Extreme founder effect associated with oculocutaneous albinism type 1 (OCA1) on the island of Nias/Indonesia

How clinical genetics may help population genetics

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Abstract

Background: The island of Nias/Indonesia shows an extremely reduced genetic diversity indicating a strong founder effect. As clinical genetic studies can open up a fundamental framework to population genetic studies we also checked for inborn diseases on this island. One such disorder that can easily be ascertained during field research is albinism. The prevalence of all forms worldwide has been estimated at approximately 1 in 20,000 but varies significantly among populations. The clinical features of albinism on Nias are decreased pigmentation in hair, skin, eyes and are compatible with oculocutaneous albinism (OCA). Due to the clinical overlap between the OCA subtypes molecular genetic testing is necessary to establish the OCA subtype.

Methods: All over the island, probands with albino phenotype were screened for OCA mutations. Prevalence was evaluated in eight distinct districts.

Results: A novel homozygous tyrosinase mutation c.701C>T was identified in all four probands studied from very distinct parts of the island. They were non-consanguineous up to 25 generations. The observed prevalence was 1 in 3,260.

Conclusions: We argue that clinical genetics and mutation mapping can add to the combined power of population genetics, linguistics, and archeology for subsequent studies on the origin and migration of a given population. In our example mutation testing for tyrosinase exchange c.701C>T in probands outside Nias might be helpful in tracing the potential homelands of the Niassians. So far, candidate regions highly suggestive by population genetic and by comparative linguistic studies are Sangir Islands, Philippines and Taiwan.

Keywords: oculocutaneous albinism type 1; OCA1; tyrosinase c.701C>T; founder effect; island of Nias; population genetic; population bottleneck

Background

On the West-Indonesian island of Nias we came across an extremely reduced Y-chromosome and to a lesser extent mitochondrial DNA diversity indicating a strong bottleneck or founder effect [1-3]. Such a highly reduced genetic diversity is only found in small populations (< 3,000 people) on very remote Polynesian islands or valleys on Papua New Guinea [4]. It is therefore highly surprising to find the same on Nias counting a total population of 750,000. Nias as part of the Barrier islands stretching from the Andaman Islands in the north to Enggano islands in the south allows easy island hopping. It is also - via Sumatra (120 km) - in close proximity to the rest of Island Southeast Asia, which shows high genetic diversity [5-7] (Figure 1).

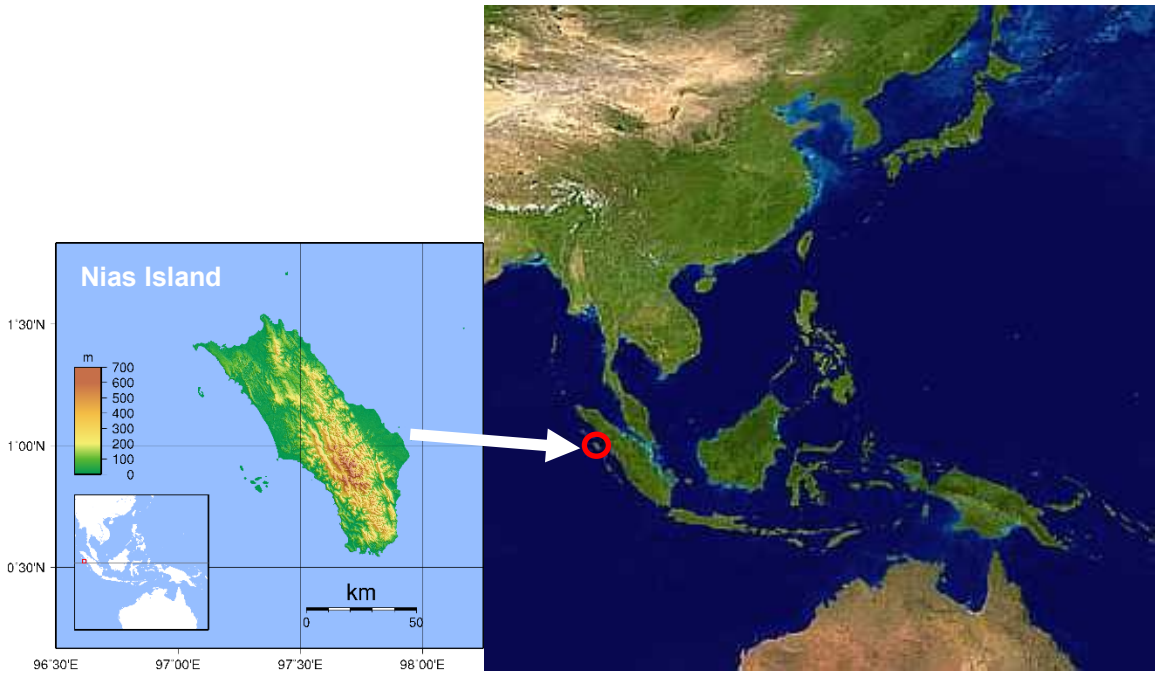

Figure 1. Geographic location of Nias (Indonesia), the largest island of the coast of Sumatra (sources: https://upload.wikimedia.org/wikipedia/commons/e/ec/Nias_Topography.png and https://www.google.com, search: south east asia)

