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Genetic Association between WZRD-1 Variants and the Variation in Magical Abilities in Canadians

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Current literature lacks a consensus on the mechanism of biological inheritance of magical abilities. Past findings have suggested that the presence of restriction enzyme digestion sites in *WZRD-1* are associated with variations in human magical abilities. In this study, Next Generation Sequencing (NGS) was performed to characterize the genetic sequence of *WZRD-1* in 610 Canadian subjects. These individuals were divided into three cohorts as determined by their magical abilities and blood status. Four genetic variants within *WZRD-1* were identified, and the genotypic and allelic frequencies were determined for each cohort. The genetic variant, *g.[1049_1050insGCACGGTAC]*, was predominately found in wizards and witches with magical heritage, whereas the genetic variant, *g.[1073_1074ins9C]*, was most commonly identified in muggle-born individuals. Furthermore, the genetic variant, *g.[1049_1050insGCACGGTAC;1052_1053ins6A]*, was identified primarily in squibs, however, was also seen among muggle and magical individuals. These findings suggest a potential role for the *WZRD-1* gene in the development of magical abilities, and supports further investigation into the biological function of this region. Further, the identified genetic variants may serve as a possible genetic marker for determining blood status.

Keywords: WZRD-1, Genetic Maker, Genetic Variant, Witch, Wizard, Muggle, Inheritance of Magic Abilities, Next Generation Sequencing.

Introduction

The hereditary nature of magical power has been a mystery for many generations of wizards, witches, and muggles. While it is largely accepted that magical power is inherited from parental witches and wizards to their offspring, there are many notable exceptions to this rule. For example, the capacity for pure bloods to be able to produce squib offspring (lacing magical ability), and the ability of muggles to produce offspring with magical capacities, escape traditional Mendelian genetics.

Generally, the wizarding population can be subdivided into three categories; pure-bloods, half-bloods, and muggle-borns. Pure-bloods are classified as families or individuals with no muggles or muggle-born individuals in their bloodline, however, nearly all witches and wizards have a non-magical ancestor if one traces far back enough in their family-tree. Thus, it is more common to consider one as “pure-blood” if their parents and grandparents are not muggles, nor muggle-born. Alternatively, a witch or wizard with a muggle or muggle-born parent or grandparent is considered a half-blood. Muggle-borns are witches and wizards who were born to two non-magical parents. Their magical abilities are not affected by non-magical parentage. A fourth, and proportionally infrequent, demographic in the wizarding world are called “squibs”. Squibs are non-magical individuals born to at least one magical parent. At most, squibs compose 1% of the wizarding world. It is thought that squibs married to muggles are ancestors of muggle-borns who demonstrate an unexpected resurfacing of magical abilities generations later, however, the mechanism of this phenomena has yet to be elucidated.

Current literature lacks any exploration of the wizarding genome and how it varies among magical and non-magical populations. Notably, there have been some groups that have theorized on the inheritance pattern of the wizarding ability. Craig et al. (2005) were the first group to publish their idea that the wizarding ability is inherited in a Mendelian fashion, with the wizard allele (W) being recessive to the muggle allele (M). Thus, witches and wizards would theoretically possess two copies of the wizard allele (WW), muggle-borns descend from WM parents and possess WW genotypes, and half-bloods descend from one WW parent and one MW parent. However, under this logic, the existence of squibs would be much more common

as they would be the exclusive result of WW and MM parents.

Recent findings have pointed to the *WZRD*-1 gene as playing a potential role in the regulation of magical abilities. *WZRD-1* spans 77-base pairs and is located on the p-arm of the human chromosome 9 (*WZRD-1 g.1026_g.1103*). No known protein is encoded by *WZRD-1*.

The *WZRD-1* region, *g.1047_1082*, is known to be highly polymorphic. Past literature demonstrated that *WZRD-1* polymorphisms could contribute to the observed phenotypes of magical abilities. Using XbaI, EcoRI, and MspI restriction enzymes, Scamander et al. (2001) identified various digestion sites in the *WZRD-1* region that pointed to a possible association between these markers and magical abilities in a population of British witches and wizards. Although the evidence from Scamander's study (2001) fell under much scrutiny, this suggests that *WZRD-1* polymorphisms may have potential to be a predictor of magical abilities. Notably, and despite this polymorphic quality, literature has shown that the flanking sequences of *WZRD-1* are widely conserved among individuals.

