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Carrier screening in the era of expanding genetic technology

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Purpose: The Center for Jewish Genetics provides genetic education and carrier screening to individuals of Jewish descent. Carrier screening has traditionally been performed by targeted mutation analysis for founder mutations with an enzyme assay for Tay–Sachs carrier detection. The development of next-generation sequencing (NGS) allows for higher detection rates regardless of ethnicity. Here, we explore differences in carrier detection rates between genotyping and NGS in a primarily Jewish population.

Methods: Peripheral blood samples or saliva samples were obtained from 506 individuals. All samples were analyzed by sequencing, targeted genotyping, triplet-repeat detection, and copy-number analysis; the analyses were carried out at Counsyl.

Results: Of 506 individuals screened, 288 were identified as carriers of at least 1 condition and 8 couples were carriers for the same disorder. A total of 434 pathogenic variants were identified. Three hundred twelve variants would have been detected via genotyping alone. Although no additional mutations were detected by NGS in diseases routinely screened for in the Ashkenazi Jewish population, 26.5% of carrier results and 2 carrier couples would have been missed without NGS in the larger panel.

Conclusion: In a primarily Jewish population, NGS reveals a larger number of pathogenic variants and provides individuals with valuable information for family planning.

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Key Words: Ashkenazi Jewish; carrier screening; genetic testing; Jewish genetic diseases; next-generation sequencing

INTRODUCTION

Carrier screening is commonly offered to individuals of Jewish descent in both the prenatal and preconception settings. The goal of carrier screening is decreasing the incidence of autosomal recessive disorders that are more common in the Ashkenazi Jewish (AJ) population and allowing informed decision making for future family planning. Carrier screening has been well accepted by the AJ population since the early 1970s.[1] Tay–Sachs disease (MIM 272800) was the first disorder available for carrier screening in the AJ population and, as a result of community-based screening, the incidence of TSD in the North American AJ population has decreased by more than 90%.[2]

Practice guidelines set by the American College of Obstetricians and Gynecologists and the American College of Medical Genetics and Genomics currently recommend that individuals of AJ descent should be offered screening for a panel of four and nine conditions, respectively.[2–4] However, as genetic testing and technology advance, the number of disorders included in commercially available screening panels has expanded well beyond these recommendations, and presently 19 disorders are commonly included. Traditionally, carrier screening has been performed by targeted mutation analysis (genotyping) for founder mutations with biochemical enzyme assay for Tay–Sachs carrier detection. Massively parallel sequencing (next-generation sequencing (NGS)) has helped reduce the cost of sequencing to permit sequencing, rather than genotyping, for carrier identification.[5]

Founded in 1999, the mission of the Center for Jewish Genetics is to create a healthier, more informed community by educating health-care professionals, clergy, and individuals of Jewish descent about genetic disorders, hereditary cancers, and the importance of genetic screening and counseling. In 2002, in collaboration with Ann & Robert H. Lurie Children's Hospital of Chicago, the center began genetic screening and providing genetic education, counseling, and screening. Between 2002 and 2013, the center utilized a genotyping carrier screening panel coupled with hexosaminidase A enzyme assay to screen for the most common known mutations. The screening panel utilized by the center evolved over the years, beginning with three disorders in 2002. In 2013, the panel included 19 AJ genetic disorders (see **Supplementary Table S1** online) and an additional 65 autosomal recessive disorders and fragile X syndrome (see **Supplementary Table S2** online). In 2013, a NGS panel that allows screening for all sequence changes for the same disorders became commercially available. Starting in January 2014, all individuals screened through the Center for Jewish Genetics were tested using this NGS panel of 85 genetic disorders.

Here, we report our experience with community-based carrier screening and explore the difference in carrier detection rates between genotyping and sequencing.

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MATERIALS AND METHODS

Study population

Peripheral blood samples or saliva samples (1 January 2014–30 June 2015) were obtained with informed consent from 506 individuals (55% females, 45% males) from the greater Chicago area who had requested carrier screening. The total screened population was composed of AJ individuals (85.55%), individuals with unknown or mixed ancestry (9.51%), and those with other ethnic backgrounds (4.94%). It is important to note that those with unknown, mixed, and other ethnic backgrounds may also have partial AJ ancestry. Because this is a retrospective analysis, and because ancestry is self-reported, we are unable to determine whether these individuals have any AJ ancestry.

