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Establishment and characterization of a primary human chordoma xenograft model:

Laboratory investigation

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Abstract

Object—Chordomas are rare tumors arising from remnants of the notochord. Because of the challenges in achieving a complete resection, the radioresistant nature of these tumors, and the lack of effective chemotherapeutics, the median survival for patients with chordomas is approximately 6 years. Reproducible preclinical model systems that closely mimic the original patient's tumor are essential for the development and evaluation of effective therapeutics. Currently, there are only a few established chordoma cell lines and no primary xenograft model. In this study, the authors aimed to develop a primary chordoma xenograft model.

Methods—The authors implanted independent tumor samples from 2 patients into athymic nude mice. The resulting xenograft line was characterized by histopathological analysis and immunohistochemical staining. The patient's tumor and serial passages of the xenograft were genomically analyzed using a 660,000 single-nucleotide polymorphism array.

Results—A serially transplantable xenograft was established from one of the 2 patient samples. Histopathological analysis and immunohistochemical staining for S100 protein, epithelial membrane antigen, and cytokeratin AE1/AE3 of the primary patient sample and the xenografts confirmed that the xenografts were identical to the original chordoma obtained from the patient. Immunohistochemical staining and western blot analysis confirmed the presence of brachyury, a recently described marker of chordomas, in the tumor from the patient and each of the xenografts. Genome-wide variation was assessed between the patient's tumor and the xenografts and was found to be more than 99.9% concordant.

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Disclosure

Author contributions to the study and manuscript preparation include the following. Conception and design: Gallia. Acquisition of data: Siu, Salmasi, Binder, Tran. Analysis and interpretation of data: Gallia, Siu, Salmasi, Orr, Zhao, Hann. Drafting the article: Gallia, Siu, Salmasi, Orr, Hann. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Gallia. Study supervision: Gallia.

Portions of this work were presented at the Third International Chordoma Research Workshop in Bethesda, Maryland, March 18, 2011.

Conclusions—To the best of their knowledge, the authors have established the first primary chordoma xenograft that will provide a useful preclinical model for this disease and a platform for therapeutic development.

Keywords

animal model; brachyury; chordoma; xenograft; oncology

Chordomas are rare, locally infiltrative tumors that occur in the axial skeleton, with cranial chordomas accounting for 32% of all chordoma cases in the US.¹² The current standard of care for chordomas is resection followed by high-dose radiotherapy.¹ Complete resection, however, is difficult due to the location and infiltrative nature of these tumors. Additionally, chordomas are resistant to radiotherapy and chemotherapy. To identify novel and efficacious therapies for the treatment of chordomas, preclinical animal model systems are necessary. Currently, there are only a few primary chordoma cell lines that have been established. The first chordoma cell line, U-CH1, derived from a recurrent sacrococcygeal chordoma after radiotherapy, was reported in 2001.¹⁸ Several other lines have since been reported, including 3 lines derived from clival chordomas,¹⁷ a metastatic lesion from a soft-tissue neck mass,¹⁴ a primary sacral chordoma (CCL-3),¹³ a recurrent lumbar spine chordoma (CH 8),²¹ and a scapular tumor (EACH-1).⁶ In general, chordoma cell lines have been difficult to establish, grow slowly in vitro,^{18,21} and in some instances undergo crises with continued passaging.⁶ Of the reported cell lines, only 2, EACH-1 and U-CH1, have been grown as xenografts in athymic nude mice.^{6,16} To the best of our knowledge, a primary human chordoma xenograft model has not been established.

Numerous previous studies have demonstrated that primary xenografts more closely resemble the parental tumors, genotypically and phenotypically, than do cell lines derived from the parental tumor. In one study, karyotype and gene expression analysis performed on a primary breast carcinoma as well as a cell line and xenograft derived from that sample showed the greatest similarity between the xenograft and the primary tumor.⁹ Recently, in a model of small cell lung cancer, the patient sample and primary xenograft shared expression of tumor-specific genes, whereas that expression signature was lost upon in vitro culturing.⁵ A similar phenomenon occurs in glioblastomas, where amplification of the epidermal growth factor receptor in primary glioblastomas is not reflected in standard adherent glioblastoma cell culture models but is maintained in vivo when passaged as a primary serial xenograft.¹⁵ These findings strongly support the need to establish xenografts from primary tumors that better retain the fidelity of the original tumors, and therefore produce more clinically relevant model systems. Here, we describe the establishment and characterization of a serially transplantable primary chordoma xenograft model, JHH-2009-011.