This study attempts to use Next Generation Sequencing (NGS) to characterize the *WZRD-1* genetic sequence in Canadian witches and wizards, muggles, and squibs. We aim to test the hypothesis that there may be a predictive relationship shared between *WZRD-1* genetic variance and magical abilities.

Methods

Materials

Common chemicals and reagents were purchased either Sigma-Albus (London, UK) or Thermo Fisher Scientific (Waltham, MA).

Experimental Design

This study was reviewed and approved by the Research Ethics Board of the University of British Columbia and the Committee of Ethical Research on Non-Magical Beings of the Canadian Ministry of Magic. The experimental design was carried out according to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (2018). Informed consent was obtained from all subjects prior to their participation in the study.

A total of 610 subjects, 324 males and 286 females, participated in this study (n= 610). For the purpose of this study, the subjects were separated into three groups: subjects with magical abilities (Magical), subjects without magical abilities (Muggle), and squibs. The criteria for being designated as a subject with magical abilities was dependent on current or previous enrollment to an Institution for Magic Education. 307 witches and wizards alumni with Canadian citizenship from the Ilvermorny School of Witchcraft and Wizardry (160 wizards and 147 witches), and 10 squibs were recruited from various Muggle institutions within Canada, and their family history of magical lineage was traced and verified through the Canadian Ministry of Magic. No magical spells were performed on muggle subjects for recruitment purposes. 293 muggles (158 males and 135 females) who had volunteered at designated Canadian Ministry of Magic locations across Canada were recruited for the study.

DNA Collection

Human buccal cells were collected by gently rinsing inside the subject's mouth using saline. The QIAamp DNA Kit was used to extract genomic DNA (gDNA) from the samples according to the manufacturer's protocol. Nucleic acid concentrations were measured using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA).

Polymerase Chain Reaction (PCR) and Amplification of WZRD-1 Region

PCR was performed on all samples to amplify the *WZRD-1* region using the QIAGEN Multiplex PCR Kit. Primers used in this study were purchased from Sigma-Albus (London, UK). The sequence of the forward and reverse primers used were 5'-ACG GCG GCC AGG ACT GGC GAG-3' and 5'-GCG CCA TCG CGC CGA GCG TGC-3', respectively. PCR was conducted in a total volume of 16.5 µL, containing 7.5 µL QIAGEN Multiplex PCR Master Mix, 2.5µL primer mix, 1.75 µL RNase-free water and 5 µL of DNA sample. The PCR cycling program involved: initial denaturation at 95°C for 15 min, 35 cycles of denaturation at 95°C for 30s, annealing at 55°C for 60s, and extension at 72°C for 60s, and a final extension step at 60°C for 30 min. We confirmed the success of PCR reactions by gel electrophoresis using the MuggleBio Precast Agarose Gel system from Sigma-Albus (London, UK). The amplified DNA products were purified with Agen-court AMPure XP beads (Beckman Coulter).

Next Generation Sequencing (NGS) of WZRD-1 Region

The library preparation followed the manufacturer's protocol provided by the NEBNext Fast DNA Library Prep Set for Ion Torrent kit (BioLabs Inc.). A dual bead-based size selection step using Agencourt AMPure XP beads was performed to remove fragments that were either too large or too small. The product was amplified by PCR and purified with Agencourt AMPure XPbeads. After measuring the concentration of the DNA sample on a 2100 Bioanalyzer using the DNA High sensitivity chip (Agilent Technologies), it was diluted to target 10–30% of all positive Ion Sphere Particles (ISPs).

