric cancer family [23]. This may cool-down our hyper-expectation on NGS (exactly WES) to identify new responsible genes. The genes found by WES may be sporadic or anecdotal ones.

Like the history of searches for CDH1 changes in Japan, the copy number changes in other gene loci would be possible culprit for familial occurrence of gastric cancer. Understanding the human

genome variations [24] showed us there are numerous copy number variations in human genome. The copy numbers variations, from amylases to glutathione-S-transferases, are related to from our evolutionally fixed eating styles to inter-individual differences in xenobiotics adaptations, but the true significance of the most of them awaits further investigation. Inferring these genetic changes to the association of any disease require robust replication and control study. Actually some of the single nucleotide variations are found in our “control” DNAs (Table 2), thus the analyses of the “robust” controls are necessary. On the other hand some deletions (1-30 kb, small exonic CNVs) have been revealed to be responsible autism disorder [25]. Gastric cancer like autism spectrum disorders is relatively common, and occurs in modestly earlier stage of human life. It is obviously a very environmental disease according to the immigrant studies and the trends of the incidence observed in the past decades. Several platforms to survey the genome-wide alterations of copy numbers including NGS became available in genetic test labs for clinical use to detect large, moderately large, and exonic size deletions in the genome. When these platforms become more popular and economically feasible, the more numbers of endoscopists in practice are encouraged to refer any strange cases, such as early-onset, multiplicity, signet ring cell type, and of course, familial aggregation.

Nucleotide change	Amino acid change	Minor Allele Count	MAF	dbSNP ID	1000 Genomes*	
					Minor Allele Count	MAF
c.546A>C	p.Lys182Asn	1	0.003	rs201141645	1	0
c.2494G>A	p.Val832Met	2	0.005	rs35572355 (“pathogenic allele”)	2	0.001
Duplication of exon 11	unknown	2	0.005	NA	NA	NA

**Table 2:** Single nucleotide variations of CDH1 gene found in 189 healthy aged Japanese controls (Yamada H et al. unpublished results) MAF: Minor allele frequency; NA: not available \*<http://www.1000genomes.org/announcements/may-2011-data-release-2011-05-12>

At this moment, a greater question how this initiating genetic change brings about a full-blown gastric cancer remains to be investigated. Genetic and epigenetic changes at CDH1 locus are known, but the precise profile of the consequence in this particular disease, not like in common gastric cancer [26], awaits further investigation. The mouse model [27] and omics analysis including tumor microenvironment [28] may provide the clue.

## Acknowledgement

We greatly appreciate the grants from the Ministry of Health, Labour and Welfare (21-1, 10103838), from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) (221S0001), and from the Smoking Research Foundation.

## References

- Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, et al. (1998) E-cadherin germline mutations in familial gastric cancer. *Nature* 392: 402-405.
- Caldas C, Carneiro F, Lynch HT, Yokota J, Wiesner GL, et al. (1999) Familial gastric cancer: overview and guidelines for management. *J Med Genet* 36: 873-880.
- Brooks-Wilson, Kaurah AR, Suriano P, Leach G, Senz S, et al. (2004) Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. *Journal of medical genetics* 41: 508-517.
- Lynch HT, Kaurah P, Wirtzfeld D, Rubinstein WS, Weissman S, et al. (2008) Hereditary diffuse gastric cancer: diagnosis, genetic counseling, and prophylactic total gastrectomy. *Cancer* 112: 2655-2663.
- Fitzgerald RC, Hardwick R, Huntsman D, Carneiro F, Guilford P, et al. (2010) Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. *Journal of medical genetics* 47: 436-444.
- Matsukuma KE, Mullins FM, Dietz L, Zehnder JL, Ford JM, et al. (2010) Hereditary diffuse gastric cancer due to a previously undescribed CDH1 splice site mutation. *Hum Pathol* 41: 1200-1203.
- Ascaño JJ, Frierson H Jr, Moskaluk CA, Harper JC, Roviello F, et al. (2001) Inactivation of the E-cadherin gene in sporadic diffuse-type gastric cancer. *Mod Pathol* 14: 942-949.
- Bacani JT, Soares M, Zwingerman R, di Nicola N, Senz J, et al. (2006) CDH1/E-cadherin germline mutations in early-onset gastric cancer. *J Med Genet* 43: 867-872.
- Shah MA, Salo-Mullen E, Stadler Z, Ruggeri JM, Mirander M, et al. (2012) De novo CDH1 mutation in a family presenting with early-onset diffuse gastric cancer. *Clin Genet* 82: 283-287.
- Benusiglio PR, Malka D, Rouleau E, De Pauw A, Buecher B, et al. (2013) CDH1 germline mutations and the hereditary diffuse gastric and lobular breast cancer syndrome: a multicentre study. *J Med Genet* 50: 486-489.
- Ikonen T, Matikainen M, Mononen N, Hyytinen ER, Helin HJ, et al. (2001) Association of E-cadherin germ-line alterations with prostate cancer. *Clin Cancer Res* 7: 3465-3471.

