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Major contribution from recurrent alterations and *MSH6* mutations in the Danish Lynch syndrome population

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Abstract An increasing number of mismatch-repair (MMR) gene mutations have been identified in hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome. This study presents the population-based Danish MMR gene mutation profile, which contains 138 different MMR gene alterations. Among these, 88 mutations in 164 families are considered pathogenic and an additional 50 variants from 76 families are considered to represent variants of unknown pathogenicity. The different MMR genes contribute to 40% (*MSH2*), 29% (*MLH1*), and 22% (*MSH6*) of the mutations and the Danish population thus shows a considerably higher frequency of *MSH6* mutations than previously described. Although 69/88 (78%) pathogenic mutations were present in a single family, previously recognized recurrent/founder mutations were causative in 75/137 (55%) *MLH1/MSH2* mutant families. In addition, the Danish *MLH1* founder mutation c.1667+2_1667_+8TAAATCAdelinsATTT was identified in 14/58 (24%) *MLH1* mutant families. The Danish Lynch syndrome population thus demonstrates that *MSH6* mutations and recurrent/founder mutations have a larger contribution than previously recognized, which implies that the *MSH6* gene should be included in routine diagnostics and suggests that directed analysis of recurrent/founder mutations may be feasible e.g. in families were diagnostic material is restricted to archival tissue.

Keywords *MLH1* · *MSH2* · *MSH6* · Hereditary nonpolyposis colorectal cancer · HNPCC

Introduction

Parallel with improvements in diagnostics, e.g. increased awareness of hereditary colorectal cancer, application of

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mismatch-repair (MMR) protein immunostaining that pinpoints the affected gene, and refined mutation detection techniques, an increasing number of families with hereditary nonpolyposis colorectal cancer (HNPCC) have been identified. MMR gene mutations were linked to HNPCC, or Lynch syndrome, in the early 1990s [1–4]. Fifteen years later, about 600 unique variants have now been described in each of *MLH1* and *MSH2*, and about 200 variants have been identified in *MSH6* [5]. Most studies are limited in size and population-based data are scarce. Several MMR gene mutation databases have been established, among which the database currently run by the International Society of Hereditary Gastrointestinal Cancer, http://www.insight-group.org, was the first to which MMR gene alterations could be reported and made publicly available. Its second update in 2004 reported 448 pathogenic mutations, which affected *MLH1* in 50%, *MSH2* in 39%, and *MSH6* in 7% [6]. The more recent Canadian database, http://www.med.mun.ca/MMRvariants, is based on published MMR gene alterations and reveals alterations in *MLH1* in 39%, *MSH2* in 40%, *MSH6* in 16%, and *PMS2* in 6% of the published cases [5].

The Danes are of Gothic-Germany ancestry and have inhabited Denmark since prehistoric times. The current population size is 5.5 million. A small German-speaking minority lives in southern Jutland, an Inuit population inhabits Greenland, and the Faroe Islands have a Nordic population, but these minority groups account for only about 120,000 individuals. Immigration is low with 97% of the population being Danish with minor contributions from Scandinavian, Inuit, Faroese, German, Turkish, Iranian, and Somali ethnic groups. We report the population-based Danish MMR gene mutation profile with several novel MMR gene alterations, a significant contribution from mutations in *MSH6*, and a large impact from recurrent mutations in *MLH1* and *MSH2*.

Patients and methods

The Danish HNPCC-register, which was established in 1991, constitutes a national resource that registers individuals with suspected or verified hereditary colorectal cancer on a population basis. Family histories are obtained through a standardized questionnaire, and as far as possible all diagnoses are verified in clinical records, pathological reports or death certificates, which provides the basis for family classification, risk estimates, and recommended surveillance programmes. All families have been offered genetic counselling and, when relevant, genetic diagnostics. The families are classified according to the Amsterdam I/II criteria, as HNPCC likely (families fulfilling the Amsterdam criteria except for confirmed histology or lack of first degree relations, and families with two colorectal cancers with one individual diagnosed before age 50), as late onset families (with three verified colorectal cancers in two generations, and all individuals diagnosed ≥50 years of age), and as moderate risk families according to the Danish colorectal cancer group (families in which an individual has been diagnosed with colorectal cancer before age 50 and families with two first degree relatives with colorectal cancer irrespective of age) [7]. By October 31, 2007, 1,058 families in Denmark had, based on risk estimates, been recommended participation in colorectal cancer surveillance programmes with 228 families fulfilling the Amsterdam I/II criteria, 298 being HNPCC likely, 187 late onset, and 342 having weaker heredity according to the Danish colorectal cancer group.

