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Genomic analysis to assess disease progression and recurrence in patients with oral squamous carcinoma – a preliminary study.

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Ethics statement/confirmation of patient permission:

All patients gave informed consent prior to treatment (ethics REC ref. numbers 07/Q1206/30 and 08/H1306/127).

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Abstract

Introduction

Squamous cell carcinoma (SCC) is the commonest oral malignancy. Due to the ease of access, the progression from dysplasia to invasive carcinoma and subsequent second primaries or locoregional recurrences, can be extensively studied.

Methods

Patients with recurrent disease were included. Samples were sequenced, between one and six per patient. DNA samples were prepared and libraries were multiplexed to between 40-80 samples per lane of an Illumina HiSeq 3000 and sequenced with 2x100bp paired end sequencing. Copy number data was generated by CNAnorm.

Results

The recurrent SCC of the patients examined presented with unique patterns of descent when compared to earlier samples from the primary SCC, and three main classes of patterns emerged. Four patients showed convincing evidence that the latter lesion was directly descended from cells from the first lesion. Four patients shared no detectable genomic events between the two lesions. Three patients had some shared events between the early and later lesions, but with enough differences to deduce that the two lesions had a shared ancestor, but were not directly descended from each other. The patient characteristics were presented in detail including the overall survival from each group.

Conclusions

There are three groups of patients with a distinct genomic pattern demonstrated after a second clinical episode of SCC. A larger study with similar methodology and with a longer duration may provide reliable conclusions with respect to survival. Using novel techniques, genomic data can be available to the clinical team, at the time of treatment planning.

Key words: Oral cancer, Squamous cell carcinoma, Recurrence, Oral dysplasia, DNA sequencing

Introduction

Cancer is a genetic disease that demonstrates evolutionary principles and may result from the accumulation of genomic aberrations [1]. Understanding these events will help clinicians to control disease progression and guide therapeutic interventions. Head and Neck Squamous Cell Carcinoma (HNSCC) is one of the most prevalent cancers in the world and among the

main causes of cancer death [2]. Oral squamous cell carcinoma (OSCC), a subgroup of HNSCC, is primarily attributed to alcohol consumption and tobacco use. Local recurrence and / or regional neck node metastases are significant prognostic indicators of survival for OSCC [3]. Distant metastasis is relatively rare in OSCC compared to other cancer types. The identification of genomic factors associated with high risk of a new disease may be useful for the proper selection of patients that will benefit from specific interventions such as an elective neck dissection or adjuvant chemoradiotherapy. OSCCs show a high degree of inter-patient heterogeneity [4, 5]. The introduction of next generation sequencing (NGS) has allowed researches to sequence large number of genes at a time through fast and relatively inexpensive whole exome and genome sequencing [6]. Data from such work may be extensive but often involved tissue from single disease episodes that lacked continuity. Intra-tumour heterogeneity and sub-clonal structure of OSCC are poorly understood, due to studies using only a single tissue sample per patient, as the use of a single tumour biopsy severely hinders the analysis of spatial intra-tumour heterogeneity [7]. A molecular progression model was previously described, to elucidate the transition from normal mucosa to HNSCC (8). The difference in this work is that we are looking at the genomic changes of the recurrence / second primary and we can compare those (in the same patient) with the genomic changes seen in dysplasia / OSCC at its first presentation several years before. We previously published work showing extensive clonal variation in spatially separated samples [9] and the genomic changes leading from dysplasia to carcinoma [A] in OSCC in a cohort of patients. 13 of these patients (subgroup of the initial cohort) again developed disease. Now we are able to examine the recent disease in this subgroup of the cohort and compare the findings. Using low coverage whole genome sequencing, we could examine genomic copy number for every sample, allowing us to study the genomic progression from a first presentation dysplasia / OSCC carcinoma to subsequent disease progression or recurrence. This early work demonstrates what information can be available to the clinical team with advancing technology.

Methods

Patients:

Following a previous collection of 200 consecutive patients with either oral dysplasia or cancer [10], we identified 13 patients for further study. These were patients that initially had an area of dysplasia that progressed to OSCC or patients that presented in a maxillofacial clinic with a primary OSCC (with no history of dysplasia), and received the relevant

treatment (curative intent) before 2010, who then developed another area of dysplasia or OSCC several months or years later. All patients gave informed consent prior to treatment (ethics REC ref. numbers 07/Q1206/30 and 08/H1306/127). 11 of these patients produced enough good quality DNA to sequence. Their clinical details are shown in table 1. In total, 28 post 2010 samples were sequenced, between one and six per patient

DNA extraction:
The formalin-fixed paraffin embedded tissue blocks were obtained from Leeds Pathology archive. The areas of highest tumour/dysplasia cell content were identified by a head and neck pathologist. These were micro-dissected and the DNA extracted using Qiagen QIAamp DNA micro kit (Qiagen, UK).