12. Salahshor S, Hou H, Diep CB, Loukola A, Zhang H, et al. (2001) A germline E-cadherin mutation in a family with gastric and colon cancer. *Int J Mol Med* 8: 439-443.
13. Frebourg T, Oliveira C, Hochain P, Karam R, Manouvrier S, et al. (2006) Cleft lip/palate and CDH1/E-cadherin mutations in families with hereditary diffuse gastric cancer. *J Med Genet* 43: 138-142.
14. Kluijdt I, Siemerink EJ, Ausems MG, van Os TA, de Jong D, et al. (2012) CDH1-related hereditary diffuse gastric cancer syndrome: clinical variations and implications for counseling. *International journal of cancer. Journal international du cancer* 131: 367-376.
15. Bunyan DJ, Eccles DM, Sillibourne J, Wilkins E, Thomas NS, et al. (2004) Dosage analysis of cancer predisposition genes by multiplex ligation-dependent probe amplification. *British journal of cancer* 1155-1159.
16. Oliveira C, Senz J, Kaurah P, Pinheiro H, Sanges R, et al. (2009) Germline CDH1 deletions in hereditary diffuse gastric cancer families. *Hum Mol Genet* 18: 1545-1555.
17. Yamada H, Shinmura K, Ito H, Kasami M, Sasaki N, et al. (2011) Germline alterations in the CDH1 gene in familial gastric cancer in the Japanese population. *Cancer Sci* 102: 1782-1788.
18. Shinmura K, Kohno T, Takahashi M, Sasaki A, Ochiai A, et al. (1999) Familial gastric cancer: clinicopathological characteristics, RER phenotype and germline p53 and E-cadherin mutations. *Carcinogenesis*, 20: 1127-1131.
19. Fujita H, Lennerz JK, Chung DC, Patel D, Deshpande V, et al. (2012) Endoscopic surveillance of patients with hereditary diffuse gastric cancer: biopsy recommendations after topographic distribution of cancer foci in a series of 10 CDH1-mutated gastrectomies. *The American journal of surgical pathology* 36: 1709-1717.
20. Sugimoto S, Yamada H, Takahashi M, Morohoshi Y, Yamaguchi N, et al. (2013) Early-onset diffuse gastric cancer associated with a de novo large genomic deletion of CDH1 gene. *Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association*
21. Yamada M, Fukagawa T, Nakajima T, Asada K, Sekine S, et al. (2013) Hereditary diffuse gastric cancer in a Japanese family with a large deletion involving CDH1. *Gastric cancer : official journal of the International Gastric Cancer Association and the Japanese Gastric Cancer Association*
22. Majewski JJ, Kluijdt I, Cats A, Scerri TS, de Jong D, et al. (2013) An E-catenin (CTNNA1) mutation in hereditary diffuse gastric cancer. *J Pathol* 229: 621-629.
23. Schuetz JM, Leach S, Kaurah P, Jeyes J, Butterfield Y, et al. (2012) Catenin family genes are not commonly mutated in hereditary diffuse gastric cancer. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 21: 2272-2274.
24. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, et al. (2006) Global variation in copy number in the human genome. *Nature* 444: 444-454.
25. Poultney CS, Goldberg AP, Drapeau E, Kou Y, Harony-Nicolas H, et al. (2013) Identification of small exonic CNV from whole-exome sequence data and application to autism spectrum disorder. *Am J Hum Genet* 93: 607-619.
26. Yasui W, Ito H, Peek R, Tahara E (2012) Recent progress in carcinogenesis, progression and management of upper GI cancer: the 21st Hiroshima Cancer Seminar--the 5th Three Universities' Consortium International Symposium, 6 November 2011, International Conference Center Hiroshima. *Japanese journal of clinical oncology*, 42: 351-356.
27. Mimata A, Fukamachi H, Eishi Y, Yuasa Y (2011) Loss of E-cadherin in mouse gastric epithelial cells induces signet ring-like cells, a possible precursor lesion of diffuse gastric cancer. *Cancer Sci* 102: 942-950.
28. Hirayama A, Kami K, Sugimoto M, Sugawara M, Toki N, et al. (2009) Quantitative metabolome profiling of colon and stomach cancer microenvironment by capillary electrophoresis time-of-flight mass spectrometry. *Cancer Res* 69: 4918-4925.