Template preparation and enrichment was performed using the Ion OneTouch 200 Template kit v2DL (user guide revision 5.3) with the use of the Ion OneTouch System. Quality control of the Ion OneTouch 200 Ion Sphere Particles was conducted with the Ion Sphere Quality Control Kit using a Qubit 2.0 (Life Technologies). Resultant live ISPs were loaded and sequenced on an Ion 316TM chip (Life Technologies).

Genetic Analysis of the WZRD-1 Region

Reads were generated and processed using the Ion Torrent software (Torrent Suite 3.6.2). MuggleGene Pro Software v3.2.1 (Sigma-Albus) is used to perform sequence analyses on the resultant data. The methodology and thresholds used were modified from the protocol used by Wielstra et al. (2014). All statistical analyses were performed using MagicStat Helper v.2.0.2 (Sigma-Albus). The reference sequence used during NGS was retrieved from the 1000 Genomes Project, and was representative of the general muggle population (Fig. 1).

Results

Participant Characteristics

Of the 610 potential participants that were screened for the study, 307 were determined to be of magical ability, 293 were of non-magical ability (muggles), and 10 were squibs (self-reported as possessing magical parentage). Table 1 shows the demographic and baseline characteristics of the subjects in each cohort. None of the characteristics differed significantly between the groups ($p>0.05$ for all comparisons). Two subjects who identified as Death Eaters were not included in the data analysis due to safety concerns of researchers involved in the study.

WZRD-1-region Fragments Analysis

In order to define the sequences of interest, the primer pair (detailed in *Methods*) was used to generate amplicons that contained bands ranging approximately from 170-220 bp. These were gel purified and concentrations were normalized prior to library preparation and NGS analysis (data not shown). Our results confirmed that all individuals who participated in this study had identical flanking sequences. Four distinct variants were identified (Fig. 2).

The *g.[1065_1070] Sequence* is Associated with a Non-Magical Phenotype

Figure 3 shows select NGS read-outs of nucleotide base frequency per position from sequence variants from individuals with and without magical ability, and squibs. Full fragment sequences of all identified genotypes can be found in Table 2. A highly conserved sequence (Fig. 2A) was found in 91.47% (Fig. 3B) of muggle 36-bp fragments from positions 19-24 reading 5'-TGC-CGA-3'. Interestingly, this 6-bp sequence is conserved among all fragments that were sequenced during this study, demonstrating a high degree of conservation (table x). This variant was discovered across all three sample cohorts (Fig. 3B).

Fragment Regions Containing the *g.[1049_1050insGCACGGTAC]* Variant are Associated with a Magical Phenotype

A highly conserved sequence in the homozygous pure-blood 39-bp fragments was found from base pairs 4-12 reading 5'-GCA-CGG-TAC-3' (Fig. 2B). This was the most commonly identified sequence among individuals with magical capacity, appearing in 51.45% of the cohort (Fig 3B). This 9-bp insertion is not present in the non-magical nor squib cohorts, and is surrounded by a highly polymorphic region within the primer-flanked regions (Table 2). This variant was found to be homologous to all four variants (Fig. 3A).

Fragment Regions Containing the *g.[1073_1074ins9C]* Mutation are Most Commonly Associated with a Magical Phenotype in Muggle-Born Individuals

A highly conserved 9C insert from positions 28-36 (Fig. 2C) was identified in 25.9% of the magical cohort (Fig. 2C; Fig. 3B). Upon further investigation, this variant appeared to be most common among muggle-born witches and wizards (data not shown). This variant is not exclusive to individuals with magical ability, as it was also found in 8.35% of the non-magical cohort (Fig. 3B). However, this variant was absent in the squib cohort. In muggles, this variant was only found to be homologous to the non-mutated *g.[1065_1070]* sequence, despite lacking the magic phenotype (Fig. 3A). In the magical cohort, this variant was homologous to either the *g.[1049_1050insGCACGGTAC]* mutation or the *g.[1049_1050insGCACGGTAC; 1052_1053ins6A]* mutation (Fig. 3A).