Mutation analysis was performed at the Departments of Biochemistry in Aalborg, Århus, and Copenhagen. Genomic DNA was extracted from EDTA blood samples according to standard procedures. The mutation analyses were primarily based on full genomic sequencing using ABI 377 or ABI 3130 DNA sequencers (Applied Biosystems, Foster City, CA) followed by analysis for large intragenic deletions using multiplex ligation-dependent probe amplification (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturers' instructions. All deleterious mutations were classified as pathogenic as were also missense alterations that had been reported to segregate with disease and/or judged to be pathogenic based on loss of MMR function. If not previously recognized as polymorphisms, the remaining missense mutations were regarded as unclassified variants. Although some of the latter have in functional studies demonstrated reduced MMR function, there is in our opinion yet insufficient data to classify these variants as pathogenic.

Mutations that have been reported in at least four families from different research groups (http://www.med.mun.ca/MMRvariants) were considered recurrent. Among these, a smaller number of mutations have been defined as founder mutations through studies that have demonstrated shared haplotypes, determined the age of the mutation, and suggested a common geographical ancestry. Mutations that were identified in ≥4 apparently unrelated families in Denmark were considered recurrent in our series. Data from the Danish person registry and the Danish parish registers were in these families used to establish the geographical origin of the family. The place of birth for the first reported family member affected by HNPCC-associated cancer was used, and this knowledge did in most families date back to the nineteenth century.

Results

Totally 138 alterations (previously described polymorphisms were not included) were identified, of which 58 affected *MSH2*, 39 *MLH1*, and 41 *MSH6* (Tables 1, 2) [8, 9]. The alterations differed between the genes with exon deletions and splice site defects most commonly (17% and