Low coverage genome sequencing:
All samples were processed using updated versions of previously published protocols [11]. DNA samples were prepared for sequencing using NEBnext DNA Ultra library preparation kits (NEB). Samples were labelled during library preparation using unique 6bp indexes. Libraries were multiplexed to between 40-80 samples per lane of an Illumina HiSeq 3000 and sequenced with 2x100bp paired end sequencing.

Data analysis:
Sequencing reads were trimmed of adapters using cutadapt [12] and aligned to the human genome (hg19) using BWA [13]. Copy number data was generated by CNAnorm [14], using a pooled control of 20 British individuals downloaded from the 1000 genomes project [15]. Breakpoints were called using DNAcopy [16].

Results

Between 3,957,318 and 186,861,910 sequencing reads per sample were produced (median 14,579,601). These were compared to 37 samples previously sequenced from the matched earlier disease (between one and nine samples per patient). Table 1 indicates the pathology and dates of initial presentation as well as the dates of the subsequent disease. Details of treatment are presented. The time difference between the first presentation and the recent pathology is presented in table 2. As each patient presented with a unique clinical history and pattern of genomic damage, they are described briefly as individual cases, to demonstrate the variety of ways in which the early and later lesions were related to each other. The Sloan

binary grading system has been used to classify the dysplasia as low or high grade (17). Patient PG001 presented with three episodes of low-grade dysplasia (LGD) between 2005 and 2008, high-grade dysplasia (HGD) in 2008 and SCC in 2009. This was followed in 2014 by a new case of LGD and then SCC. One LGD sample from 2006, two from 2008, plus and HGD and SCC sample from 2008/9 were compared to LGD and SCC samples from 2014. The copy number profiles of the 2006/2008 samples were all very similar to each other. Most changes were shared between all samples, with each sample showing some additional unique events. This indicates a shared common ancestor, but with none of the samples being directly descended from another. The samples from the recent pathology (after 2010) were also similar to each other, but with the difference that all the events seen in the LGD sample were also seen in the SCC, but not vice-versa, indicating that the LGD was the direct ancestor of the SCC. When comparing the 2006-2008 disease with the 2014 disease, only one event was shared, a small deletion on chromosome 9. This indicates that there was a shared ancestor of all the samples, but that the 2014 disease is not closely related to the 2006-2008 samples. If the 2014 lesion was a direct recurrence, it would be expected to contain most, if not all of the copy number events shared between the early disease. Since it did not, it was most likely descended from an earlier field cancerisation. The changes in this patient are illustrated in figure 1.

Patient PG025 had HGD and SCC samples from 2009 and SCC from a neck metastasis in 2011. The HGD sample had no copy number changes, so nothing could be inferred from this sample. The 2009 SCC sample had only one event, a small deletion on chromosome 5. The metastatic SCC had a much more disrupted genome, and also contained this deletion, confirming that it was a genuine metastasis of the 2009 disease, and that its genome had continued to evolve since.

Patient PG071 had HGD and SCC samples from 2010, followed by three episodes of HGD (from which 5 samples were taken, plus adjacent normal tissue) in 2016. The 2010 HGD and SCC samples had identical copy number profiles. The 2016 HGD samples were all similar to each other, and shared two events from 2010 (chromosome 7 deletion and a focal amplification on chromosome 11). The normal sample had no changes. As the later HGD samples shared only a small fraction of the changes seen in 2010, the later lesions can be considered to share a common ancestor with the earlier disease, but not to be a recurrence. As the adjacent normal sample did not display any changes, it can be surmised that the putative

field effect linking the lesions is only in a small number of apparently normal cells, with most normal cells in the region containing no genomic changes.

Patient PG099 had SCC in 2010. An SCC which provided two samples occurred in 2016. All samples showed considerable genomic damage. The two 2016 samples were identical, but shared nothing with the 2010 disease. There was no evidence that this was progression, so the 2016 lesion can be considered a fresh primary. If any field change linked the two lesions to a common ancestor, it did not contain any copy number changes.

Patient PG105 had two LGD, three HGD and three SCC samples from a lesion taken in 2010 and two SCC samples from a lesion in 2016. The multiple samples from 2010 had regional variation, with each SCC sample most closely resembling the nearest dysplasia sample, rather than more distant SCCs. This indicates a complex sub-clonal development of the initial disease. The 2016 samples were identical to each other, but shared nothing with any 2010 sample, indicating that it was a new primary lesion, not progression.