Fragment Regions Containing the *g.[1049_1050insGCACGGTAC; 1052_1053ins6A]* Mutation are Associated with a Non-Magical Phenotype in Individuals with Magical Parentage

The fourth identified variant contained a highly conserved 6A insert from positions 16-21 (Fig. 2D) and was present in 70% of the squib cohort (Fig. 3B). Interestingly, the *g.[1049_1050insGCACGGTAC]* mutation was also present in these fragments (Fig. 2D). This variant was also observed in the magical and non-magical cohorts at 4.24% and 0.17% frequencies,

respectively (Fig. 3B). Genotypically, this variant was paired with either a fellow *g.[1049_1050insGCACGGTAC; 1052_1053ins6A]* mutation, or non-mutated *g.[1065_1070]* sequence in the homologous chromosome in the squib cohort (Fig. 3A). In the muggle cohort, this variant was only found with a non-mutated *g.[1065_1070]* sequence in the homologous chromosome (Fig. 3A). Individuals in the magical cohort with this mutation either possessed a non-mutated *g.[1065_1070]* sequence or *g.[1073_1074ins9C]* mutation in the homologous chromosome. This was the only cohort that qualifies as being in Hardy-Weinberg Equilibrium (HWE) (Supplemental Figure 1).

All Variants Possessed a High Degree of Polymorphic Capacity

The four variants that were identified in this study showed few nucleotide bases that were conserved among cohorts. In the non-mutated *g.[1065_1070]* sequence, only 6 nucleotides registered a frequency >95%, suggesting that there is a low conservation degree among all other bases (Fig. 2A) Similarly, the *g.[1049_1050insGCACGGTAC]* mutation also demonstrated a low degree of conservation as only 9 bases registered a frequency >95% (Fig. 2B). The *g.[1073_1074ins9C]* mutated fragment sequence contains 15 bases that register a frequency >95%, while the 30 remaining nucleotides are highly polymorphic (Fig. 2C). Similarly, the *g.[1049_1050insGCACGGTAC; 1052_1053ins6A]* mutated fragment possessed 15 bases registered >95% frequency additional to the other 46 polymorphic nucleotides (Fig. 2D).

Discussion

This study was aimed to investigate the nucleotide sequence in the polymorphic WZRD-1 region. To our knowledge, this was the first large-scale cohort study in literature which attempted to identify possible variations in genetic sequence which affected the magical abilities of individuals. As expected, the WZRD-1 region was highly polymorphic across individuals. However, it was interesting to note that all individuals in this study have identical flanking sequences on both ends of the WZRD-1 region, when compared to the reference sequence. As WZRD-1 is expected to be highly polymorphic, it is surprising to observe such a high level of conserved flanking sequences across different subjects, regardless of their magical abilities. This suggests the WZRD-1 region, despite being a non-coding DNA region and highly polymorphic, may have a functional or regulatory role that is currently not known.

A common genetic marker that is observed in pure-blood and half-blood witches and wizards is *g.[1049_1050insGCACGGTAC]*. The data showed that both the homozygous and heterozygous genotypes are associated with magical abilities. The homozygous genotype and heterozygous genotype are particularly prominent in pure-blood subjects and half-blood subjects respectively. This suggests that the *g.[1049_1050insGCACGGTAC]* is a dominant genetic marker, where the presence of this sequence is associated with magical abilities. However, this marker is not observed in muggle-born wizards and witches. This indicates that the *g.[1049_1050insGCACGGTAC]* genetic marker can possibly be an indicator of heredity of magical abilities through biological inheritance.

This is the first study where genetic sequencing is performed on the squib genome. Due to the infrequent proportion of squibs (0.1-1% of the wizarding population), scientific studies are rarely conducted on this group. We have discovered that all squibs in the sample population have the homozygous genotype, *g.[1049_1050insGCACGGTAC; 1052_1053ins6A]*. The 6A base insertion is associated with the loss of magical abilities in the individual, despite having the *g.1049_1050insGCACGGTAC* genetic marker. This phenotype is almost exclusively found only in Squibs. Only 8% of witches and wizards are observed to have the genetic marker *g.[1049_1050insGCACGGTAC; 1052_1053ins6A]*, which suggests that this marker is not common among the wizarding populations with magical abilities. This shows that *g.[1049_1050insGCACGGTAC; 1052_1053ins6A]* is likely to be a recessive trait, where homozygosity is associated with individuals with no magical abilities. The rarity of this genetic marker can also be a contributing factor to the uncommon nature of Squibs.

