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Genomic alterations in ameloblastoma and ameloblastic carcinoma – a collaborative study

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Background

Ameloblastoma is a benign odontogenic neoplasm exclusively located in the jawbones. It is characterized by local invasiveness, with propensity for gross facial deformity, tooth displacement, a high rate of recurrence, occasional metastasis and malignant transformation. It is the most common odontogenic tumour in Nigeria, accounting for 63% of all odontogenic tumours[1]. A recent Nigerian hospital based study found the mean age of occurrence as 34.2±14.3 years, the male to female ratio as 1:1.1, the mandible as the most involved jawbone and the mean tumour evolutions as 42.9±41.8 months[2]. Although benign, its biological aggression and typical late hospital presentation in Nigerian patients poses a significant therapeutic challenge. Surgical intervention is currently the method of treatment and results in significant facial deformity with compromise of function and quality of life in many Nigerian patients that cannot afford appropriate reconstruction. Even with surgical intervention, the recurrence rates reported span 10-40% for tumours treated with wide excision and conservative approaches respectively[3].

Identification of altered molecular pathways in ameloblastoma may allow us to identify novel targets for chemotherapeutic intervention or other molecular targeted therapy and so prevent or limit extensive surgical intervention. However, no study has characterized the genomic profile of Ameloblastoma (never mind African cases of ameloblastoma) with a view to developing chemotherapeutic alternatives. We therefore aim to characterize the whole genome of Ameloblastoma and ameloblastic carcinoma in Nigerian patients as a first step in identifying such

novel targets. Although array studies of the ameloblastoma transcriptome has been done in the Caucasian population[4,5], and there have been investigations at a number of individual genomic loci, the genomic basis for the frequency of this tumour in black Africans needs investigation, especially as possible molecular targets for therapy are already being recognized.

A study like this not only aims to provide scientific and clinical information, but also allows for new collaborative links to be made. At present there are no ongoing links between Oral Pathology in the UK and in Nigeria, and this study will therefore allow for mobility between the UK and Nigeria which will be to the benefit of both parties: the Unit of Oral and Maxillofacial Pathology, University of Sheffield and the Department of Oral Pathology, Ibadan. The prominence of Oral Pathology in West Africa is on the rise with the recent IAOP Africa regional Oral Pathology congress (http://www.iaopafricadivision.org/) and the mobility and science funded by this project will allow us to make useful collaborative links and lay the basis for many other projects.

Study aims and Objectives

We will undertake genome-wide copy number and loss-of-heterozygosity (LOH) profiles in a cohort of ameloblastomas and ameloblastic carcinomas in order to describe:

1. Copy number alterations
2. The number and type of alterations present
3. How the genomic landscape alters in malignant tumours

Methods

A total of 29 tumours fixed in formalin and embedded in paraffin (FFPE), of which 13 tumours also had tumour stored in RNAlater™ solution, were selected for this study. New H&E stained slides of all of the cases were cut and examined to confirm the diagnosis. DNA was extracted using the QIAamp® DNA FFPE Tissue Kit (QIAGEN, Germany) and the DNeasy® Tissue Kit (QIAGEN, Germany) for the FFPE and RNAlater samples respectively. DNA concentrations were obtained using a Nanodrop spectrophotometer (ThermoFischer, UK) and confirmed by Qubit fluorometric quantitation (ThermoFischer, UK). Due to problems with the DNA yield obtained from the FFPE samples, a High Sensitivity DNA Assay (Agilent, USA) was also performed. This confirmed a poor yield and quality of the DNA obtained from the FFPE samples. Because of this, only the samples kept in RNAlater storage solution were used for the genomic analysis presented in this report.

Whole genome analysis was performed using the Oncoscan™ FFPE Assay Kit (Affymetrix, USA) following the manufacturer's instructions. Quality control gels were performed after each PCR (two

in total) before moving forward with the array. After passing the quality controls gels, the samples were loaded into the arrays and hybridized for 16-18 hrs. After this, the arrays were washed, stained and scanned using a GeneChip® Scanner (Affymetrix, USA). Data was analysed using the Oncoscan Console 1.3, Nexus Express for Oncoscan 17.0 and the Somatic Mutation Viewer 1.0.1 (ThermoFischer, UK). DNA from a SCC cell line with known genomic changes was used as a positive control.