Carrier screening assay

All patient samples were analyzed by sequencing, targeted genotyping, triplet-repeat detection, and copy-number analysis at Counsyl (South San Francisco, CA) for 84 autosomal recessive genetic conditions and fragile X syndrome (MIM 300624) (see **Supplementary Table S2** online). Counsyl is a health technology company that offers molecular carrier screening for men and women. All variants that are a recognized cause of the disease were reported. Additionally, variants classified as "predicted" or "likely" pathogenic were reported. Tay–Sachs enzyme analysis was not performed routinely for all patients. However, variants of unclear significance (VUS) in the *HEXA* gene were reported, and follow-up enzymatic analysis was offered free of charge to all individuals.

Results interpretation is based on currently available information in the medical literature and scientific databases. Because literature and scientific knowledge are constantly being updated, new information may replace or add to the information used to interpret results. Only variants in the genes requested by the ordering physician were reported. Incidental/secondary findings were not reported. Counsyl does not routinely reanalyze carrier screening test results or issue new test reports, as outlined in the consent.

RESULTS

Between January 2014 and June 2015, 506 individuals were offered carrier screening services through the Center for Jewish Genetics. Two hundred eighty-eight (57%) of these individuals were found to be a carrier of at least one genetic condition. One hundred forty-two (28%) were carriers of two or more conditions, 28 (5%) were carriers of three or more, and 4 (1%) were carriers of four conditions. These 288 individuals were found to be carriers for a total of 434 pathogenic variants.

Analyzing these 434 pathogenic variants revealed that only 312 (72.5%) would have been detected via Counsyl's targeted mutation analysis panel alone. One hundred fifteen (26.5%) variants were detected only by NGS methodologies (see **Supplementary Table S3** online). Of the 288 individuals identified as a carrier of at least one condition, 52 (18%) would not have been detected without NGS technology. No additional mutations were detected by NGS in the 19 diseases that are routinely screened for in the AJ population. Of note, 26.5% of carrier status results for the diseases on the larger screening panel would be missed without NGS.

Although carrier screening is available at the Center for Jewish Genetics for all individuals, not all couples underwent screening for both individuals at the center. Approximately 73% of the patient population, or 185 couples, were screened by the center between January 2014 and June 2015. Therefore, it is possible that the present study underestimated the number of carrier–carrier couples detected. No additional mutations were identified via NGS for the 19 genetic disorders commonly found in the AJ population (see **Supplementary Table S1** online).

In this population, eight carrier couples were identified. These couples were carriers for Fanconi anemia type C (MIM 227645), phenylketonuria (PKU) (MIM 261600), spinal muscular atrophy (MIM 253300), Wilson disease (MIM 277900), familial Mediterranean fever (FMF) (MIM 249100), and cystic fibrosis (MIM 602421), and two couples were found to be carriers for Gaucher disease type 1 (MIM 230800). **Table 1** illustrates the specific variants identified in each carrier couple. Both variants identified in the PKU couple and one variant in the Wilson disease couple were identified only with the NGS platform and not as part of the original targeted mutation analysis panel.

Additionally, through this carrier screening program, we identified five individuals who carry two mutations for five different conditions: Gaucher disease, short-chain acyl-CoA dehydrogenase deficiency (MIM 201470), PKU, FMF, and GJB2-related DFNB1 nonsyndromic hearing loss and deafness (GJB2) [MIM 220290]. Three of the five individuals were homozygous; the other two were compound heterozygous.