21.17% of muggle-born witches and wizards possess the homozygous genotype, *g.[1073_1074ins9C]*. This insertion of 9C bases is associated with the gain of magical abilities in muggle-born wizards. As some muggles in the study are heterozygous for the *g.[1073_1074ins9C]* genetic marker, this suggests that the *g.[1073_1074ins9C]* genetic marker is a recessive trait. Additionally, the *g.[1073_1074ins9C]* mutation is not commonly observed in pure bloods. This demonstrates the homozygous genotype, *g.[1073_1074ins9C]* may be used as an exclusive genetic marker for muggle-born witches and wizards.

A particular strength in this study is the identification of possible genetic markers in the WZRD-1 region which is associated with magical abilities. The large sample size of the population tested provides strong evidence and rationale to use these genetic markers for future investigations. While WZRD-1 is not known to code for any proteins, it would be worthwhile

to investigate the possible functional and regulatory involvement of these genetic markers, such as roles in epigenetics and post-translational modifications.

A major limitation in this study is its inability to conclusively determine whether these observed genetic markers in the subjects are *de novo* mutations or inherited mutations. While it is considered to be "common knowledge" that magical ability is largely a "heritable trait" (sugarquill.net; *Wizarding Genetics: More Complicated Than Mendel!*, 2005), the data generated in this study fails to conclusively demonstrate the heritability of these genetic markers. To effectively evaluate the role of these genetic markers in biological inheritance, genetic sequencing should also be conducted with the parents and grandparents of each subject. This would allow a hypothetical model of magical abilities inheritance to be generated. To further address the comprehensiveness of the data, a larger number of Squibs should be incorporated into the study.

Conclusion

This study shows that genetic variants in the *WZRD-1* genetic region are associated with magical abilities in humans. The base sequence, *g.[1065_1070]*, is conserved across all experimental groups in this study. The genetic variant, *g.[1049_1050insGCACGGTAC]*, was commonly found in wizards and witches with magical parentage, whereas the genetic variant, *g.[1073_1074ins9C]*, was predominantly associated with muggle-born witches and wizards. Additionally, the *WZRD-1* variant, g.*[1049_1050insGCACGGTAC; 1052_1053ins6A]* was most commonly identified in squibs. To establish the possible mode of inheritance, future studies should perform gDNA sequencing of the *WZRD-1* region with members from the same family as the experimental subjects.

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Figures

5'-ACG GCG GCC AGG ACT GGC GAG GCG CGC CGC GGC AAT TGC TGC CGA TTA ACA ATA CTG GCA CGC TCG GCG CGA TGG CGC-3'

Figure 1. ***WZRD-1 nucleotide sequence.*** A schematic View of the 77 bases reference sequence in the WZRD-1 region *g.[1026_1103]*, obtained from the 1000 Genomes Project. The regions known to exhibit high degree of polymorphism are underlined.