Results:

A total of 13 tumours (all of which had been stored in RNALater) were analysed using the Oncoscan™ FFPE Assay Kit (Affymetrix, USA). Ten tumours were ameloblastomas and three were ameloblastic carcinomas (Table 1: details of the 29 FFPE samples are available, but not shown). In terms of genome aberrations, the ameloblastomas showed a mean genome change of 9.7%, with a mean of 88.7 copy number (CN) aberrations and 7.5% of loss of heterozygosity (LOH), whereas the ameloblastic carcinomas had a mean genome change of 6.8% with a mean of 87.3 copy number (CN) aberrations and 3.6% of loss of heterozygosity (LOH) (table 2, Figure 1 A and B). As shown in table 3, ameloblastomas and ameloblastic carcinomas shared somatic mutations in BRAFV600E, EGFR, KRAS and PTEN genes. One ameloblastoma showed also a mutation in the TP53 gene which was not observed in any other tumour (benign or malignant) and two (66.7%) ameloblastic carcinomas showed a somatic mutation in the PIK3CA gene, which was not observed in the ameloblastoma cohort. When analysing the regions of CN gain, CN loses and LOH among the samples, it was observed that all tumours (benign and malignant) presented with a CN gain in chr8q23.3, affecting the CSMD3 gene. Other common affected regions included chr1p34.2-p34.1, chr2q11.2, among others. More details of these aberrations can be seen in table 3.

ID	Case No	Diagnosis	Gender	Age	Location	Evolution
20	1	Follicular ameloblastoma	Male	30	Mandible	5 years
54	2	Ameloblastic carcinoma	Male	30	mandible	10 months
61	3	Plexiform ameloblastoma	Female	30	maxilla	2 years
71	4	Acanthomatous ameloblastoma	Female	43	mandible	13 years
14	5	Ameloblastic carcinoma	Male	28	mandible	8 months
24	6	Cystic ameloblastoma	Female	64	Mandible	NK
63	7	Recurrent Multycistic ameloblastoma	Female	47	mandible	NK
76	8	Solid multicystic ameloblastoma	Female	28	Mandible	7 years

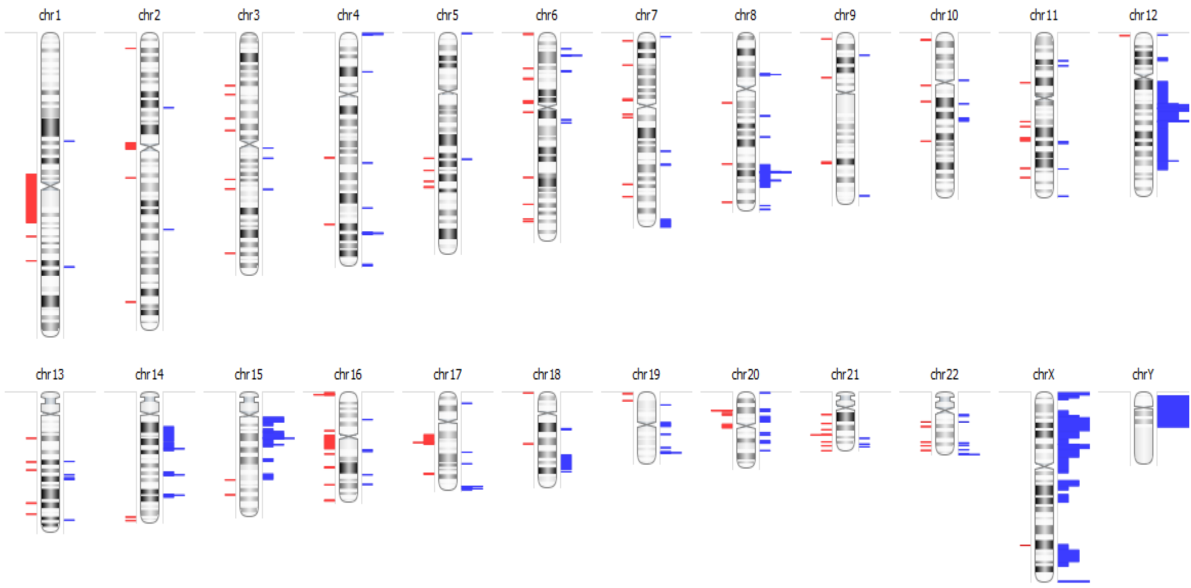
79	9	Ameloblastic carcinoma	Male	4	Mandible	4 months
80	10	Recurrent solid multicystic ameloblastoma	Female	25	Mandible	7 years
82	11	Cystic ameloblastoma (mural)	Male	18	Mandible	5 months
83	12	Atypical ameloblastoma	Male	35	Mandible	3.5 years
85	13	Atypical acanthomatous ameloblastoma	Male	26	Mandible	6 years