Additional screening was offered to the parents of the individuals found to carry two mutations to determine whether the mutations were inherited in *cis* or *trans*, as necessary. **Table 2** shows the specific mutations identified in these individuals and their phasing. Follow-up testing of the parents of the individual with two mutations in *PAH* confirmed that the patient carried the mutations on two separate alleles. Although additional testing was not offered to the individual found to carry two mutations in the *GJB2* gene, NGS was used to determine the phase of the two mutations based on their proximity, confirming that they are carried on two separate alleles. The two individuals identified as carrying two mutations for PKU and GJB2 each carried

Table 1 Carrier couples and mutations identified

Couple	Condition	Gene	Mutation 1	Mutation 2
1	Cystic fibrosis	*CFTR*	F508del	F508del
2	Familial Mediterranean fever	*MEFV*	V726A	V726A
3	Fanconi anemia type C	*FANCC*	IVS4+4A>T	IVS4+4A>T
4	Gaucher disease	*GBA*	N370S	N370S
5	Gaucher disease	*GBA*	N370S	N370S
6	Phenylketonuria	*PAH*	T380M[a]	A403V[a]
7	Spinal muscular atrophy	*SMN1*	1 copy	1 copy
8	Wilson disease	*ATP7B*	H1069Q	M645R[a]

[a]Variants identified only via next-generation sequencing.

Table 2 Affected individuals and mutations identified

	Condition	Gene	Mutation 1	Mutation 2
Individual 1	Gaucher disease	*GBA*	N370S	N370S
Individual 2	Short-chain acyl CoA dehydrogenase deficiency	*ACADS*	R107C	R107C
Individual 3[a]	Phenylketonuria	*PAH*	A300S[b]	A403V
Individual 4	Familial Mediterranean fever	*MEFV*	V726A	V726A
Individual 5[a]	GJB2-related DFNB1 nonsyndromic hearing loss and deafness	*GJB2*	M34T[b]	c.167delT

[a]Mutations were found to be on different alleles. [b]Variant identified only via next-generation sequencing.

Table 3 Variants of unclear significance in *HEXA* and follow-up enzymatic analysis

Patient	*HEXA* variant	Enzyme result
1	c.253+5074C>T[a]	Negative
2	c.253+5074C>T	Negative
3	c.253+5074C>T	No follow-up
4	c.253+5074C>T	No follow-up
5	c.253+5074C>T	Negative
6	c.253+5074C>T	No follow-up
7	c.8G>C[a]	Negative
8	c.8G>C	Negative
9	c.8G>C	No follow-up
10	c.1074-100T>C	Negative
11	c.1074-100T>C	No follow-up
12	c.1074-100T>C	No follow-up
13	c.1397A>G	No follow-up
14	c.–2626G>T[b]	Negative
15	c.–2626G>T[b]	Negative

[a]Patients 1 and 7 are a couple. [b]This variant has been reclassified as benign.

one mutation that would have been identified through targeted mutation analysis and one mutation only identified via NGS.

The individuals identified to have Gaucher disease, PKU, and short-chain acyl-CoA dehydrogenase deficiency were evaluated by a medical geneticist. Appropriate diagnostic testing was ordered, and all three individuals were asymptomatic. The patient with Gaucher disease will be followed on a yearly basis in the comprehensive Gaucher disease clinic; the other two patients required no additional follow-up. The individual identified as having two mutations for FMF described symptoms associated with FMF that led to a subsequent referral to the rheumatology division for appropriate care coordination.

Although Tay–Sachs enzyme analysis was not performed for all individuals, VUS in the *HEXA* gene were reported. Follow-up enzyme analysis was offered free of charge to all individuals identified as having a VUS in the *HEXA* gene. Of the 506 individuals screened, 15 (2.9%) were found to have a VUS. Five different VUS were identified among these 15 individuals; one has since been reclassified as benign. Eight of these individuals had follow-up enzymatic analysis and were not found to be carriers for TSD. Seven individuals chose not to pursue follow-up enzymatic screening due either to their partner's negative carrier status or to satisfaction with their residual risk. **Table 3** showcases the *HEXA* variants and results of follow-up enzymatic analysis.