Methods

Establishment of Xenograft

Under a Johns Hopkins Institutional Review Board–approved protocol, primary tumors from 2 different patients were each implanted subcutaneously into two 5–8-week-old female athymic nude mice (NCI-Frederick) as previously described.¹⁵ Briefly, the surgical specimen was mechanically dissociated using scalpels into small mince, mixed in a 1:1 ratio with growth factor–reduced Matrigel (BD Biosciences CA), and injected subcutaneously into the flanks of athymic nude mice with 200 μ l used for each injection. All animal protocols and procedures were performed in accordance with the Johns Hopkins Animal Care and Use Committee guidelines. The mice were housed in standard facilities and given free access to Baltimore City water and chow. Animals were monitored frequently for signs of tumor growth. Xenografts were passaged in a similar fashion as described above.

Clinical Information

The tumor from which the serially transplantable primary xenograft was established in this study was derived from a male patient who received his initial diagnosis at 56 years of age. He presented in September 2006 with double vision and was found to have a large skull base mass involving the superior aspect of the clivus with extension into the sellar and suprasellar space. He initially underwent a left frontotemporal craniotomy in September 2006, and the pathological examination revealed a chordoma. He then underwent a sublabial transsphenoidal approach in November 2006 for disease in the sella and clivus, and in February 2007 he completed fractionated radiotherapy. In November 2008, he underwent a second sublabial transsphenoidal resection for progressive disease. His tumor subsequently progressed and, in May 2009, he underwent an expanded endonasal endoscopic approach and resection. The primary tumor used to establish the described xenograft was from this resection, that is, the patient's fourth surgical procedure.

Histopathological and Immunohistochemical Analyses

All histopathological and immunohistochemical analyses were performed using tissue fixed in 10% formalin and embedded in paraffin. Tissue was obtained from patient samples after appropriate approval was obtained from the Johns Hopkins University Institutional Review Board. Tissue sections were evaluated from the original patient sample (P0), 2 first generation xenografts (P1-A and P1-B), and 1 second generation xenograft (P2). Paraffin-embedded sections were cut at 5 μ m, deparaffinized, and stained with either H & E or immunohistochemical stains as specified. Immunohistochemical analysis was performed using antibodies specific for S100 protein (dilution 1:6000, Dako USA), cytokeratin AE1/AE3 (dilution 1:4000, Chemicon/Millipore), EMA (dilution 1:1000, Ventana), and brachyury (dilution 1:50, Santa Cruz Biotechnology). For brachyury immunohistochemical analysis, citrate buffer (Biogenex) was used for antigen retrieval. Detection of antibody binding was achieved using a biotinylated secondary antibody and either horseradish peroxidase-conjugated streptavidin (Ventana) for S100 protein, cytokeratin AE1/AE3, and EMA or horseradish peroxidase-conjugated anti-goat polymer (Dako USA) for brachyury. Immunostaining was visualized using the chromogen 3',3'-diaminobenzidine (DAB) (Dako USA).