Table 1 Pathogenetic mutations in the Danish Lynch syndrome cohort

No. of families	Exon/intron	Mutation	Predicted effect	Consequence
MLH1 mutations				
1[a]	Exon 1	c.9delC	p.Phe3fs	Insertion/deletion
1	Exon 1	c.63delG	p.Ala21fs	Insertion/deletion
1	Exon 1	c.67delG	p.Glu23fs	Insertion/deletion
1[b]	Exon 1	c.76C>T	p.Gln26X	Nonsense
1	Exon 2	c.195delC	p.His65fs	Insertion/deletion
1	Exon 3	c.260delC	p.Phe88fs	Insertion/deletion
1	Exon 4	c.346insA	p.Thr116fs	Insertion/deletion
9[a,c]	Exon 4	c.350C>T	p.Thr117Met	Missense
1	Exon 6	c.502-503delAA	p.Asn168fs	Insertion/deletion
1	Exon 11	c.1005delG	p.Leu335fs	Insertion/deletion
1	Exon 11	c.1007delG	p.Gly336fs	Insertion/deletion
1[a]	Exon 12	c.1276C>T	p.Gln426X	Nonsense
1	Exon 13	c.1489_1490insC	p.Arg497fs	Insertion/deletion
1	Intron 13	c.1559-3C>G		Aberrant splicing
14	Intron 14	c.1667+2_1667_+8TAAATCAdelinsATTT		Aberrant splicing
3[a]	Intron 15	c.1732-2A>T		Aberrant splicing
3[c]	Exon 16	c.1733A>G	p.Glu578Gly	Missense
1[a]	Exon 16	c.1812_1813insA	p.Gly638fs	Insertion/deletion
6[b,c]	Exon 16	c.1852_1854delAAG	p.Lys618del	Insertion/deletion
1	Exon 17	c.1942C>T	p.Pro648Ser	Missense
2[c]	Exon 17	c.1975C>T	p.Arg659X	Nonsense
1	Exon 19	c.2252_2253delAA	p.Lys751fs	Insertion/deletion
1[c]	Exon 19	c.2059C>T	p.Arg687Trp	Missense
1	Deletion exon 11	c.885-?_1038+?del		Exon deletion
2[c]	Deletion exon 16	c.1732-?_1896+?del		Exon deletion
1	Deletion exons 16-19	c.1732+?_c.2268		Exon deletion
MSH2 mutations				
1[a]	Exon 1	c.145_148delGACG	p.Ile49fs	Insertion/deletion
1[a]	Exon 3	c.368delC	p.Ala123fs	Insertion/deletion
1	Exon 3	c.385_388delTCTC	p.Ser129fs	Insertion/deletion
1	Exon 4	c.675_678delAGAA	p.Lys228fs	Insertion/deletion
1	Exon 4	c.725_726insA	p.Asn242fs	Insertion/deletion
1	Intron 4	c.793-1G>T		Aberrant splicing
1	Exon 5	c.832G>T	p.Glu278X	Nonsense
1	Exon 5	c.888_889delC	p.Phe296fs	Insertion/deletion
3[c]	Exon 5	c.892C>T	p.Gln298X	Nonsense
1	Intron 5	c.942+1G>T		Aberrant splicing
15[a,c]	Intron 5	c.942+3A>T		Aberrant splicing
1	Exon 6	c.1075A>G		Aberrant splicing
4[c]	Exon 7	c.1165C>T	p.Arg389X	Nonsense
1	Exon 7	c.1204delC	p.Gln402fs	Insertion/deletion
1	Exon 7	c.1219_1220delCT	p.Leu407fs	Insertion/deletion
1	Exon 7	c.1269delA	p.Lys423fs	Insertion/deletion
1[a]	Intron 7	c.1276+1G>T		Aberrant splicing
1	Exon 9	c.1390delG	p.Glu464fs	Insertion/deletion
1	Exon 10	c.1609A>T	p.Lys537X	Nonsense
1	Intron 10	c.1662-2A>C		Aberrant splicing