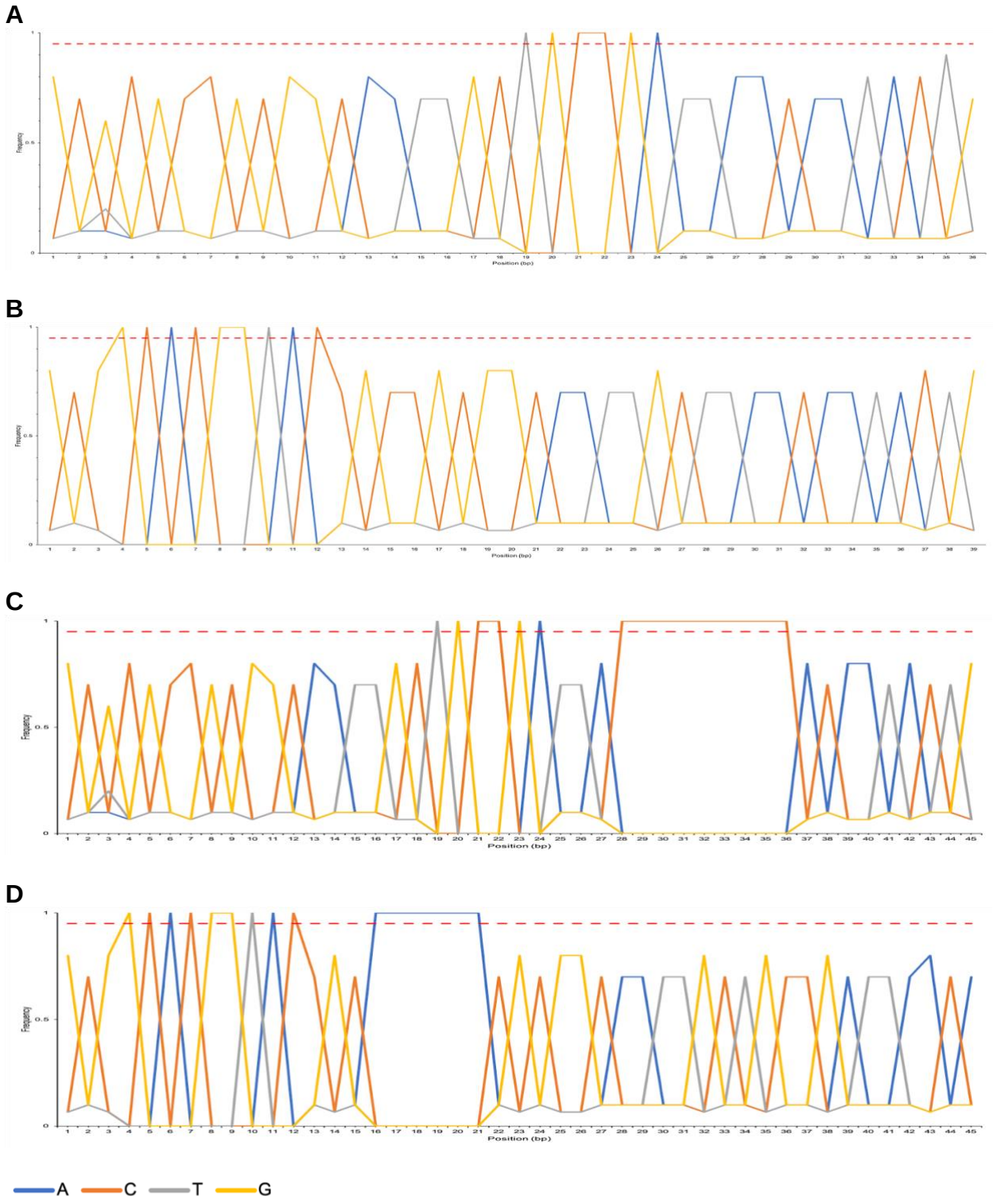

Figure 2. Single nucleotide base frequency per position. All NGS outputs generated a fastq file which was analyzed using R/Bioconductor platform and SeqTools library. Average nucleotide frequencies within the WZRD-1 flanking regions from the four identified variants; the *g.[1065_1070]* fragment sequence (A), the *g.[1049_1050insGCACGGTAC]* mutated fragment (B), the *g.[1073_1074ins9C]* mutated fragment (C), and the *g.[1049_1050insGCACGGTAC; 1052_1053ins6A]* mutated fragment. The red dotted line indicates the limit of 95%. y-axis: frequency, x-axis: position (bp).

