WZRD1 Gene, A Possible Marker for Squibidity Disorder in *Homo Sapiens*

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From the limited number of studies previously done on Squibidity, there is great potential for advancements that would further our understanding of this disorder, and which would benefit both magical and non-magical people. Squibidity, is one of the most mysterious conditions to affect the Wizarding world. It is estimated that approximately 1 out of every 1200 individuals, who are born into a Wizarding family, are affected. The severity of this condition varies, but most individuals with this phenotype lack the ability to perform magic of any kind, although they may still able to observe magic as well as interact with certain magical objects and creatures. In our experiment, we look to find a link between genetics and Squibidity. In particular, three genes have shown promise to be strong indicators of Squibidity; *MGC3, QDCH12*, and *WZRD1*. Examination of all three genes, shows that there is great between the magical population and squib populations. As expected, genes for individuals are shown to be more similar to individuals of the same corresponding group, with *WZRD1* showing 72% similarity within Squibs, *QDCH12* showing 82%, and *MGC3* with 35% similarity. FISH experiments performed on the *WZRD1* gene shows that all 32 Squibs that were sampled in this study had a common copy number deletion in the 22q18.4-q19.3 of Chromosome 22. Given this result, we believe that the *WZRD1* gene could be a good potential marker for Squibidity.

Keywords: Squibidity, Magic, *WZRD1*, Gene Marker

Introduction

Squibidity is a very rare and unusual disorder in the Wizarding world, appearing in only a handful of children every generation or so. It appears indiscriminately in Wizarding populations, regardless of pureblood or half-blood status. Squibs cannot deliberately practice magic, yet they are distinct from Muggles in that their magical heritage still: (1) grants them immunity from Muggle-repelling charms (Boilsworthamus *et al.*, 1824); (2) gives them access to magical objects; and (3) allows them to keep magical pets and familiars. Unfortunately, their inability to practice magic cripples these children so severely that they often cannot function as citizens in our society where they are often regarded as outcasts. In fact, this condition has been demonstrated to be psychologically harmful with an increased likelihood of suicide for those affected (Sudsore, 2009). Hence the reason why many Wizarding families have adopted the

practice of sending their Squib children off to Muggle boarding schools at a very young age - a kindness since the alternative is usually discrimination and prejudice.

The Society for the Support of Squibs reports two suspected Squib births in the past five years, seven confirmed Squib cases in the past two decades, and thirty-two registered Squibs with their organization at present (Longbottom, 2014). Statistics suggest that approximately 1 in every 1200 individuals born to a Wizarding family will be diagnosed as a Squib around the age of 10 and confirmed at age 11, the nominal age when lack of affinity for a wand is observed or when the child is not offered admission to a magical school (Frickle *et al.,* 1721).

Although these numbers appear to be insignificant at first glance, every Squib birth results in large consequences for the family. As such, St. Mungo's pioneered an research program to investigate Squibidity in 1942, and has since identified a set of genes from the *WIZARDOUS* gene family to play a role in magical ability (Dumbledore *et al.,* 1963). Of these genes, three have been selected as possible Squibidity-causing candidates due to their high degree of variance when compared between the Wizarding population and the Squib population: *WZRD1*, *MGC3* and *QDCH12*. In this paper, these three genes are further characterized and compared between and within the two aforementioned populations using exome resequencing and FISH analysis.

Materials and methods

Whole genome sequencing data for the Squib and Wizarding populations were taken from unrelated individuals and obtained as part of the Magical Genome Sequencing Project (Dumbledore, 1991). 32 Squib and 120 Wizarding sequences were analyzed. These data were generated with Illumina, Roche 454 and Life Technologies sequencing technology platforms. The reads were aligned using ELAND. 76 base-paired end reads were generated with >10x coverage.

Exon Capture and Probe Design

Prior to performing exon capture, we using oligonucleotide baits targeting the exons, promoter and intergenic control regions of the *WIZARDOUS* gene family as identified by Dumbledore (1963). A total of 133,780 baits of 120bp in length were designed using CertainChoice eArray software, covering a total of 13.4 MB genomic regions. The baits targeted more than 12,000 of the 19,000 loci containing coding regions and exons; whereas 32,000 baits specifically targeted 250bp regions upstream of the putative promoter regions. The control regions were roughly evenly distributed through Chromosomes 3, 6, 8 and 22, and selected at random. Resequencing was done using the CertainChoice All Human Exon Capture System, following the manufacturer's standard protocol with minor variations in that the agarose gels in step 12 were omitted.