Western Blotting

Whole-cell extracts were made from primary tumor, xenografts, and the short-term cultures using a standard radio-immunoprecipitation assay lysis buffer supplemented with protease and phosphatase inhibitor cocktails. Lysates were incubated on ice for 30 minutes and then centrifuged at 14,000 rpm at 4°C for 10 minutes to remove debris. Forty micrograms of protein, as determined by the BCA protein assay kit (Pierce Biotechnology), was heated for 5 minutes with 2.5% β -mercaptoethanol/lithium dodecyl sulfate sample buffer (Invitrogen) and loaded onto 4%–12% NuPAGE Bis-Tris gels (Invitrogen). Gels were transferred to a polyvinylidene difluoride membrane (Bio-Rad) and probed according to standard procedures. Briefly, the membrane was blocked for 1 hour with 0.1% Tween/5% nonfat dry milk and probed with the goat polyclonal anti-brachyury antibody clone C-19 (Santa Cruz Biotechnology) at a dilution of 1:1000 in blocking buffer overnight at 4°C. The membrane was then washed and incubated for 1 hour at room temperature with anti-goat secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology). Signals were visualized by chemiluminescence according to the manufacturer's instructions (SuperSignal West Femto and SuperSignal West Pico, Thermo Scientific).

Establishment of Short-Term Cultures From JHH-2009-011

Harvested xenografts were mechanically and enzymatically dissociated with collagenase type IV (GIBCO) and underwent red blood cell lysis with lysing buffer according to the manufacturer's instructions (BD Biosciences). Tumor cells were resuspended in DMEM (Invitrogen) supplemented with 10% fetal calf serum (Gemini Bio-Products) and 1% penicillin-streptomycin (Invitrogen) and incubated at 37°C with 5% CO₂ in a humidified incubator. When cultures reached 80%–90% confluence, they were subcultured with trypsin-EDTA (Invitrogen). The growth rate was ascertained by plating various numbers of cells and using Alamar Blue (Invitrogen) to monitor cell growth daily over a 5-day period.

Single-Nucleotide Polymorphism Genotyping

DNA was extracted from the patient's primary tumor (P0) and the xenografts from both the P1-A and P2 generations using the Qiagen DNeasy kit (Qiagen) following the manufacturer's instructions. Genotyping was then performed at the Johns Hopkins University SNP Center using the Illumina Human660W-Quad v1 DNA analysis Beadchip (Illumina).

Analysis of Genotype Data

The Illumina Human660W Beadchip was used to analyze copy number variances in tumor samples. All SNP positions were based on the hg18 (Human Genome, NCBI Build 36) version of the human genome reference sequence. Fluorescence intensity image files were processed using Illumina BeadStation software to provide normalized experimental intensity values (R) for each SNP position. For each SNP, the R value was compared with the intensity values for that SNP from a training set of normal samples and represented as a ratio (called the "Log R ratio") of $\log_2(R_{\text{experimental}}/R_{\text{training set}})$. Single-nucleotide polymorphism concordance analyses were performed to assess the percentage identity of the original patient tumor (P0) and the subsequent xenograft passages 1 (P1-A) and 2 (P2). Briefly, the heritability/reproducibility analysis was performed using the GenomeStudio software to analyze SNP concordance in a pairwise fashion between P0 and P1-A and between P0 and P2. The result of this analysis was the "reported error rate," which represented SNPs that differed between the 2 samples in the analysis. Thus, the percentage SNP concordance rate was calculated using the following formula: % SNP concordance = $100 \times (1 - \text{reported error rate})$.

Homozygous deletions were defined as 3 or more consecutive SNPs with a Log R ratio value of -2.0 or less. As all 3 samples were from the same origin, we assume that most of the allele status would be concordant across these tumor samples. As such, at one SNP locus, the scenario of Log R ratio value -2.0 or less in 1 sample and -1.8 or less in the other 2 samples was also considered acceptable. The first and last SNPs of the homozygous deletion region were considered to be the boundaries of homozygous deletions. To eliminate chip artifacts and potential copy number polymorphisms, we removed all homozygous deletions that were also detected in 2 normal control samples. Adjacent homozygous deletions separated by 3 or fewer SNPs were considered to be part of the same deletion, as were homozygous deletions within 100,000 bp of each other. To identify the target genes affected by homozygous deletions, we compared the location of genes in the RefSeq dataset with the genomic coordinates of the observed homozygous deletions. Any gene with boundaries overlapping with a homozygous deletion was considered to be affected by the deletion.