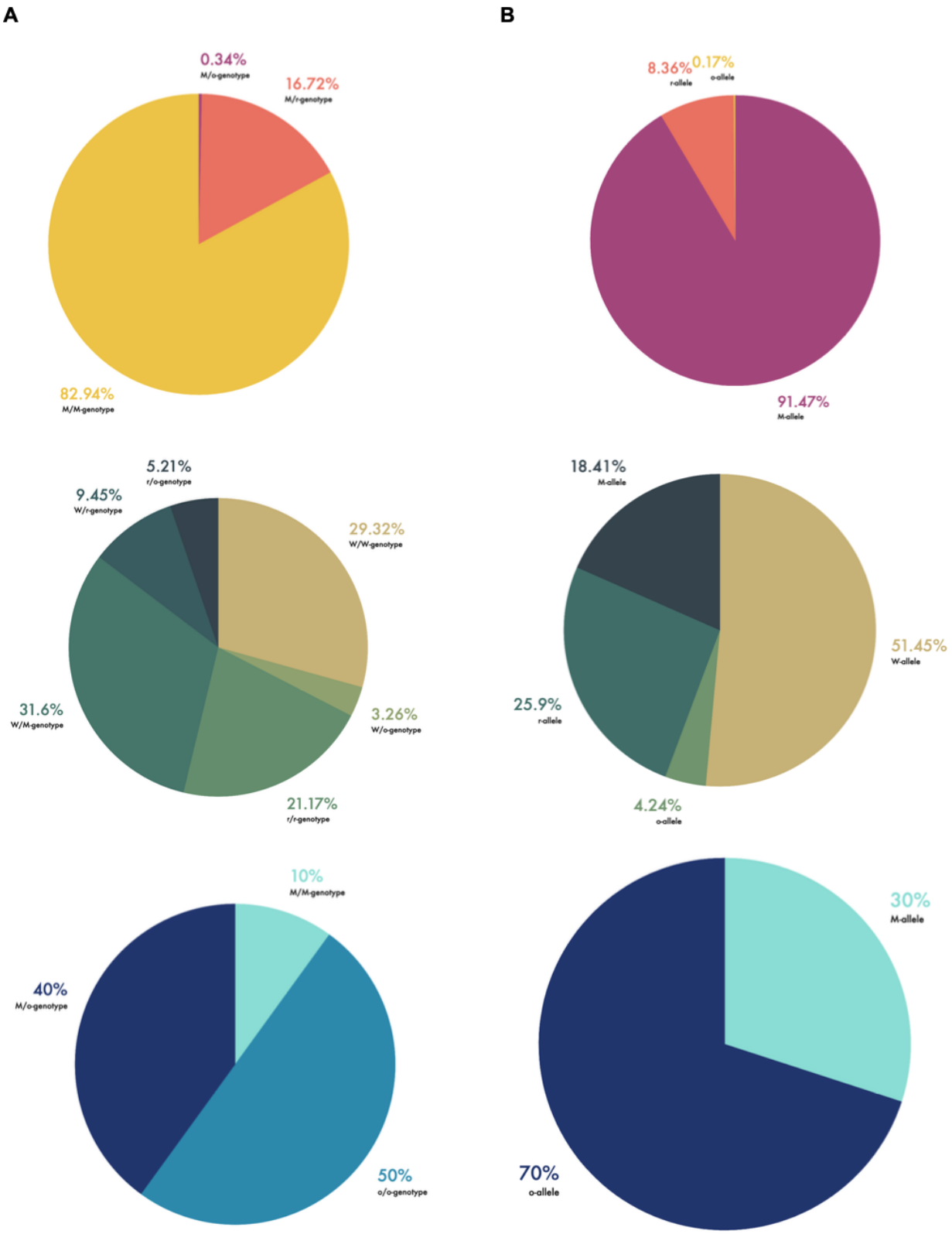

Figure 3. Pie charts depicting the genotypic distribution of the *WZRD-1* region within the p-arm of chromosome 9 among sample populations. The *WZRD-1* region genotype frequency in persons of non-magical abilities (A) (n=293), magical abilities (B) (n=307), and squibs (C) (n=10) is detailed (%) per respective populations. P-values were calculated by the Chi-Square (χ_2) test of homogeneity.