Target Enrichment and Library Prep

We enriched the targeted exonic regions using Moldywart's CertainChoice technology for Illumina paired-end sequencing. The library was prepared according to the Moistboil Certain Choice protocol, version 1.2; 3.0 ug of DNA (from Squib or Wizarding) was sheared, end-repaired, ligated, and amplified before purification. Library quality was determined using Moistboil DNA chips. 680 ng of the prepped libraries were used in solution with the RNA baits for hybridization, which was carried out at 65C in a PCR machine. The target regions were then purified on magnetic beads, and then post-hybridization amplified. Captured libraries were quantified using real-time PCR and a NanoDrop Spectrophotometer. Quality control was performed on a Mudsore Bioanalyzer. The final concentration of the library samples was 50 nM; they were then sequenced using Illumina in a 2x100 paired-end format.

Data Analysis and FISH

Data analysis was performed by Biggins and Baggins at St. Mungo's for hybridization similarity between the *WZRD1, MGC3* and *QDCH12 genes* of both populations (See File 1: WMQSim_X3). FISH was performed at St. Mungo's, with a probe designed by Madam Pomfrey. The probe labelled 83D9 was designed specifically for the Chromosome 22 region 22q18.4-q19.3, generated by microdissection and DOP-PCR. Chromosomes were taken from peripheral blood lymphocyte cultures at the metaphase stage. DOP-PCR and Microdissection protocol was taken from Boyle *et al*. (2013) and the FISH protocol was taken from Murkywater *et al.* (2009).

Results

Exon Resequencing Hybridization

MGC3
The gene MGC3 was found to be significantly different between the Wizarding population and the Squib population ($p<0.05$). This gene was 74% similar amongst the wizard genomes, and 35% amongst Squibs' genome.

WZRD1
The gene WZRD1 was also identified to be significantly different between the two populations ($p<0.0001$), but was found to be 84% similar within Wizarding population and 72% similar within the Squib sample population (Figure 1a,b). This suggests that mutations contributing to the significant difference between the two populations are likely to be shared between Squibs and, therefore, could contribute to the development of the disorder.

QDCH12
Lastly, QDCH12 showed significant difference between the two populations ($p<0.05$), with only 56% similarity within the Wizarding population, but an impressive 82% similarity within the Squib populations. The QDCH12 baits did not show a clear or

consistent pattern of hybridization in the Wizarding population, but it hybridized on chromosomes 3A, 3B12A, and 12B in 20 Squibs out of the 32 sampled population.

FISH Analysis

An interesting hybridization pattern was observed when both populations' chromosomes were introduced to the 83D9 probe. This probe hybridized to two loci for both chromosome 22 copies in all Wizarding samples, but hybridized to the same two loci of chromosome 22 in only 7 squibs out of the 32 (22%) sampled population. This suggests a possible deletion in the *WZRD1* gene contributing to Squibidity. Subsequent FISH, using a probe specific for the chromosome region 22q18.4-q19.3, revealed deletion in both copies of chromosome 22 in all 32 samples of the squibs population (Figure 1C). This suggests that region 22q18.4- q19.3 is essential in giving wizards and witches the ability to use magic and, could possibly be a marker for the Squibidity disorder.

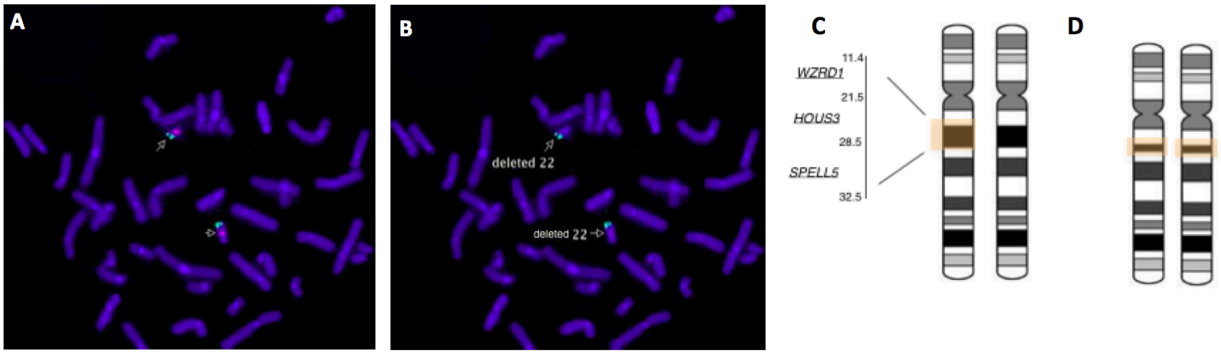

Figure 1. Deletion of the 22q18.4-q19.3 locus within the *WZRD1* gene in squib samples. A - B. Images of fluorescence in situ hybridization with probe 83D9 specific for the chromosome region q18.4-q19.3. **A.** Sample obtained from 1 individual of the squibs population, and **B.** from 1 individual of the wizards population. **C.** Proposed schematic diagram of the human chromosome 22 showing the *WZRD1* gene locus found in Wizarding population. **D**. Proposed schematic diagram of the human chromosome 22 showing the deletion in *WZRD1* gene locus found in Squibs population.