Patient PG109 had LGD, HGD and SCC samples from 2010 following by SCC in 2014. The 2010 SCC and the adjacent LGD are similar to each other but completely different to the other 2010 dysplasias, which are themselves identical to each other and to the 2014 SCC. Therefore, the 2014 lesion is not a recurrence of the 2010 SCC, but is instead derived from the nearby dysplastic cells.

Patient PG113 had SCC in 2010 followed by another SCC yielding four samples in 2014. The 2014 samples were all identical, but shared nothing with the 2010 sample, apart from a break at the chromosome 3 centromere. This is an extremely common event in OSCC, so is not a reliable indicator of common ancestry. The 2014 disease can be considered a new primary, not a recurrence.

Patient PG118 had an SCC and accompanying HGD sample from 2010 followed by two samples from a 2012 SCC. The 2010 HGD had no genomic changes. The 2012 SCCs were identical to each other and shared most events with the 2010 SCC. However, both the 2010 and 2012 lesions had events which were not present in the other. Since the 2010 HGD sample had no changes, the common ancestor of both the 2010 and 2012 SCC sample was probably within the 2010 lesion, most likely a portion of the SCC which was not sampled. This can be considered a recurrence of the original SCC, and is illustrated in figure 2.

PG123 had LGD, HGD and SCC in 2010 followed by neck metastasis in 2011. The 2010 LGD had no genomic changes. The HGD genome was mostly undamaged, but it did share a gain in chromosome 5p with all SCC samples. The 2010 SCCs showed regional variation. The 2011 metastasis had a mixture of events from the 2010 SCC samples. It had all the

shared events, and some new ones, but some of the 2010 regional variation was either missing or present at sub-clonal levels. It seems likely that this was a multi-clonal metastasis.
Patient PG156 had HGD and SCC in 2011, followed by HGD and SCC six months later in 2012, which was judged at the time to probably be a metastasis. The 2011 lesion showed regional variation. The 2011 HGD had several events not seen in the SCC samples, indicating shared ancestry, but not direct descent. The 2012 samples (including the HGD) were identical to each other and had all the events seen in some of the 2011 samples, indicating from which part of the 2011 lesion they metastasised. The 2012 samples also had new events not seen in 2011. Examples from this patient are shown in figure 3.
Patient PG196 had SCC in 2008, LGD in 2011, followed by HGD and SCC in 2015. The 2008 lesion was unavailable. The 2011 LGD had no genomic damage. The 2015 SCC samples had everything in the 2015 HGD sample, plus some local, unique events. As the 2008 sample was missing, and the 2011 sample had no changes, nothing can be inferred about progression from this sample.

Discussion

The aim of this pilot work was not to bring any major new insights into the biology of oral cancer, but rather to show what data could be obtained with advancing technology. As an exemplar, we assessed the similarities and differences between the genomic profiles of HNSCC samples and earlier disease from the same patients. To this end we sequenced dysplasia and carcinoma samples from two or more matched lesions, separated in time. In this way, we were able to ascertain whether the second lesion was a recurrence or metastasis of the first lesion, or whether it could be considered a second primary.
The disease of the patients examined developed in a slightly different way, with unique patterns of descent from the earlier lesion to the later disease, however, three main classes of patterns emerged. Group 1 (PG025, PG118, PG123 and PG156), showed convincing evidence that the second lesion was directly descended from cells in the first lesion. It is of note, that disease recurred within 2 years of the first treatment with curative intent (Table 2). Most, if not all of the genomic changes shared across samples in the early lesions were seen again in the later lesions. Interestingly, these four patients included the three metastatic samples (PG025, PG123, PG156). Although this is a small sample size, it does appear that metastatic disease can be traced back to an earlier primary.

Group 2 (PG099, PG105, PG113 and PG196), shared no detectable genomic events between the two lesions. PG196 had no changes to the early lesion, but the other three had multiple changes in the early lesion that were not seen in the second lesion, and multiple events in the second lesion that were new. Since no trace of the earlier lesions are seen in the makeup of the later lesions, it can be surmised that the curative therapy for these patients was successful, in that it removed all cancerous tissue. The later lesions in these cases can probably be considered as genuine second primaries, not recurrences. These 'new' cancers developed many years later from the first treatment; in 3 out of the 4 patients it was more than 5 years later (Table 2).