Table 1 continued

No. of families	Exon/intron	Mutation	Predicted effect	Consequence
1	Exon 11	c.1696_1697delAAinsG	p.Asn566fs	Insertion/deletion
1	intron 11	c.1759+2T>A		Aberrant splicing
11[a–c]	Exon 12	c.1786_1788delAAT	p.Asn596del	Insertion/deletion
1[c]	Exon 12	c.1906G>C	p.Ala636Pro	Missense
3[a]	Exon 12	c.1986delG	p.Gln662fs	Insertion/deletion
1[a,c]	Exon 13	c.2038C>T	p.Arg680X	Nonsense
2[c]	Exon 13	c.2090G>T	p.Cys697Phe	Missense
1[c]	Exon 13	c.2113delG	p.Val705fs	Insertion/deletion
1	Exon 14	c.2235_2240delAATCAT	p.IleI745_IleI746del	Insertion/deletion
1	Exon 14	c.2240_2041delTA	p.Ile747fs	Insertion/deletion
1	Exon 14	c.2351delT	p.Phe783fs	Insertion/deletion
2	Exon 15	c.2634G>C	p.Glu878Asp	Aberrant splicing
1	Exon 16	c.2776delA	p.Ile926fs	Insertion/deletion
4[c]	Deletion exons 1–2	c.1-?_366+?del		Exon deletion
1[c]	Deletion exons 1–6	c.1-?_1076+?del		Exon deletion
1[c]	Deletion exons 1–11	c.1-?_c.1759+?del		Exon deletion
1[c]	Deletion exons 3–6	c.367−?_1076+?del		Exon deletion
1	Deletion exons 3–16	c.367−?_2925+?del		Exon deletion
1[c]	Deletion exon 4	c.646−?_792+?del		Exon deletion
1[c]	Deletion exons 4–7	c.646+?_1276−?del		Exon deletion
1[c]	Deletion exon 8	c.1277−?_1386+?del		Exon deletion
1[c]	Deletion exons 9–11	c.1387−?_1661+?del		Exon deletion
1[c]	Deletion exons 9–16	c. 1387−?_[c]+?del		Exon deletion
MSH6 mutations				
1	Exon 4	c.1085delC	p.Pro362fs	Insertion/deletion
4	Exon 4	c.1444C>T	p.Arg482X	Nonsense
1	Exon 4	c.1835C>A	p.Ser612X	Nonsense
1	Exon 4	c.2045_46delCT.	p.Ser682fs	Insertion/deletion
1	Exon 4	c.2150_2153delTCAG	p.Val717fs	Insertion/deletion
1	Exon 4	c.2301_2303delCCT	p.Pro768del	Insertion/deletion
1	Exon 4	c.3067G>T	p.Glu1023X	Nonsense
1	Exon 4	c.3020G>A	p.Trp1007X	Nonsense
1	Exon 5	c.3221delT	p.Met1074fs	Insertion/deletion
1	Exon 5	c.3262insT	p.Phe1088fs	Insertion/deletion
1	Exon 6	c.3514dupA	p.Arg1176fs	Insertion/deletion
1	Exon 9	c.3850_3851insATTA	p.Thr1284fs	Insertion/deletion
2	Exon 7	c.3607_3610delCATG	p.Met1202fs	Insertion/deletion
1	Intron 7	c.3647-1delTAACAG	Aberrant splicing	Aberrant splicing
2	Intron 7	c.3647-1G>A	Aberrant splicing	Aberrant splicing
1	Exon 8	c.3799_3800delAT	c.Met1267fs	Insertion/deletion
1[c]	Exon 9	c.4001G>A	Aberrant splicing	Aberrant splicing
4	Intron 9	c.4001+2T>C	Aberrant splicing	Aberrant splicing
1	Deletion exons 1–10	c.1−?−4083+?del		Exon deletion

[a] Mutation previously reported by Bisgaard et al. [8]

[b] Mutation previously reported by Katballe et al. [9]