Discussion

Ever since Dumbledore *at al*. (1963) discovered the *WIZARDOUS* gene family and hypothesized its role in the genetics behind magic, many studies have been done to investigate the genetic makeup of the Wizarding population. Indeed, the Magical Genome Sequencing Project (Dumbledore et al., 1991) was pioneered with the intention to determine what exactly made witches and wizards tick. Only recently has this interest been expanded to include Squibs as a subpopulation of the Wizarding world. A previous analysis of Dumbledore et al.'s study (1963) listed three candidate genes for involvement in the Squib disorder: *WZRD1*, *MGC3* and *QDCH12.* To eliminate common

germline mutations from consideration, we limited the list of variants to those above 5% minor allele frequency common in both Squib and Wizarding groups.

The criteria for determining the best candidates was as follows: the genetic content must be shared within the two populations independently of one another; the variance must be significant and shared within the Squib population. Thus we were left with the 3 aforementioned genes as the best candidates since they are significantly different in content and sequence between both populations. Exon resequence hybridization was done to determine the similarity of these genes between the two populations (Squib and Wizarding), and then those genes were further analyzed to see how similar they were within the two populations as well.

The most likely candidate for a Squibidity marker is by far the *WZRD1* gene. Even though there is some genetic variance across both populations for that gene, the Wizards all have two copies of the 22q18.4-q19.3 region on chromosome 22, whereas the Squibs have suffered a deletion so that there is only one copy on the chromosome (Figure 1C). This copy number deletion is present across all Squibs in our sample population, suggesting that it may play a significant role in causing the disorder, or at least identifying Squibitity as a genetic marker. Future studies could investigate a possible frameshift effect on the downstream *HOUS3* and *SPELL5* genes, interrupting their functions and thus promoting the progress of Squibidity.

The gene *WZRD1* was also identified to be significantly different between the two populations ($p<0.0001$), but was found to be 84% similar within Wizarding population and 72% similar within the Squib sample population (Figure 1 A,B). This suggests that mutations contributing to the significant difference between the two populations are likely to be shared between Squibs and, therefore, contribute to the development of the disorder.

MGC3, was significantly different between the two populations which indicates that the *MGC3* gene might contribute to Squibidity disorder. However, the *MGC3* gene is unlikely to be a major contributor to the cause for Squibidity because it was only found to be 35% similar within the Squib genomes. Because it has been reasonably conserved in the Wizarding population (74% similarity), the huge variance across the 32 samples Squibs lends credit to the belief that a lack of overall conservation of MGC3 probably plays a role in Squibidity without being the single defining cause of the disorder.

Future studies should be done regarding the *MGC3* gene because it is a member of the family gene *MAGIC*, which functions in developing the wizard's ability to use magical objects such as wands, brooms and more unique artifacts like the *deluminator*. Previous studies have found the possibility of some sort of distinct pheromone released by the magical individual is detected by sensors in the wand (Lovegood *et al.,* 2002). Although the *MGC3* gene is probably not significant in terms of causing Squibidity or not, it may be significant in the severity of the disorder.

The *QDCH12* gene varies significantly between the two sample populations, but it is surprisingly better conserved in the Squibs than it is in the Wizards. The *QDCH12* baits hybridized on chromosomes 3A, 3B12A, and 12B in 20 Squibs out of 32. Perhaps

the low similarity within the Wizarding population is because there is a non-coding region that is silent, but a de novo gene has arisen in that same region within Squibs that interferes with their magical abilities. Future studies should definitely be conducted in that vein.

Magic is a very complex trait. There are many genes and many gene families that are involved in Wizarding genetics, not to mention the genetics in non-human magical creatures such as centaurs and giants. Squibidity is a rare yet life altering disorder that has always been present in the Wizarding world. The frequency of this disorder has remained very low due to breeding selection against it, but the fact remains that Squibidity appears in perfectly healthy Wizarding families as a seemingly very random phenomenon. The loss of magical ability in any individual is detrimental to our society, since we have such small numbers to begin with. There is much research to be done in this line in order to preserve these abilities and this research represents a potential first step in allowing us to diagnose the disorder and possibly explore preventative measures.

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