Table 1. Details of the clinical cohort. NK = not known

Ameloblastoma			
ID	**Total CN aberrations**	**%LOH**	**% Genome Changed**
20	99	3.3	6.0
61	83	4.3	1.5
71	82	4.0	2.4
24	136	4.5	10.9
63	98	6.3	23.1
76	90	8.9	6.5
80	84	9.2	16.0
82	60	8.3	7.5
83	93	2.7	9.3
85	62	23.6	13.9
Average	**88.7**	**7.5**	**9.7**

Ameloblastic carcinomas			
ID	**Total CN aberrations**	**%LOH**	**% Genome Changed**
54	67	1.8	3.3
14	113	2.9	10.2
79	82	6.0	7.0
Average	**87.3**	**3.6**	**6.8**

Table 2. Common Genome aberrations of ameloblastomas and ameloblastic carcinomas.



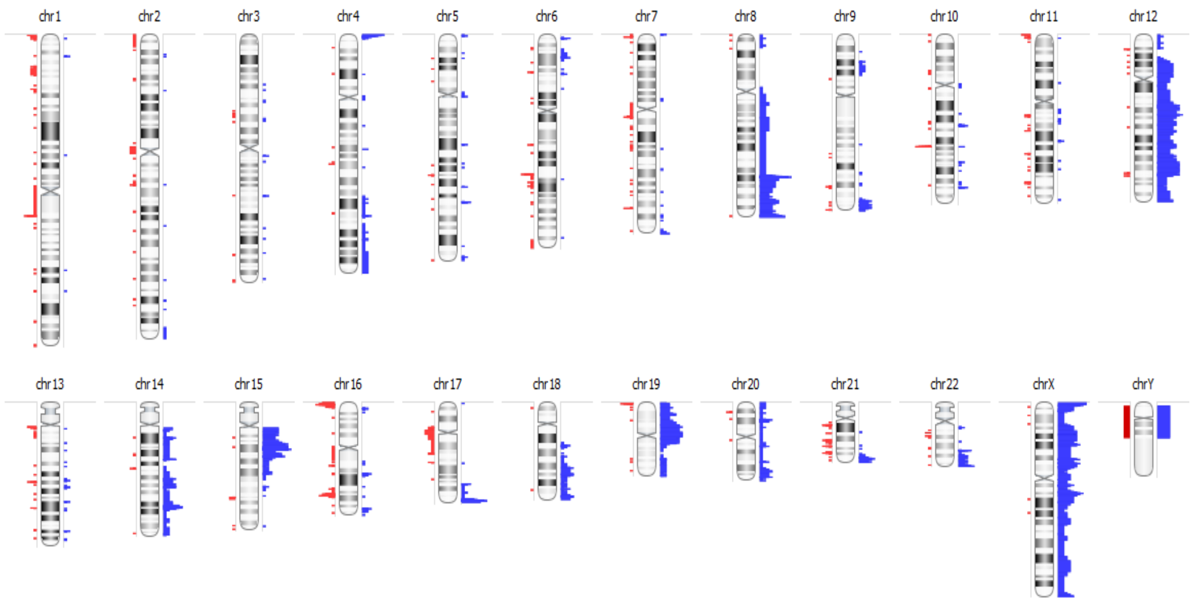


Figure 1A Genomic aberrations of ameloblastomas (10 samples) 1B) Genome aberrations of ameloblastic carcinomas (3 samples). Red: CN loss, Blue: CN gain

ID	BRAF	EGFR	KRAS	PIK3CA	PTEN	TP53	Histology	Gender	Location	age	Evolution (months)
20	x		x				Follicular	male	mandible	30	60
61		x					Plexiform	female	maxilla	30	24
71	x		x				Acanthomatous	female	mandible	43	156
24		x					Cystic	female	mandible	64	NK
63	x	x	x				Recurrent Multycistic	female	mandible	47	NK
76		x			x		Solid Multycistic	female	mandible	28	84
80		x	x		x		Recurrent solid multicystic	female	mandible	25	84
82	x	x					Cystic	male	mandible	18	5
83	x	x					Atypical	male	mandible	35	42
85	x		x			x	Atypical acnthomatous	male	mandible	26	72
	60%	70%	50%	0%	20%	10%					

ID	BRAF	EGFR	KRAS	PIK3CA	PTEN	TP53	Histology	Gender	Location	age	Evolution (months)
54	x		x	x			Ameloblastic carcinoma	male	mandible	30	10
14	x	x	x	x			Ameloblastic carcinoma	male	mandible	28	8
79		x	x		x		Ameloblastic carcinoma	male	mandible	4	4
	66.70%	66.70%	100%	66.70%	33.3%	0%					

Table 3. Somatic mutations identified in ameloblastomas (top) and ameloblastic carcinomas (bottom). NK = not known.