Group 3(PG001, PG071 and PG109), had some shared events between the early and later lesions, but enough differences to conclude that the two lesions had a shared ancestor, but were not directly descended from each other. This conclusion is not due to what proportion of changes are shared, but reached if there are events which are ubiquitous across all samples in the first lesion, which are completely absent in the second lesion. Amongst those patients, the later disease in PG071 was dysplasia only. Since this was not descended from the earlier SCC, the clinicians involved could feel more confident in the decision not to treat. The second lesion in PG109 appeared to be descended from dysplastic cells from the first lesion, which raises interesting questions as to what constitutes a recurrence, and what counts as field effect. It is possible that the four patients with no detected shared genomic changes did in fact share common ancestry between lesions, but that this field effect could only be detect using point mutations. However, if multiple events are observed in all samples of an early lesion, but are absent in a second lesion, we can be confident that direct descent has not occurred.

Tables 3 and 4 present details relating to survival. The sample is too small and the follow up interval in some patients is not long enough for us to make meaningful conclusions. At present it is difficult to link this genomic data with clinical care, in oral cancer. The presented features need to be investigated further in order to validate their potential of predicting long term prognosis following recurrence of OSCC . We provided data of three different patterns of disease progression. As our understanding is improving, future work may elucidate which mutations are relevant and of practical use in the management of the disease. Further work in a larger scale with similar patients and methodology may be able to provide patient specific practical guidance in clinical care. A larger study with a longer follow up interval will provide a more detailed assessment of survival after disease recurrence.

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References

1. Merlo LM, Pepper JW, Reid BJ, Maley CC: Cancer as an evolutionary and ecological process. Nat Rev Cancer 2006, 6:924-935.
2. Leemans CR, Braakhuis BJ, Brakenhoff RH: The molecular biology of head and neck cancer. Nature reviews Cancer 2011, 11:9-22.
3. Hakeem AH, Pradhan SA, Kannan R, Tubachi J: Clinical outcome of surgical treatment of T1-2 N0 squamous cell carcinoma of oral tongue with observation for the neck: Analysis of 176 cases. Ann Maxillofac Surg 2016, 6:235-240.
4. Kandoth C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA, et al: Mutational landscape and significance across 12 major cancer types. Nature 2013, 502:333-339.
5. Hedberg ML, Goh G, Chiosea SI, Bauman JE, Freilino ML, Zeng Y, Wang L, Diergaarde BB, Gooding WE, Lui VW, et al: Genetic landscape of metastatic and recurrent head and neck squamous cell carcinoma. J Clin Invest 2016, 126:169-180.
6. Tabatabaeifar S, Kruse TA, Thomassen M, Larsen MJ, Sorensen JA: Use of next generation sequencing in head and neck squamous cell carcinomas: a review. Oral Oncol 2014, 50:1035-1040.
7. Tabatabaeifar S, Thomassen M, Larsen MJ, Larsen SR, Kruse TA, Sorensen JA: The subclonal structure and genomic evolution of oral squamous cell carcinoma revealed by ultra-deep sequencing. Oncotarget 2017, 8:16571-16580.
8. Califano J1, van der Riet P, Westra W, Nawroz H, Clayman G, Piantadosi S, Corio R, Lee D, Greenberg B, Koch W, Sidransky D. Genetic progression model for head and neck cancer: implications for field cancerization. Cancer Res 1996,56(11):2488-92.
9. Wood HM, Conway C, Daly C, Chalkley R, Berri S, Senguven B, Stead L, Ross L, Egan P, Chengot P, et al: The clonal relationships between pre-cancer and cancer revealed by ultra-deep sequencing. J Pathol 2015, 237:296-306.
10. Wood HM, Daly C, Chalkley R, Senguven B, Ross L, Egan P, Chengot P, Graham J, Sethi N, Ong TK, MacLennan K, Rabbitts P, Conway C. The genomic road to invasion-examining the similarities and differences in the genomes of associated oral pre-cancer and cancer samples. Genome Med 2017 Jun 7;9(1):53.

11. Wood HM, Belvedere O, Conway C, Daly C, Chalkley R, Bickerdike M, McKinley C, Egan P, Ross L, Hayward B, et al: Using next-generation sequencing for high resolution multiplex analysis of copy number variation from nanogram quantities of DNA from formalin-fixed paraffin-embedded specimens. Nucleic Acids Research 2010, 38.
12. Martin M: Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnetjournal 2011, 17.
13. Li H, Durbin R: Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009, 25:60.
14. Gusnanto A, Wood HM, Pawitan Y, Rabbitts P, Berri S: Correcting for cancer genome size and tumour cell content enables better estimation of copy number alterations from next-generation sequence data. Bioinformatics 2012, 28:40-47.
15. Genomes Project C, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR: A global reference for human genetic variation. Nature 2015, 526:68-74.
16. Venkatraman ES, Olshen AB: A faster circular binary segmentation algorithm for the analysis of array CGH data. Bioinformatics 2007, 23:657-663.
17. Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol. 2006 Nov;42(10):987-93.