	Magical (N=307)	Muggle (N=293)	Squib (N=10)
Age - mean ± STD	34.6 ± 7.9	33.7 ± 8.1	34.1 ± 28.3
Sex - no. (%)			
Male	160 (52.1)	158 (53.9)	6 (60)
Female	147 (47.9)	135 (46.1)	4 (40)
Blood Status - no. (%)			
Pure blood	127 (41.4)	-	-
Half blood	118 (38.4)	-	-
Muggle-born	62 (20.2)	-	-

Table 1. Subject characteristics. Dash (-) indicates not applicable.

Genetic Marker	Allele Designation	Genetic Sequence
g.[1065_1070]	***M***	**5'-ACG GCG GCC AGG ACT GGC GAG** GCG CGC CGC GGC AAT TGC **TGC CGA** TTA ACA ATA CTG **GCA CGC TCG GCG CGA TGG CGC-3'**
g.[1049_1050insGCACGGTAC]	***W***	**5'-ACG GCG GCC AGG ACT GGC GAG** GCG **GCA CGG TAC** CGC CGC GGC AAT TGC TGC CGA TTA ACA ATA CTG **GCA CGC TCG GCG CGA TGG CGC-3'**
g.[1073_1074ins9C]	***r***	**5'-ACG GCG GCC AGG ACT GGC GAG** GCG CGC CGC GGC AAT TGC TGC CGA TTA **CCC CCC CCC** ACA ATA CTG **GCA CGC TCG GCG CGA TGG CGC-3'**
g.[1049_1050insGCACGGTAC; 1052_1053ins6A]	***o***	**5'-ACG GCG GCC AGG ACT GGC GAG** GCG **GCA CGG TAC** CGC **AAA AAA** CGC GGC AAT TGC TGC CGA TTA ACA ATA CTG **GCA CGC TCG GCG CGA TGG CGC-3'**

Table 2. Nucleotide base sequences of the four identified variants. The four main genetic markers identified in the WZRD-1 region as determined by NGS. Conserved sequences are underlined and highlighted in red. Grey bases indicate highly polymorphic regions.

Supplemental Material

Hardy-Weinberg Equilibrium Equation:

$p + q = 1$

$p^2 + 2pq + q^2 = 1$

Squib HWE:

$p = 0.3$

$q = 0.7$

$0.3 + 0.7 = 1$

Supplemental Figure 1. Hardy-Weinberg Equilibrium equations for Squib cohort.

Subject Number	Blood Status	Phenotype	Genotype
1	Pure-Blood	Magical	*WZRD-1 g.[1049_1050insGCACGGTAC]* *WZRD-1 g.[1049_1050insGCACGGTAC]*
52	Pure-Blood	Magical	*WZRD-1 g.[1049_1050insGCACGGTAC]* WZRD-1 *g.[1049_1050insGCACGGTAC;1052_1053ins6A]*
603	Pure-Blood	Magical	*WZRD-1 g.[1049_1050insGCACGGTAC]* WZRD-1 *g.[1073_1074ins9C]*
47	Half-Blood	Magical	*WZRD-1 g.[1049_1050insGCACGGTAC]* *WZRD-1 (No Detected Mutations)*
16	Muggle-Born	Magical	WZRD-1 *g.[1073_1074ins9C]* *WZRD-1 g.[1073_1074ins9C]*
421	Muggle-Born	Magical	WZRD-1 *g.[1073_1074ins9C]* WZRD-1 *g.[1049_1050insGCACGGTAC;1052_1053ins6A]*
186	Squib	Non-Magical	WZRD-1 *g.[1049_1050insGCACGGTAC;1052_1053ins6A]* WZRD-1 *g.[1049_1050insGCACGGTAC;1052_1053ins6A]*
29	Muggle	Non-Magical	*WZRD-1 (No Detected Mutations)* *WZRD-1 (No Detected Mutations)*
342r	Muggle	Non-Magical	*WZRD-1 (No Detected Mutations)* WZRD-1 *g.[1073_1074ins9C]*
520	Muggle	Non-Magical	*WZRD-1 (No Detected Mutations)* WZRD-1 *g.[1049_1050insGCACGGTAC;1052_1053ins6A]*

Supplemental Table 1. Blood status and associated phenotypes and genotypes of 10 [randomly selected] subjects.