Chromosome	Cytoband Location	Event	Frequency in AC %	Frequency in Ameloblastoma %
chr8	q23.3	CN Gain	100	100
chr1	p34.2 - p34.1	LOH	100	80
chr2	p11.2	LOH	100	50
chr2	q11.2	LOH	100	90
chr16	p13.3	LOH	100	70
chr17	q12 - q21.2	LOH	100	70
chr1	p13.3 - p13.2	LOH	66.7	80
chr2	p25.2 - p25.1	LOH	66.7	80
chr11	q24.3 - q25	LOH	66.7	60
chr17	q23.2 - q24.1	LOH	66.7	70

Table 4. Common altered genome regions in ameloblastoma and ameloblastic carcinoma (AC).

Discussion and conclusion

This is the first presentation of whole genome assessment of genomic changes in ameloblastoma. The data generated demonstrates that ameloblastoma appear to be relatively genomically stable, with very little consistent change in the genome. This also is seen in the small number of ameloblastic carcinomas analysed. There is a surprising lack of genomic alterations as has been demonstrated in many other malignant tumour types.

We have also identified a novel area of copy number gain that is present in ameloblastoma and ameloblastic carcinoma. This locus (8q23) encodes for a single gene, CSMD3. This is a very large protein whose function is poorly understood. However, it and other CSMD family members, have been associated with a number of malignant tumours, including phyllodes tumours (Lae et al, 2016) and non-small cell lung cancer, where it is commonly mutated (Liu et al, 2012). We shall seek to validate this result in the UK and Nigerian ameloblastoma material we have and seek funding to investigate its function in a number of ameloblastoma cell lines which we have in the department. There are also a number of other consistent genomic alterations, many of which contain numerous genes, which will require validation and further exploration to identify candidate genes.

The mutational landscape in our series is very similar to that seen in the literature. A high prevalence of BRAFV600E mutations has been reported by many authors (Gultekin et al, 2018; Diniz et al, Sweeney et al, 2014). We have also identified a number of other mutations in our cohort, some of

which have been described before, including EGFR and KRAS. PIK3CA mutations were only identified in ameloblastic carcinoma. No mutations in SMO were identified.

Achievement of stated outcomes:

The cohort size has been modified due to the poor quality of the DNA retrieved from the FFPE tissues.

We have:

1. Identified copy number alterations in ameloblastoma and ameloblastic carcinoma
2. Described the number and type of alterations present
3. Shown that the genomic landscape alters very little in the small number of malignant tumours tested.

Future work

We have a small number of the arrays remaining and will continue analysis using DNA from some of the FFPE samples which passed basic QC. This is technically challenging, but we do hope to obtain useful data from this. The issues we have faced with this material reinforces the need for standardisation and QC in FFPE tissues. This may be more challenging in the developing world where facilities and infrastructure are less well developed.

Will also seek to continue this work in order to validate the alterations identified in the genome screen. We plan to do this on a collaborative basis between Sheffield and Nigeria, and may utilise Masters level projects for this ongoing research. Once validated, more substantive funding will be required.

We plan to present this interim data at the IAOP Congress in Vancouver in June 2018 and will present a more complete analysis in a future PathSoc meeting (probably in 2019).

Report from the Incoming Researcher

I arrived Sheffield in the fall and it was immediately clear to me that I had not prepared well for the weather but my co-researcher had a winter jacket waiting for me and I was indeed grateful for his thoughtfulness and kindness. I had a very warm welcome and reception by the staff of the oral pathology department of the University of Sheffield. It was a great honor for me to meet oral pathologists who had formulated classification schemes and written chapters in books that I had read as a student. Everyone was willing to show me the practice of oral pathology at its best and I thoroughly enjoyed the histopathology review sessions, the grossing of neck dissection specimens, the student teaching tutorials and the multidisciplinary tumor board meetings. I also had the privilege of observing some cell culture work and learned a great deal from this experience. I made new friends and contacts that I'm sure will influence my academic and research progress positively.

During my stay in Sheffield I was accommodated at the Halifax Hall, a great place to stay, and I was also extremely pleased at its proximity to the University Hospital and to other very interesting places in town. This meant that I could easily walk to University facilities and any other place of interest fairly quickly. My culinary experience was splendid as I sampled full English meals, Greek dishes and Chinese meals. This made me appreciate the multi-cultural nature of the town. Some environmental wonders that I experienced for the first time in my life were the reduction of daytime by taking the clock back by one hour and the falling of delicate flakes of snow for one day. Overall, the Sheffield experience was truly remarkable and I sincerely hope to visit again at some point in the future.

Dr A Adisa, February 2017

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