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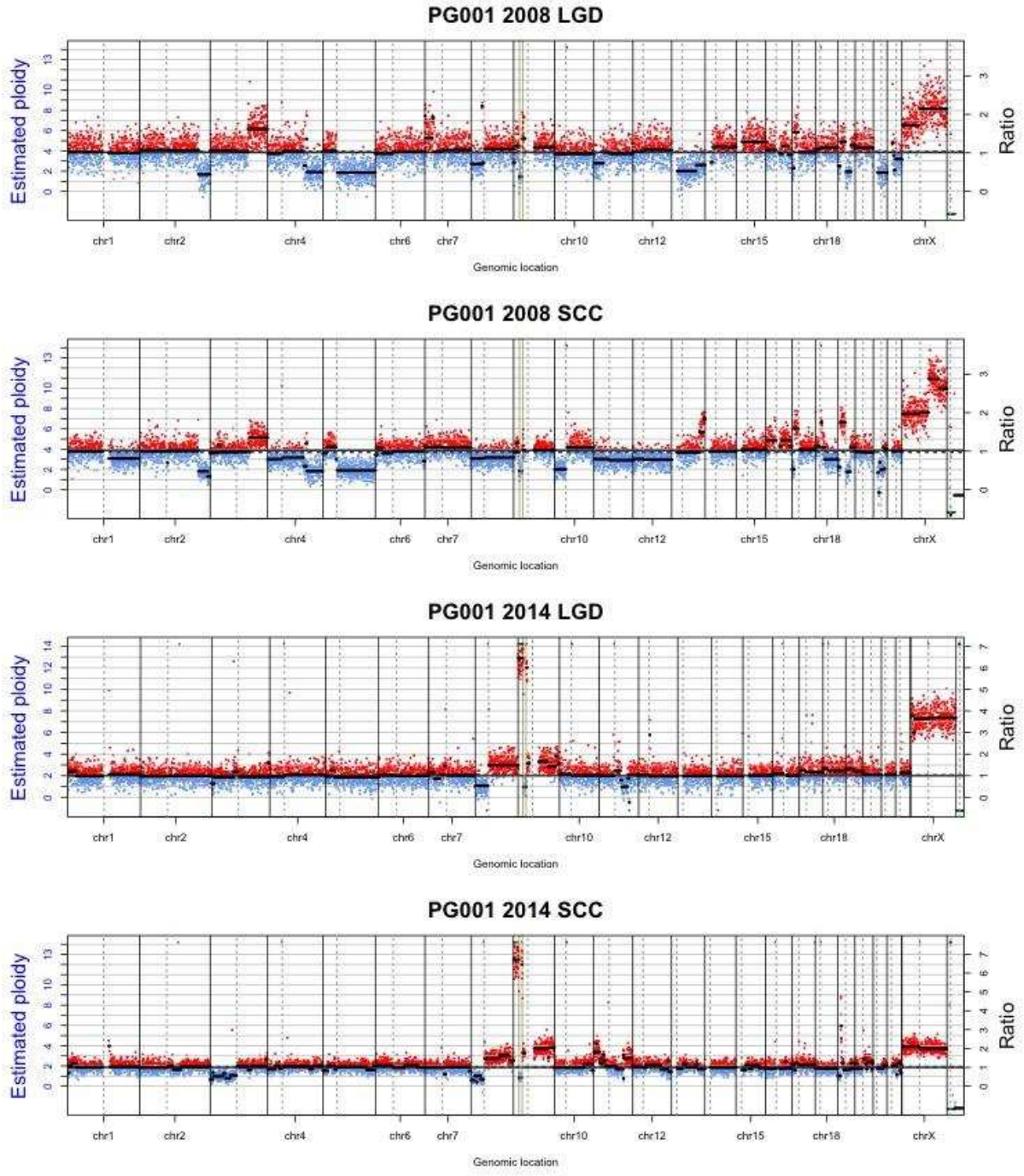

Figure 1 – Example copy number results from patient PG001, showing common ancestry but not direct recurrence. Dysplasia and SCC samples are shown from 2008 and 2014. For each, genomic position is shown along the x-axis, and estimated copy number on the y-axis. Each data point represents the copy number in a 400Kb region. The black horizontal lines represent the averaged, segmented signal. Regions with a copy number higher than normal are coloured red, and those lower than normal coloured blue. In this patient, the two 2008 samples are very similar, as are the two 2014 samples, sharing most events. The only event shared across the two years is a small deletion in chromosome 9 (highlighted). The shared event shows

common ancestry, but the multiple differences show divergence since that time, with no direct descent.