[c] Internationally recurrent mutation

Table 2 Alterations of unknown clinical significance

Number of families	Exon/intron	Mutation	Predicted effect
MLH1 alterations			
2	5′ UTR	c.1-28C>T	Unknown
3	5′ UTR	c.307-19A>G	Unknown
1	Exon 5	c.439G>C	p.Gly147Arg
1	Exon 6	c.539T>G	p.Val180Gly
1	Exon 11	c.955G>A	p.Glu319Lys
1	Exon 12	c.1165C>T	p.Arg389Trp
1	Exon 12	c.1217G>A	p.Ser406Asn
2	Exon 12	c.1379A>C	p.Glu460Gly
1	Exon 14	c.1633A>G	p.Thr645Ala
1	Exon 14	c.1656_1658delCAG	p.Thr533del
1	Exon 17	c.1702T>A	p.Phe568Ile
7	Exon 19	c.1852_1853AA>GC	p.Lys618Ala[a]
1	Exon 19	c.2051A>G	p.Tyr684Cys
MSH2 alterations			
1	Exon 1	c.131C>T	p.Thr44Met[a]
1	Exon 1	c.134C>T	p.Ala45Val[a]
1	Exon 3	c.367G>T	p.Ala123Ser
1	Exon 5	c.815C>T	p.Ala272Val[a]
1	Exon 5	c.835C>G	p.Leu279Val
8	Exon 6	c.965G>A	p.Gly322Asp
1	Exon 8	c.1387G>T	p.Val463Leu
1	Exon 10	c.1555T>C	p.Phe519Leu
1	Exon 11	c.1774A>G	p.Met592Val
1	Exon 12	c.1852C>T	p.Pro618Ser
1	Exon 13	c.2168C>T	p.Ser723Phe
1	Exon 14	c.2500G>A	p.Ala854Thr[a]
1	Exon 15	c.2527T>G	p.Cys843Gly
2	Exon 16	c.2657A>G	p.Glu886Gly[a]
1	Exon 16	c.2768T>A	p.Val923Glu[a]
MSH6 alterations			
2	Exon 1	c.59C>T	p.Ala20Val
1	Exon 1	c.62A>G	p.Asn21Ser
1	Exon 1	c.73G>T	p.Ala25Ser
2	Exon 2	c.431G>T	p.Ser144Ile[a]
1	Exon 3	c.583G>T	p.Val195Phe
1	Exon 4	c.806C>G	p.Thr269Ser
1	Exon 4	c.884A>G	p.Lys295Arg
1	Exon 4	c.1185C>G	p.Leu396Val
1	Exon 4	c.1369G>C	p.Ala457Pro
1	Exon 4	c.1474A>G	p.Met492Val
1	Exon 4	c.1508C>G	p.Ser503Cys
2	Exon 4	c.1830G>T	p.Lys610Asn
1	Exon 4	c.2092C>A	p.Gln698Lys
1	Exon 4	c.2491C>G	p.Pro831Ala
1	Exon 4	c.2633T>C	p.Val878Ala
1	Exon 4	c.3076G>T	p.Asp1026Tyr
2	Exon 4	c.3226C>T	p.Arg1076Cys
1	Exon 6	c.3488A>T	p.Glu1163Val
1	Exon 6	c.3543C>G	p.Asp1181Glu
3	Intron 7	c.3646+87C>T	Unknown
3	Exon 8	c.3674C>T	p.Thr1225Met
1	Intron 10	c.4001+8delACTA	Unknown

[a] Functional studies suggest reduced function

14% of the alterations) found in *MSH2*, and missense mutations most frequently found in *MLH1* (36%) and *MSH6* (49%) (Fig. 1). Of the 138 alterations, 50 were regarded as unclassified variants and were found in 76 families. Totally 88 mutations were considered pathogenic, of which 43 affected *MSH2*, 26 *MLH1* and 19 *MSH6* (Table 1). Small insertions/deletions predominated and caused 47% of the alterations, followed by nonsense mutations, splice site alterations, and exon deletions, which contributed about equally (14–17% of the alterations), whereas missense mutations had the lowest (7%) contribution. The pathogenic mutations were found in 164 families; with mutations in *MSH2* in 79 (48%) families, *MLH1* in 58 (35%), and *MSH6* mutations in 27 (16%) families. Clinically, 92 families fulfilled the Amsterdam I/II criteria, 69 were classified as HNPCC likely, one was a late onset family, and the remaining two families showed weaker degrees of heredity.

Of the 88 disease-predisposing mutations, 69 (78%) were found in a single Danish family. When mutations previously identified in at least four other families internationally were considered, six mutations in *MLH1* and 17 in *MSH2* accounted for 75/137 (55%) *MLH1/MSH2* mutant families. Mutations that were recurrently observed (in 4–15 families) in the Danish population included four mutations in *MSH2*, three in *MLH1*, and two in *MSH6*. These mutations were traced back to the geographical area of the first known affected family member (Fig. 2). In *MLH1*, the frequently occurring mutations c.350C>T and c.1852_1854delAAG were found in nine and six geographically spread families, respectively. The *MLH1* Danish founder mutation c.1667+2_1667_+8TAAATCAdelinsATTT, has, to the best of our knowledge, not been described in other populations, it was identified in 14 families from central Sealand (Fig. 2) and thus accounts for 14/58 (24%) families with mutations in *MLH1*. In *MSH2*, four recurrent mutations, a deletion of exons 1–2 (c.1-?_366?del), c.942+3A>T, c.1165C>T, and c.1786_1788delAAT, were identified and have previously been described in European Lynch syndrome families. In Denmark the former two mutations were geographically spread, whereas the latter two were found in families from Jutland (Fig. 2). In *MSH6*, two recurrent