Results

Establishment of Primary Chordoma Xenograft

Tumors from 2 different patients were implanted into the flanks of 2 athymic nude mice per tumor. The first tumor specimen implanted did not form any xenografts. A serially transplantable xenograft was established from the second surgical specimen implanted into nude mice (JHH-2009-011). This specimen was taken from a patient with a recurrent clival chordoma (Fig. 1). Two animals (P1-A and P1-B) were implanted from this patient's surgical specimen (P0) with both animals forming xenografts (100% tumor take). The P1-A was a faster growing tumor and, interestingly, the times between subsequent passages derived from the P1-A lineage have been decreasing with successive passaging, from 215 days for the first passage after implantation of the surgical specimen to an average of 103 days for the last passage. This xenograft lineage has been growing as primary xenografts that have been serially passaged for more than 520 days in vivo and is currently in passage 4 (P4) with successful tumor growth in 13 of 14 implantations (93% tumor take) in this generation. Figure 2A shows a nude mouse with bilateral flank tumors 120 days after implantation.

Serially Passaged Primary Chordoma Xenografts Maintain Histological and Immunohistochemical Profile as Well as Brachyury Expression of the Original Patient Tumor

All xenografts were examined macroscopically, and grossly, the xenografts had a lobulated appearance (Fig. 2B). The second patient's original surgical specimen (P0), xenografts from both P1-generation animals (P1-A and P1-B), and a xenograft from one of the P2 generation animals (derived from P1-A) were examined microscopically. On H & E-stained sections, the patient's tumor (P0) showed a lobulated architecture with individual lobules separated by fibrous septae. The tumor was composed of bland epithelial cells, many of which contained prominent vacuoles in the cytoplasm ("physaliphorous cells"). The tumor cells had round nuclei with minimal pleomorphism and were associated with a myxoid stroma (Fig. 3). No mitotic figures were identified, and immunohistochemical analysis for Ki 67 (MIB-1) revealed a relatively low percentage of immunopositivity (approximately 1%, data not shown). The xenografted tumors showed a similar histological appearance with a variable proportion of physaliphorous cells. Immunoperoxidase stains specific for S100 protein, and cytokeratins (AE1/AE3) were intense and diffusely positive in the primary tumor and the xenografts (Fig. 3). Immunoperoxidase stains specific for EMA showed surface staining in all specimens (data not shown).

We next investigated the expression of brachyury, an immunohistochemical marker with high specificity for chordoma.²⁰ Both the patient sample and the xenograft-derived tumors showed immunoreactivity limited to the nuclei (Fig. 4A). Taken together, the histopathological and immunohistochemical characteristics of the tumor samples were diagnostic for chordoma. Importantly, the morphology and staining characteristics were consistent in the patient sample and the xenografts. Furthermore, the appearance and immunoprofile of the tumor were maintained in the xenografts throughout multiple passages in vivo.

The expression of brachyury was next examined by western blot analysis. All tumors including the original patient tumor and each xenograft had strong brachyury expression (Fig. 4B). Interestingly, the xenografts had higher brachyury expression than the primary tumor.

Short-Term Cultures

We were able to establish short-term adherent cultures from the JHH-2009-011 xenografts (Fig. 5). The cultures were a mixture of at least 2 cell types. The most notable were smaller cells, which had the highly vacuolated appearance consistent with the physaliphorous nature of chordoma cells (Fig. 5A); the cells in the second population were larger and nonvacuolated (Fig. 5B). The cultures grew slower than other cell lines such as glioblastoma (data not shown) and exhibited standard growth kinetics (Fig. 5C). Cells were passaged approximately every 10–14 days. The short-term cultures were positive for brachyury; however, the expression was less than that of the primary tumor (Fig. 5D).