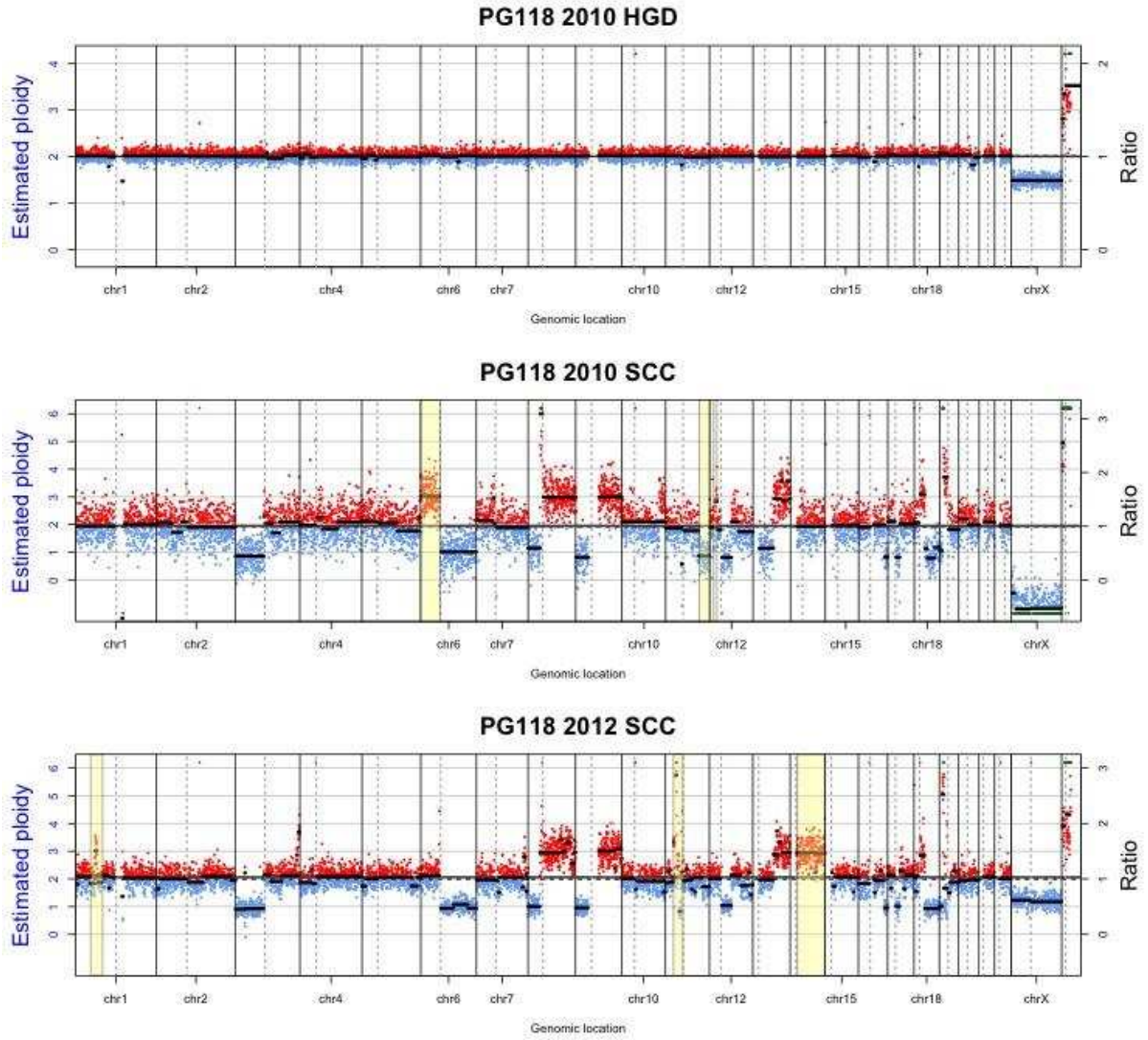

Figure 2 – Example copy number results from patient PG118, showing recurrence. The dysplasia and SCC sample from 2010 are shown, with a representative SCC from 2012. The dysplasia shows no genomic changes. Most of the events in the 2010 SCC reappear in the 2012 disease. However, each sample has a number of unique events (highlighted). The shared events indicate common ancestry, while the differences indicate divergence since that time. Since the common events are not present in the dysplasia, the common ancestor is probably part of the 2010 SCC which was not sampled at the time.