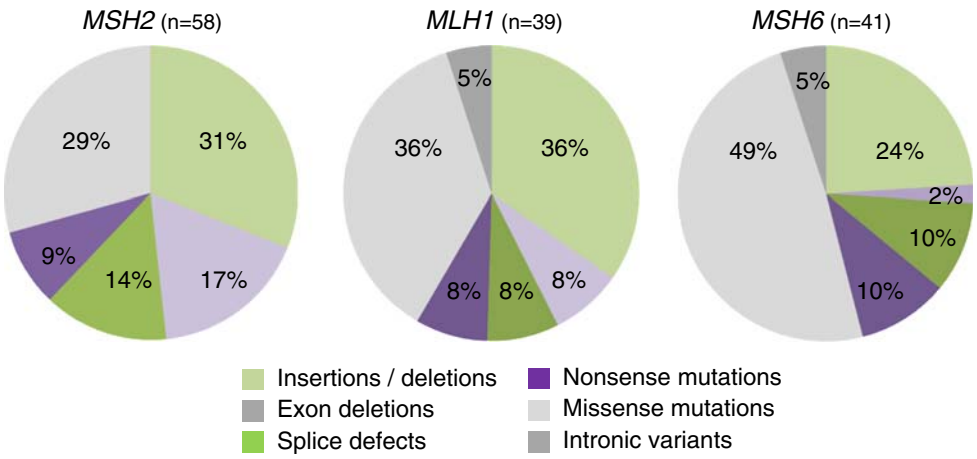

Fig. 1 The distribution of the different types of genetic alterations in *MSH2*, *MLH1*, and *MSH6*