Genomic Analysis of Original Surgical Specimen and Primary Xenografts

We next investigated the identity and copy number variation of the primary tumor (P0) and 2 passages (P1-A and P2) of its subsequent xenograft using whole-genome genotyping. This study was performed after the xenograft had been established and was growing in vivo for more than 13 months. The SNP concordance rate for P0 and P1-A was 99.96%, and the SNP concordance rate for P0 and P2 was 99.97%. There were no significant amplifications (> 10 copies) observed in either the original patient sample or the xenografts. The lack of significant amplifications in primary chordomas has been previously described.⁷ Numerous homozygous deletions were identified (Table 1). All 3 samples (P0, P1-A, and P2) were identical regarding homozygous deletions except in one location. On chromosome 4, P0 showed loss of heterozygosity, and P1-A and P2 showed a homozygous deletion. There are no genes, however, that fall in this region.

Discussion

The aim of this study was to establish a preclinical xenograft model of chordoma from primary tumors to allow for future investigation of chordoma tumorigenesis and development and evaluation of therapeutics. We injected 2 independent patient samples into athymic nude mice, and a serially transplantable xenograft was established from one of these patient samples. Our data demonstrate that this xenograft model is similar to the original patient's sample histologically, immunohistochemically, and genomically.

Historically, it has been difficult to establish chordoma cell lines, and only a few chordoma cell lines are found in the literature.^{6,13,14,17,18,21} Many of these cell lines are slow growing and consequently challenging to work with in vitro.^{18,21} We also attempted to establish cell lines during this study and were successful in establishing short-term cultures from our xenografts. These cell lines contained vacuolated cells and were positive for brachyury by western blot analysis. We are currently in the process of establishing long-term chordoma cultures.

To the best of our knowledge, only 2 xenograft models of chordoma have been reported in the literature, both of which are based on the injection of established chordoma cell lines EACH-1⁶ and U-CH1.¹⁶ Ten million EACH-1 cells were injected subcutaneously in the flanks of 3 female athymic nude mice, which led to tumor formation after approximately 4 weeks,⁶ whereas subcutaneous implantation of 5 million U-CH1 cells into the flanks of NOD/SCID/interleukin 2 receptor^γ null mice led to tumor formation 10 weeks postimplantation.¹⁶ Interestingly, this latter model appeared to be dependent on the mouse background used, as no tumors resulted from the implantation of 5 million U-CH1 cells into the flanks of CD1 nude mice.¹⁶ Our study is the first to establish a serially transplantable primary chordoma xenograft model. Notably, this xenograft was derived from a patient who had previously received radiotherapy; however, the significance of radiation therapy to engraftment in this model is unclear.

Discriminating chordoma from other tumors with similar histological appearance, such as chondrosarcoma and chordoid meningioma, often depends on immunohistochemistry for S100 protein, EMA, and cytokeratins.³ Many approaches to identify additional biomarkers for chordomas have been used including cytogenetic,^{2,18} gene expression,^{8,19} and proteomic profiling.²² These efforts resulted in the identification of 2 potential biomarkers, brachyury^{8,19} and keratin 10.²² Although still in its infancy, the use of immunohistochemical staining for brachyury is increasingly being used in the diagnosis of chordoma.

Brachyury is a T-box transcription factor that regulates notochord development^{10,11} and was found to be expressed in 53 of 54 chordomas and absent in 323 other nonchordoma tumors, including tumors that are sometimes mistakenly identified as chordomas such as chondrosarcomas and enchondroma.²⁰ It has thus been suggested that brachyury is a highly specific biomarker of chordomas.²⁰ A recent study reported frequent copy number gains in the region of *T*, the gene encoding brachyury, in a series of sporadic chordomas from 181 patients.¹⁶ In our study, we have shown by both immunohistochemistry and western blot analysis that the patient tumor and serial xenografts express brachyury. Our copy number analysis data indicate that the high level of brachyury expression in the patient tumor and successive xenograft passages is not due to amplification. Interestingly, we observed an increased expression of brachyury in our xenografts on western blot analysis compared with the original tumor sample. One possible explanation for this finding is that the patient sample contained other nonneoplastic tissue such as stromal elements that reduced the proportion of brachyury that we observed in the assay.