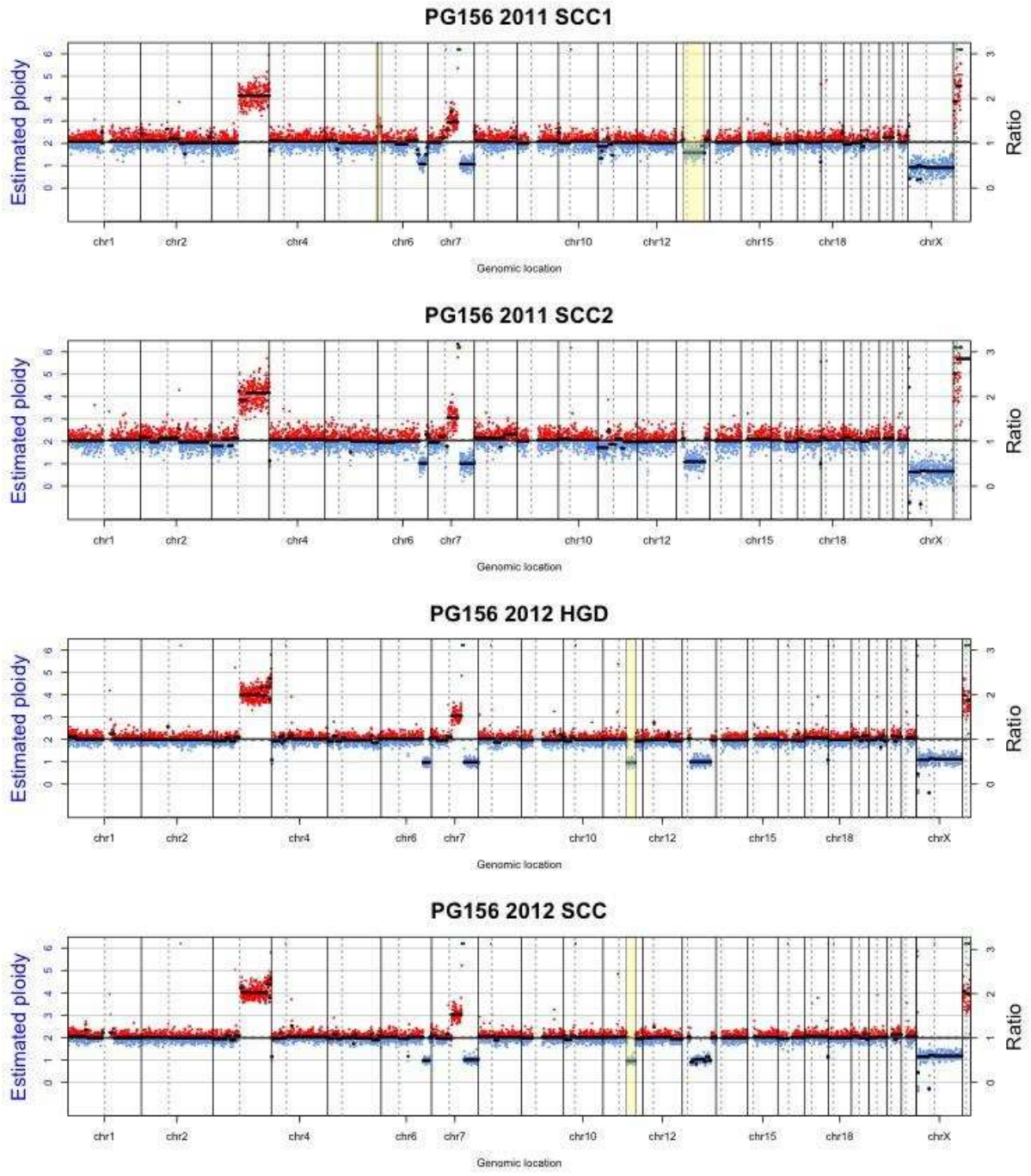

Figure 3 – Example copy number results from patient PG156, showing metastasis. Two SCC samples from 2011 are shown, with an HGD and SCC sample from 2012. 2011 SCC1 has all the genomic changes seen in SCC2, as well as two extra events (highlighted). The HGD and SCC samples from 2012 are identical, and also have everything seen in 2011 SCC2, plus one extra highlighted deletion. As the events of 2011 SCC2 are found in all the other samples, this can be considered as representative of the common ancestor, and the founder of the metastasis.

Table 1: These are the patients involved in this work. In this table, the dates are given for the pathology as well as the dates of the initial and subsequent disease. Details of treatment are presented.