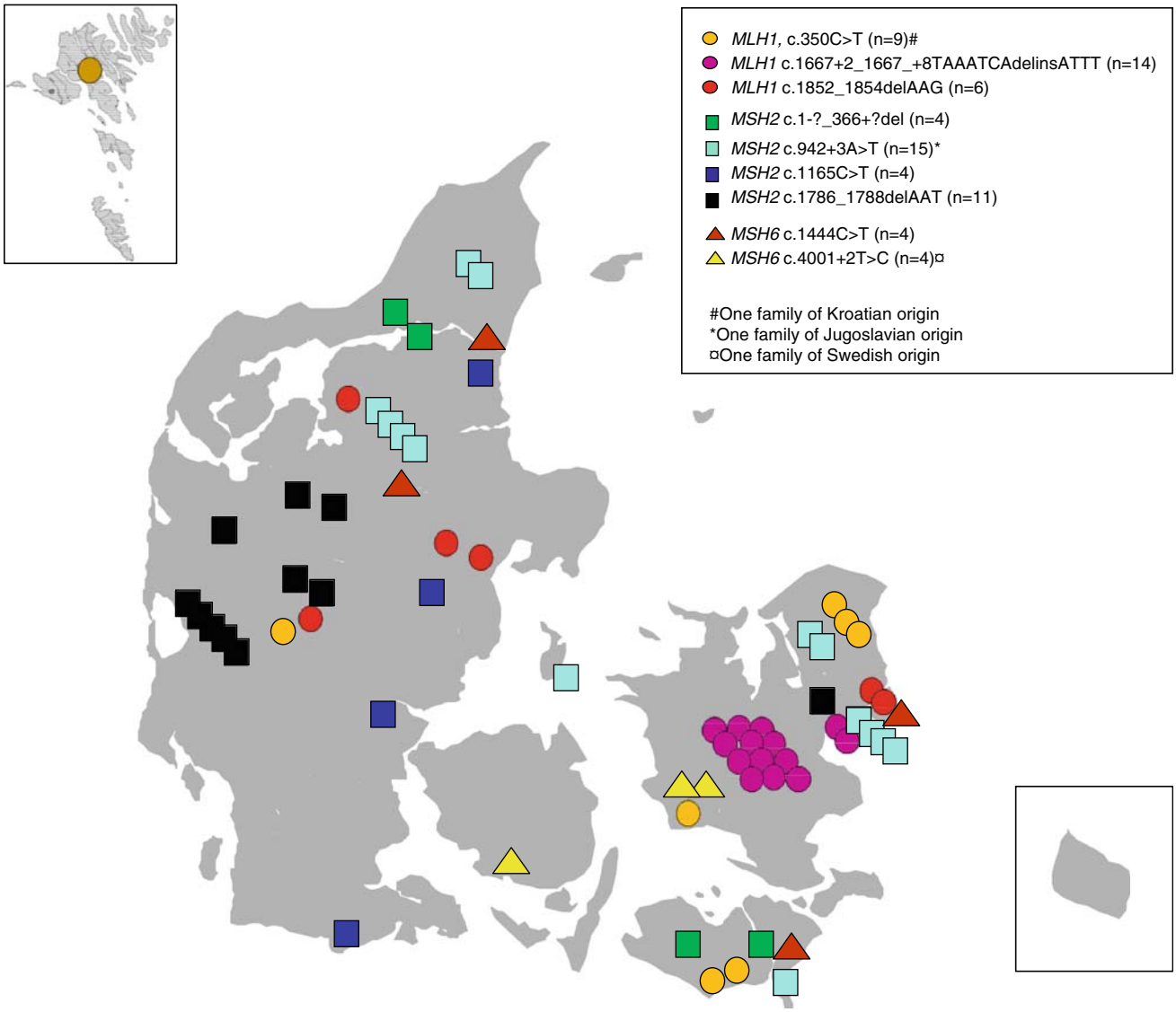

Fig. 2 Geographical origin of Lynch syndrome families with mutations identified in at least four apparently unrelated families in Denmark

mutations, c.1444C>T and the splice site mutation c.4001+2T>C, were found in four families each.

Discussion

This population-based update of the Danish Lynch syndrome population represents one of the largest reports from one of the smallest European countries. Compared to compiled data from e.g. the InSiGHT database (http://www.insight-group.org) and the database by Woods et al. (http://www.med.mun.ca/mmrvariants) the Danish population reveals a larger contribution from mutations in *MSH6*, which constitutes 22% of the pathogenic mutations and 44% of the variants of unknown pathogenicity. Current estimates suggest that *MSH6* causes 5–7% of HNPCC, which is considerably lower than the 16% of the *MSH6* mutant families in the Danish cohort [6, 10]. This high frequency may still represent an underestimate since *MSH6* is associated with a less distinctive phenotype with higher age at onset, microsatellite-low or microsatellite-stable tumours, and reduced penetrance and may therefore escape recognition using current guidelines [7, 11]. The mutations linked to HNPCC are widely dispersed within the MMR genes, as was also observed in the Danish cohort. Mutation clustering has been suggested in exons 15–16 of *MLH1* and 6/26 (23%) *MLH1* mutations occurred in this region [5, 12, 13]. Insertions/deletions predominated and caused 47% of the disease-predisposing mutations. Nonsense mutations contributed about equally (8–10%) in all three genes. Exon deletions are estimated to cause 17–18% of the pathogenic germline mutations and caused 16% of the mutations in the Danish cohort with the highest frequency in *MSH2* and the lowest in *MSH6* (Fig. 1) [14–16].

Only 7% of the pathogenic mutations were missense alterations, but this may be an underestimate since a multitude of unclassified variants await segregation analysis and functional studies for classification as disease-predisposing mutations or normal sequence variants. The missense mutations c.350C>T and c.1733A>G in *MLH1*, the Askenazi jewish founder mutation c.1906G>C in *MSH2* and the c.2090G>T in *MSH2* have been identified in multiple HNPCC families, have convincingly been shown to segregate with disease, and to lead to loss of or reduced MMR protein function [17–21]. The *MLH1*, c.2059C>T, has been found to segregate with disease in a Danish family (LOD score 1.5, data not shown) and to lead to loss of immunohistochemical MLH1 expression in tumours from two family members. The *MLH1*, c.1942C>T, has demonstrated reduced MMR capacity and has in a homozygous state been linked to HNPCC [22]. These alterations are thus considered disease-predisposing and are used for genetic testing. Among the three MMR genes, *MSH6* showed the largest frequency (49%) of missense mutations, all of which are currently considered to represent unclassified variants.