Preclinical therapeutic development relies on accurate and reproducible in vitro and in vivo model systems. Moreover, in vitro models are imperative for large-scale drug screening and biological manipulation.⁴ In a recent study, the U-CH1 chordoma cell line was demonstrated to express brachyury, and upon knockdown of brachyury, the cells senesce.¹⁶ These findings strongly support the role of brachyury in chordoma tumorigenesis. The maintenance of brachyury expression in vivo, coupled with the close genomic and histological resemblance of the serial xenografts to the patient tumor, suggests that this primary xenograft model closely recapitulates the biology of the original tumor. Therefore, the combination of this primary xenograft model with the available in vitro models of chordoma will help facilitate the identification of more efficacious therapeutics.

Conclusions

We have established the first primary chordoma xenograft model. This xenograft model is serially transplantable and genomically more than 99.9% identical to the original parental tumor by whole-genome SNP array analysis. We believe this xenograft model will be useful for understanding the biology of chordomas and as a preclinical therapeutic model.

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Abbreviations used in this paper

EMA	epithelial membrane antigen
SNP	single-nucleotide polymorphism

Fig. 1. Preoperative MR images. Preoperative sagittal T1-weighted image obtained after administration of Gd (**A**) and axial T2-weighted image (**B**) demonstrating a large clival chordoma with significant intracranial extension and mass effect on the optic apparatus and the brainstem.

Fig. 2.

Photographs of the primary chordoma xenograft. **A:** Photograph of a nude mouse with bilateral chordoma xenograft tumors. **B:** Gross appearance of the bilateral xenograft tumors harvested from the animal shown in panel A demonstrating a lobulated appearance.

Fig. 3.

Histochemical and immunohistochemical evaluation of chordoma xenografts. Paraffin-embedded and formalin-fixed sections from the patient's primary tumor and chordoma xenografts were stained with H & E (**A–D**), and immunoperoxidase stains specific for cytokeratin (AE1/AE3) (**E–H**) and S100 protein (**I–L**). Original magnification $\times 100$.

Fig. 4. Brachyury expression. Expression of brachyury in the patient tumor (P0), xenograft passage 1, mouse A (P1-A), xenograft passage 1, mouse B (P1-B), and xenograft passage 2 (P2) was determined by immunohistochemical (A) and western blot (B) analyses. A negative control of normal brain (B) was also run in the western blot analysis. Original magnification $\times 160$.

Fig. 5. Short-term cell line cultures. **A:** Cells with the typical physaliphorous features characteristic of chordoma. **B:** Non-vacuolated cells present in the chordoma cultures. **C:** Growth curves of JHH-2009-011 cultures. **D:** Western blot analysis for brachyury expression: patient tumor (P0), U87MG glioma cell line, and JHH-2009-011 culture.

TABLE 1

Homozygous deletions in the original patient's tumor and P1 and P2 xenografts

Chromosome	Lt Boundary	Rt Boundary	Genes Affected
1	12834675	12841406	<i>PRAMEF2</i>
1	17548878	17549783	<i>PADI4</i>
1	24394051	24395330	
1	59821282	59822279	<i>FGGY</i>
1	79994456	79995483	
1	105469194	105469921	<i>MIR548H3</i>
1	105820402	105824879	
1	109374972	109375907	<i>WDR47</i>
1	150822709	150853560	<i>LCE3B</i>
1	151027995	151036431	<i>LCE1D</i>
1	157915415	157916146	
1	177874005	177874559	<i>TDRD5</i>
1	241849397	241849503	<i>AKT3</i>
2	48705324	48711100	<i>STON1-GTF2A1L, GTF2A1L</i>
2	54419233	54420945	<i>C2orf73</i>
2	76627465	76628891	
2	106061528	106062279	
2	127391209	127393549	
2	176977915	176979982	
2	226873831	226879199	
3	11620935	11621509	<i>VGLL4</i>
3	32078538	32081747	
3	47960108	47960507	<i>MAP4</i>
3	53003023	53014023	<i>SFMBT1</i>
3	65092987	65094573	
3	68720227	68722130	
3	84782456	84784518	
3	131140071	131140534	
3	163995377	164108060	
3	164247727	164251658	
3	194358175	194367719	
4	1572949	1573645	
4	6703062	6703648	
4	9822425	9842122	
4	26565103	26566534	<i>STIM2</i>
4	45887802	45899264	
4	61012841	61014357	
4	61623048	61624795	
4	64381774	64392783	