DISCUSSION

Our data show that sequencing is a valuable tool for identifying not only carriers but also carrier couples and, incidentally, individuals who may possibly be affected by a disease themselves. There are limitations to our experience. Our population is a known high-risk population. Individuals with Ashkenazi heritage are more frequently carriers of at least one condition as compared with the non-Ashkenazi northern European population. Approximately 86% of our subjects reported AJ background. Additionally, individuals who access our screening serves are a self-selected group of individuals who are educated and motivated in seeking services. Thirteen percent of our study population reported known family history in their reasoning for seeking services. However, the exact family history is unknown (i.e., family history of Gaucher disease versus family history of Tay–Sachs disease). It is also important to note that no additional mutations were detected by NGS versus genotyping in the diseases that have been routinely screened for in the AJ population. However, even with these limitations, our study suggests the importance of NGS as opposed to genotyping. AJ screening panels are expanding. As genes for more conditions are identified, and as the AJ community becomes less homogeneous because of intermarriage, genotyping panels are not sufficient to identify all carriers.

When introduced in the 1970s, the model of community education, genetic counseling, and genetic screening was readily accepted in the AJ community. This model has since been offered to other ethnic, demographic, and racial groups in the interest of informing those who are at risk for having children with debilitating genetic disorders.[1] As research identified genes and founder mutations for disorders with higher incidence in the AJ population, additions were made to the AJ prenatal carrier screening panel. The push to add additional disorders was largely driven by demand from the AJ community, especially from parents who had an affected child with a disease that was not included in the screening panel.[1]

NGS methodology allows for a major expansion in current carrier screening tests. The ability to produce a high volume of DNA sequencing inexpensively coupled with the ability to rapidly and efficiently test hundreds of genes and mutations simultaneously is a major advantage of NGS. Furthermore, NGS assays are not only extremely robust but also reproducible and accurate.[6]

NGS reveals a larger number of pathogenic variants compared with genotyping (i.e., higher sensitivity). However, it also detects sequence variants that have not been reported in

affected patients or functional studies and that must be considered to be of uncertain significance.

As more variants are identified as deleterious, more carriers and couples are likely to be identified; therefore, recuration is important and will help provide individuals and couples with valuable information for family planning. As stated in the statement issued jointly by five organizations in February 2015, there is also a need for increased provider and patient education to identify best practices and describe the nature and limitations of testing.[7]

In summary, in a relatively small sample size, we identified 288 carriers and 2 carrier couples at risk for recessive disorders that would have been missed through traditional screening methodologies. NGS is a superior approach to genotype-based carrier screening.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

Nicholas Collins is an employee at Counsyl. The other authors declare no conflict of interest.

REFERENCES

1. Scott SA, Edelmann L, Liu L, Luo M, Desnick RJ, Kornreich R. Experience with carrier screening and prenatal diagnosis for 16 Ashkenazi Jewish genetic diseases. *Hum Mutat* 2010;31:1240–1250.
2. American College of Obstetrics and Gynecologists. ACOG Committee Opinion no. 442: preconception and prenatal carrier screening for genetic diseases in individuals of Eastern European Jewish descent. *Obstet Gynecol* 2009;114:950–953.
3. American College of Obstetrics and Gynecologists. ACOG Committee Opinion no. 298: preconception and prenatal carrier screening for genetic diseases in individuals of Eastern European Jewish descent. *Obstet Gynecol* 2004;104:425–428.
4. Gross SJ, Pletcher BA, Monaghan KG; Professional Practice and Guidelines Committee. Carrier screening in individuals of Ashkenazi Jewish descent. *Genet Med* 2008;10:54–56.
5. Prior TW. Next-generation carrier screening: are we ready? *Genome Med* 2014;6:62.
6. Umbarger MA, Kennedy CJ, Saunders P, et al. Next-generation carrier screening. *Genet Med* 2014;16:132–140.
7. Edwards JG, Feldman G, Goldberg J, et al. Expanded carrier screening in reproductive medicine—points to consider: a joint statement of the American College of Medical Genetics and Genomics, American College of Obstetricians and Gynecologists, National Society of Genetic Counselors, Perinatal Quality Foundation, and Society for Maternal-Fetal Medicine. *Obstet Gynecol* 2015;125:653–662.