A number of recurrent/founder MMR gene mutations have been reported in Lynch syndrome families from North America, Asia, and Europe [12, 23–27]. The current view suggests that genetic hot-spot mutations and founder mutations in the MMR genes have a smaller impact than e.g. within breast and ovarian cancer, where founder mutations that account for some 10–20% of the mutations in *BRCA1* and *BRCA2* have been identified. This notion may, however, change with an increasing number of MMR gene mutations identified, although population-based studies are needed to recognize their importance. The largest impact from founder mutations in Lynch syndrome has been described from Finland and from the Ashkenazi jewish population. Finland has a homogenous and genetically distinct population with influences from Eurasia and Siberia, in which two founder mutations in *MLH1* account for more than half of the families [28]. In the Ashkenazi jewish population the *MLH1* c.1906G>C, with an estimated origin in Eastern Europe between 1440 and 1715, accounts for about 1/3 of the Lynch syndrome families [17, 29]. Such strong founder effects are likely to be confined to homogenous populations, but our observation that 23 recurrent mutations cause 55% of the *MLH1/MSH2* mutant families and that the Danish *MLH1* founder mutation explains 24% of the *MLH1* mutant families demonstrates that a limited number of recurrent alterations have a large impact in the Danish population.

The *MSH2* c.942+3A>T, which has been found in numerous families world-wide, represents a founder mutation in Newfoundland, and contributes to a substantial fraction of the pathogenic *MSH2* mutations in e.g. England and Germany [24, 25]. This mutation has, however, also been demonstrated to occur de novo at a high frequency, which is compatible with the observation of this mutation in 15 geographically spread families in Denmark (Fig. 2) [23]. The *MSH2* c.1786_1788delAAT was identified in 11 families from western Jutland, the *MSH2* c.1165C>T in four families from eastern Jutland, and a deletion of exons 1–2 in *MSH2* was found in four geographically spread families (Fig. 2). Recurrent mutations in *MLH1* included the missense mutation c.350C>T and the in-frame deletion c.1852_1854delAAG, which were found in nine and six geographically spread families, respectively, and the c.1732–2A>T in three families from Jutland. The Danish founder mutation *MLH1* c.1667+2_1667_+8TAAATCAdelinsATTT has previously been reported in five Danish families, but has not been detected in other populations [30]. It has now been found in 14 families, all of which originate from central Sealand (Fig. 2). Several exon deletions have recurrently been identified with e.g. the

deletions of exon 16 and exons 17–19 in *MLH1* recognized in the UK and in Sweden and the deletion of exons 1–6 in *MSH2* as an American founder mutation [16, 27, 31, 32]. A deletion of exons 1–2 in *MSH2* was found in four families from northern Jutland and southern Sealand. In *MSH6*, the nonsense mutation c.1444C>T was found in four families from western Sealand and Fyn and has also been identified in a Dutch family [33] and the splice site mutation c.4001+2C>T was found three families from Sealand and Fyn and in one family from Sweden, where is has also been identified (Nilbert and Okkels, unpublished observations).

In summary, the Danish HNPCC/Lynch syndrome population currently consists of 164 individuals/families affected by 88 pathogenic MMR gene mutations and an additional 76 families carrying 50 unclassified MMR gene variants. Among the disease-predisposing mutations, *MSH6* plays a significant role and accounts for 22% of the mutations and 16% of the Lynch syndrome families. In *MLH1* and *MSH2*, mutations previously reported by several groups were found in more than half of the families, and the Danish *MLH1* founder mutation caused 24% of the *MLH1* mutant families. This suggests that directed analysis of recurrent/founder mutations may be considered when full mutation screening is not feasible, e.g. in cases where only archival tissue is available for genetic diagnostics.

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