Chromosome	Lt Boundary	Rt Boundary	Genes Affected
4	92152066	92154802	<i>FAM190A</i>
4	115397654	115401739	
4*	130865005	131284140	
4	153011104	153014151	
4	161276893	161291349	
4	162098516	162104179	
4	166222921	166224305	<i>TMEM192</i>
4	171506266	171510282	
4	173662184	173668143	<i>GALNTL6</i>
4	187330534	187335065	<i>FAM149A</i>
4	190430563	190437117	
5	97424939	97428471	
5	119407151	119410592	
5	133162928	133164240	
5	160521628	160522618	
6	8282785	8286506	
6	18510124	18510700	<i>RNF144B</i>
6	34808615	34808904	
6	35734478	35737647	<i>FKBP5</i>
6	134310701	134311350	
6	152431765	152433867	<i>ESR1</i>
7	22401556	22403183	
7	51562248	51565974	
7	75983937	75984150	<i>UPK3B</i>
7	81279641	81280110	
7	93379933	93380386	
7	118618611	118621912	
7	133435759	133448505	
7	144401329	144404870	
7	154027571	154029878	<i>DPP6</i>
7	158193566	158197503	
8	40588920	40589336	<i>ZMAT4</i>
8	97590068	97591041	<i>SDC2</i>
8	104063951	104065695	
8	115708822	115712501	
8	118943488	118944716	<i>EXT1</i>
8	137230218	137232998	
9	138812167	138812960	<i>KIAA1984</i>
9	31281381	31282343	
9	88344799	88345514	
9	95510192	95511161	
9	112065015	112069244	

Chromosome	Lt Boundary	Rt Boundary	Genes Affected
9	129854187	129854607	
9	135615086	135615756	
9	137618998	137619990	
10	4698627	4700298	<i>LOC100216001</i>
10	5930694	5932371	
10	20037056	20037505	
10	27040564	27041820	<i>PDSSI</i>
10	31483573	31484703	
10	58879868	58881790	
10	70953496	70960940	
10	107940701	107941540	
11	11779614	11780562	
11	18055211	18056598	
11	29924172	29924814	
11	42770773	42775011	
11	81181640	81194909	
11	88604167	88607231	<i>TYR</i>
11	98690793	98691890	<i>CNTN5</i>
11	124580785	124589952	<i>PKNOX2</i>
11	134108466	134112648	
12	17498732	17499001	
12	130417683	130417936	
13	38832518	38833150	<i>LHFP</i>
13	48431569	48434645	
13	71376245	71378590	
13	89660867	89662720	
13	97328242	97330914	
13	98052451	98055147	
14	21951567	21952033	
14	39683688	39687345	
14	82505002	82506817	
15	97392342	97392917	
15	98235951	98239480	
17	38872860	38873711	
18	9805285	9806173	<i>RAB31</i>
18	22825671	22826239	<i>CHST9</i>
18	33560099	33560607	
18	45950355	45952266	<i>MYO5B</i>
18	47450904	47452176	
18	65359372	65368251	<i>DOK6</i>
19	2860848	2861369	<i>ZNF57</i>
19	5461306	5461667	

Chromosome	Lt Boundary	Rt Boundary	Genes Affected
19	13637129	13637658	
19	35979673	35981028	
20	1517540	1526976	<i>SIRPB1</i>
20	39340428	39340645	<i>ZHX3</i>
21	42223268	42226436	<i>C2CD2</i>
21	44443902	44445133	
22	22606746	22606825	
22	35473443	35476816	
22	36078340	36079892	
X	77008393	77010361	<i>MAGT1</i>
X	94937533	94938060	
Y	20176596	20355649	<i>KDM5D, CYorf15B, CYorf15A</i>

* P0 showed loss of heterozygosity while P1-A and P2 showed homozygous deletion.