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Runs of homozygosity and a cluster of vulvar cancer in young Australian Aboriginal women

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HIGHLIGHTS

- We performed a genetic study of a vulvar cancer cluster in Arnhem Land women.
- No effects of genome-wide homozygosity or individual ROHs were observed.
- Prior diagnosis of CIN was associated with diagnosis of vulvar cancer or VIN.

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ABSTRACT

Objective. A cluster of vulvar cancer exists in young Aboriginal women living in remote communities in Arnhem Land, Australia. A genetic case–control study was undertaken involving 30 cases of invasive vulvar cancer and its precursor lesion, high-grade vulvar intraepithelial neoplasia (VIN), and 61 controls, matched for age and community of residence. It was hypothesized that this small, isolated population may exhibit increased autozygosity, implicating recessive effects as a possible mechanism for increased susceptibility to vulvar cancer.

Methods. Genotyping data from saliva samples were used to identify runs of homozygosity (ROH) in order to calculate estimates of genome-wide homozygosity.

Results. No evidence of an effect of genome-wide homozygosity on vulvar cancer and VIN in East Arnhem women was found, nor was any individual ROH found to be significantly associated with case status. This study found further evidence supporting an association between previous diagnosis of CIN and diagnosis of vulvar cancer or VIN, but found no association with any other medical history variable.

Conclusions. These findings do not eliminate the possibility of genetic risk factors being involved in this cancer cluster, but rather suggest that alternative analytical strategies and genetic models should be explored.

Introduction

Cancer of the vulvar is relatively rare, and usually occurs in postmenopausal women [1]. However, the incidence of vulvar cancer among Indigenous women aged less than 50 years living in remote communities in the East Arnhem district of the Northern Territory of Australia (see Fig. S1) is more than 70 times the national incidence rate in the same age group (31.1 per 100 000 compared with 0.4 per 100 000) [2]. Vulvar cancer in pre-menopausal women, as found in communities within the East Arnhem district and several communities bordering that district (hereafter referred to as Arnhem Land), is associated with persistent human papillomavirus (HPV) infection, particularly genotype 16, whereas in older women it is more usually associated with a dermatological condition called lichen sclerosus [3–5]. The precursor lesion to HPV-related invasive vulvar cancer is vulvar intraepithelial neoplasia (VIN) usual type (warty, basaloid and mixed) [6], and the incidence of VIN in this population is similarly high (34.7 per 100 000 in women aged less than 50 years) [2].

Previous work with the Arnhem Land population, however, found no evidence that higher rates of HPV infection [7] or that particularly virulent strains of HPV [8] could explain the excess incidence of vulvar cancer in this population, suggesting the possible involvement of additional environmental and/or genetic factors that may impair host immunity. Several environmental factors, including smoking and some sexually transmissible infections (such as gonorrhea and herpes simplex virus 2), have previously been found to be associated with

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increased risk of vulvar cancer [9–12]. However, these factors are unlikely to account for the cluster in Arnhem Land, as they occur at similar rates to that found in other Indigenous populations in the Northern Territory where there is no excess of vulvar cancer [13,14]. Of greater possibility is a hereditary cofactor, based on evidence of familial clustering of HPV-associated cancers observed in the Swedish population [15], combined with reports from gynecologists servicing the Arnhem district that the cases appear to occur in distinct family groups.

To date, little work has been undertaken investigating genetic risk factors involved in vulvar cancer, partly as a result of its relative rarity and the consequent difficulty in achieving an adequate sample size to detect real effects. A recent population based case–control study in the United States used a candidate gene approach to identify a suggestive association between common variants within the TNF gene region and increased risk of vulvar cancer [16]. If the cancer cluster in Arnhem Land has a genetic basis, however, the variant(s) involved is likely to be rare, to have a large effect size, and to stem from a mutation in a common ancestor. The affected communities in Arnhem Land comprise predominantly Indigenous populations, ranging in size from several hundred to approximately 2500 people. The small populations and extremely remote locations of the affected communities suggest the potential involvement of recessive effects resulting from increased parental relatedness, as disease alleles are more likely to be inherited identical-by-descent, although alternative population genetic scenarios are possible.

This mechanism could act either through recessive variants of large effect sizes, or through the cumulative influence of genome-wide homozygosity linking many recessive variants of small effect size [17]. Genetic association studies in small, isolated populations can be limited by power considerations, and by the presence of extended regions of homozygosity. However, analytical approaches utilizing these homozygous regions present a useful, and hitherto relatively neglected, complementary approach to allelic, genotypic and haplotypic association studies [18]. The possibility that the etiologies of complex diseases include recessive alleles at many genetic loci, each of small individual effect, and that increases in genome-wide homozygosity resulting from consanguineous pairings could increase the risk of these diseases, was first proposed a decade ago [19]. Since that time, a number of studies have assessed the influence of homozygosity in various diseases by employing high-density single nucleotide polymorphism (SNP) data to estimate the proportion of the autosome in runs of homozygosity (ROHs), resulting from inheriting identical haplotypes from each parent.