Patient ID	**Year**	**Description**	**Treatment**
PG001	2005	LGD	
PG001	2006	LGD	
PG001	2008	LGD/HGD	
PG001	2009	SCC - Moderately differentiated keratinising squamous cell carcinoma, T1M0N0	Surgery
PG001	2014	LGD/SCC - Well-differentiated squamous cell carcinoma, T2M0N0	Surgery/Radiotherapy
PG025	2009	HGD/SCC - Moderately-differentiated, squamous cell carcinoma, lip mucosa	Surgery
PG025	2011	SCC - Metastatic neck SCC	Surgery/Radiotherapy

PG071	2010	HGD/SCC - Moderately-differentiated, squamous cell carcinoma, T1NM0N0	Surgery
PG071	2011	3X HGD	
PG099	2010	HGD/SCC - moderately differentiated squamous cell carcinoma arising from high-grade surface dysplasia, T1M0N0	Surgery, but previous chemoradiotherapy
PG099	2016	SCC - Multifocal early invasive poorly differentiated squamous cell carcinoma, T1N0M0	Surgery and radiotherapy
PG105	2010	LGD/HGD/SCC - Moderately differentiated squamous cell carcinoma, T1 N0 M0	Surgery
PG105	2016	SCC - Poorly differentiated squamous cell carcinoma, T2N0M0	Surgery and radiotherapy
PG109	2010	LGD/HGD/SCC - Well-differentiated squamous cell carcinoma, T1 N0 M0	Surgery only but had previous radiotherapy

PG109	2014	SCC - well differentiated squamous cell carcinoma with a verrucous appearance, T1N0M0	Surgery
PG113	2010	SCC - Poorly differentiated squamous cell carcinoma on tonsil, T2N0M0	Surgery
PG113	2014	SCC - moderately differentiated squamous cell carcinoma, T3 N2 Mx	Surgery and chemoradiotherapy
PG118	2010	HGD/SCC - Poorly differentiated squamous cell carcinoma, T1N0M0	Surgery
PG118	2012	SCC - poorly differentiated focally keratinising squamous cell carcinoma, T4N0M1	Chemotherapy
PG123	2010	LGD/HGD/SCC - Poorly differentiated squamous cell carcinoma, T2N2N0M0	Surgery
PG123	2011	SCC - metastatic squamous cell carcinoma on neck, N4	Surgery and chemoradiotherapy

PG156	2011	HGD/SCC - Poorly differentiated squamous cell carcinoma on tonsil, T4N2CM0	Excision of tonsil and radiotherapy
PG156	2012	HGD/SCC Poorly differentiated squamous cell carcinoma with basaloid features.	
PG196	2008	SCC - well-differentiated squamous cell carcinoma, T1N0M0	Surgery
PG196	2011	LGD	
PG196	2015	HGD/SCC - Moderately-differentiated squamous cell carcinoma, T1N0M0	Surgery

Table 2: Time difference between the first biopsy confirmed OSCC and the subsequent more recent confirmed OSCC. Patient PG071initially had OSCC and then developed dysplasia only.

Patient number	**Time difference**
001	5 years 5 months 25 days
025	1 year 8 months 14 days
071	5 years 10 months 17 days
099	5 years 8 months 2 days
105	5 years 9 months 5 days
109	3 years 2 months 19 days
113	4 years 28 days
118	1 year 8 months 2 days
123	1 year 1 month 3 days
156	1 year 8 months 18 days
196	7 years 8 months 16 days

Table 3: Three groups of patients are presented based on the genomic relationships. Group 1 (PG025,PG118,PG123,PG156), Group 2 (PG099,PG105,PG113,PG196) and group 3 (PG001,PG071,PG109). PG123 died cancer free, from pneumonia. All other deaths related to metastatic SCC.

Patient groups	**Patient numbers**	**Survival status /Survival after second diagnosis**
Group 1	PG025	Alive / 5 years 11 months 17 days
	PG118	Deceased / 1year 11 months 24 days
	PG123	Deceased /5years 4months 15days
	PG156	Deceased / 1 year 7 months 7 days
Group 2	PG99	Deceased / 1 year 2 months 13 days
	PG105	Alive / 1 year 2 months 5 days
	PG113	Deceased / 4 months 26 days
	PG196	Alive / 1 year 5 months 20 days
Group 3	PG001	Deceased / 9 months 20 days
	PG071	Alive / 1 year 3 months 20 days
	PG109	Alive / 2 years 8 months 19 days

Table 4: This table demonstrates the overall survival from the initial biopsy confirmed SCC. PG123 died cancer free from pneumonia

Patient groups	**Patient numbers**	**Overall survival from initial diagnosis of SCC**
Group 1	PG025	7 years 8 months 14 days (alive)
	PG118	3 years 7 months 26 days
	PG123	6 years 5 months 18 days
	PG156	3 years 3 months 25 days
Group 2	PG99	6 years 10 months 15 days
	PG105	6 years 11 months 7 days (alive)
	PG113	4 years 5 months 23 days
	PG196	9 years 2 months 6 days (alive)
Group 3	PG001	6 years 3 months
	PG071	7 years 3 months 2 days (alive)
	PG109	5 years 11 months (alive)