These ROHs are more common in outbred populations than previously thought, and their average length is proportionate to the number of generations since the common ancestor; longer runs (>5 Mb) are associated with recent parental relatedness, and shorter runs are indicative of ancient parental relatedness [17]. Associations have been identified between whole-genome ROH burden and schizophrenia [20] and with intellectual disability in simplex autism [21], although other studies found no association with survival to old age [22], risk of breast and prostate cancer [23], colorectal cancer [24], or multiple sclerosis [25]. More recently, other studies have extended the ROH analysis to identify specific loci associated with disease. This homozygosity mapping approach has been successful in identifying candidate loci for rheumatoid arthritis [26], human adult height [27], schizophrenia [28], autism spectrum disease [29] and Alzheimer Disease [30,31].

Because these analytical approaches are relatively new and consensus regarding defining criteria are yet to be firmly established, these studies employed a range of thresholds for defining ROHs [32]. Howrigan and colleagues [33] went some way towards addressing this problem by using simulation to assess the power of various methods of detecting autozygosity; importantly, their work confirmed the need to prune datasets for linkage disequilibrium (LD) and provided recommendations for thresholds aimed at detecting ancient and recent autozygosity. Accurately detecting regions inherited identical-by-descent increases the likelihood of identifying rare, semi-recessive genetic variants involved in disease etiology.

The current study aims to assess the role of partially recessive, deleterious variants in the vulvar cancer cluster in young Aboriginal women in Arnhem Land by investigating autozygosity assessed by genome-wide ROH burden and single ROH association mapping. A secondary aim is to investigate possible environmental cofactors using data extracted from participants' clinical records, relating to smoking and prior diagnoses of infections and HPV-related neoplasia.

Materials and methods

Study population

A genetic case–control study of the vulvar cancer cluster among young Aboriginal women resident in Arnhem Land was undertaken in 2011–2013, in seven Indigenous communities and a number of smaller outstations identified based on our earlier work [2,7]. These sites represent the majority of the affected communities. Given the sensitive nature of the research, prior to commencing the study, the research team consulted with elders, health boards and health services in the affected communities about the nature of research, the most appropriate ways to proceed as well as appropriate research dissemination strategies, as described previously [34]. Furthermore, an Indigenous Reference Group (IRG) that had been established during our previous study was reconstituted to advise on all aspects of the study. Ethical approval was received from the Top End Human Research Ethics Committee in 2011.

Using data from the Northern Territory Cancer Registry and the Gynaecology Outreach Service colposcopy database, women were identified who met the following criteria: had been diagnosed with vulvar cancer or high-grade VIN between 1996 and 2011; identified as Aboriginal and/or Torres Strait Islander; and their usual place of residence was in a community in Arnhem Land. Of the 34 women who met these criteria and were living, 30 were recruited to the study, as well as 62 unaffected controls, matched for age and community of residence. All participants gave written informed consent and provided two saliva samples using Oragene (OG-500) DNA collection tubes (DNAGenotek, Ottowa, Ontario, Canada). Participants were asked about their smoking status (at the time of diagnosis for cases, and at the time of recruitment for controls). Medical records from community primary health centers were used to confirm participants' date of birth, and their case/control status from previous Well Women's Screening health checks (an assessment which includes a Pap smear, vulvar examination, and discussion of sexual and reproductive health issues). Medical records were also accessed to extract information about any concurrent or previous diagnosis of cervical intraepithelial neoplasia (CIN) and any concurrent or previous infection with syphilis, gonorrhea, trichomoniasis, chlamydia or other sexually transmissible infections (STI). In instances where a control had not recently participated in a Well Women's Screening health check, they were offered screening in conjunction with community primary health centers and the NT Centre for Disease Control Sexual Health coordinator.

Global comparative data for genomic ROH in different populations were sourced from the Human Genome Diversity Project (HGDP) [35], adapting the methods used by Kirin and colleagues [36]. The HGDP has a controversial history with Australian Aboriginal populations [37]. As no suitable alternative could be found, use of this data was only undertaken after consultation with the IRG, who approved its use for comparative purposes.

Genotyping

Genomic DNA was extracted from saliva samples, according to the manufacturer's directions, and DNA concentration and purity were assessed using a NanoDrop 8000 (Thermo-Fisher Scientific) and