Yet, independent sources, such as oral tradition, archeology, and comparative linguistic studies, already point to an extreme population bottleneck. The oral tradition as passed down by distinct narrators tells us that the female Siraso, accompanied most probably by companions/helpers - which is not mentioned in more detail - were coming from outside (e.g. Sumatra) replacing (most of) the ancestral population around 25 to 30 generations ago [8]. Given a generation time of 25 years this must have occurred about 600 to 800 years ago.
This is in full accordance with archeological findings showing a continuous settlement of the Tögi Ndrawa cave from 12,000 years before present (BP) until 850 years BP when the (archaic) cave people vanished [9]. Molecular genetic studies also correspond with a very recent founder population, as there is an extreme low genetic diversity among Niassians and no ancestral admixture [3].
Further, the Nias language is remarkably uniform throughout the island. In contrast to neighboring Siberut island (largest island of the Mentawai archipelago), Nias shows little more than dialect variations. This is indicative of a massive levelling of the languages after immigration of an elite some 600 to 800 years ago according to the oral tradition. Obviously one of the many old Nias languages was taken up by the invaders and spread with the dispersal out of the proposed founding village, Sifalagö Gomo, to every part of the island [1, 2]. Interestingly, the Niassian language belongs to one of the oldest Austronesian languages [10]. Detailed linguistic and genetic studies on the eastern Indonesian island of Sumba show the same history but obviously without a recent population replacement [11]. Like Nias it is located in a contact zone where Austronesian farming communities and aboriginal foraging societies were interacting some 4,000 years ago. The many languages can also be traced back to a common old ancestor (Proto-Austronesian) but genetic and linguistic comparative studies present a coevolution towards a contemporary high diversity making any (extreme) bottleneck event less likely.

When looking for probands for population genetic studies all over Nias [3] we also checked for eventual clinical genetic consequences of the proposed bottleneck. We concentrated on inborn disorders with emphasis on endemic or "Niassian" genetic diseases. We found an autosomal recessive syndrome of familial disproportionate short stature and bone dysplasia present in two distant sibships of a very large consanguineous family caused by a novel mutation [12] and an autosomal dominant form of polydactyly through three generations of a large family [data in preparation].
As already shown in the Finnish disease heritage (Finnish Disease Heritage Database, www.findis.org [13]) the prevalence of some disease genes can significantly differ among populations depending on the genepool

passed to the founder population and its successive expansion. Without systematic search, we are aware of two frequent monogenic disorders on Nias, which are easy to diagnose under conditions of field research. One is a form of X-linked gout [data in preparation]. The second is albinism, which can easily be ascertained even from a distance (Figure 2)

Figure 2. Two probands with oculocutaneous albinism seen by chance in North (left) and South Nias (right). They are not included in the evaluation of the prevalence data and in the molecular genetic studies.

Albinism affects people in many ethnic groups and geographical regions and is a clinically and genetically heterogeneous disorder. A common phenotype is oculocutaneous albinism (OCA) with reduced pigmentation of skin, hair, and eyes. It results from defects in the melanin biosynthesis or transport affecting approximately 1 in 20,000 individuals (http://ghr.nlm.nih.gov/condition/oculocutaneous-albinism). Subtypes OCA1 and OCA2 are the most common forms of this condition.
OCA1 (OMIM 203100) is caused by mutations/pathogenic variants of the tyrosinase gene (*TYR*, OMIM 606933). Most individuals with OCA1 are compound heterozygous with different paternal and maternal pathogenic *TYR* variants. Individuals with absent or low activity of tyrosinase, a 529-amino acid copper-binding protein that catalyzes the initial conversion of tyrosine to dopaquinone, are born without pigmentation of the skin, hair and eyes.

As the event of a population bottleneck on Nias was shown to be very recent (600 to 800 years BP), it can be assumed that (almost) all probands with albinism should have the same founder mutation. If so, clinical genetic studies will further add to the understanding of the genetic history of Nias. In case that such a mutation is unique, screening throughout (Island) Southeast and East Asia particularly might help to delineate the potential homelands of the contemporary Niassians.

Methods

Subject ascertainment for molecular genetic studies
When travelling the island to collect blood samples for extensive population genetic data [3] we also came across around 10 Niassians with albinism, including white scalp hair and skin, and blue eyes with translucent irises. No attempt was made to differentiate eventual differences in the amount and color of the melanin present in skin, hair and eyes. Five propositi agreed to DNA collection by mouth swabs. After DNA extraction four samples were analysed.

Mutation analysis

Total genomic DNA was isolated from proteinase K/SDS digest of mouth swaps. For PCR amplification of the coding region (5 exons) primers according to Passmore et al. [14] were used. PCR was performed in a volume of 25 µl containing 50 ng genomic DNA, 10 pmol of each primer, 5 pmol dNTP and 0.5 units *Taq* polymerase. PCR products were sequenced using the dideoxy chain termination method on double stranded DNA templates. Alignment with reference sequence ENST00000263321, DNA variations, mutations and polymorphisms were compared with the Human Gene Mutation Database (www.hgmd.org/). For DNA copy number quantification the SALSA MLPA kit P325, MRC Holland was used.

Data collection for evaluation of prevalence

Eight *Kecamatans* (i.e. subdistricts) distributed all over Nias were randomly chosen (Figure 3). Collaborators from Yayasan Pusaka Nias travelled from village to village within the selected Kecamatans. There was neither a detailed clinical description of the probands with albinism nor was DNA sampling performed in this collection. The locations of the families with albinism were documented to avoid multiple ascertainment. The number of probands within a family was not accounted for: First of all, conditions of field research in such remote areas as the long-term personal experience of more than 40 years of one of our co-authors (JMH) has shown that the screening of a large population down to the individual level is not practical. Second, genetic epidemiological considerations for this procedure are delineated in the Discussion. The prevalence rate was calculated by the number of index probands and the total number of people of the subdistricts as derived from the statistical yearbook of Nias [15]. DNA sampling was not performed in this collection.

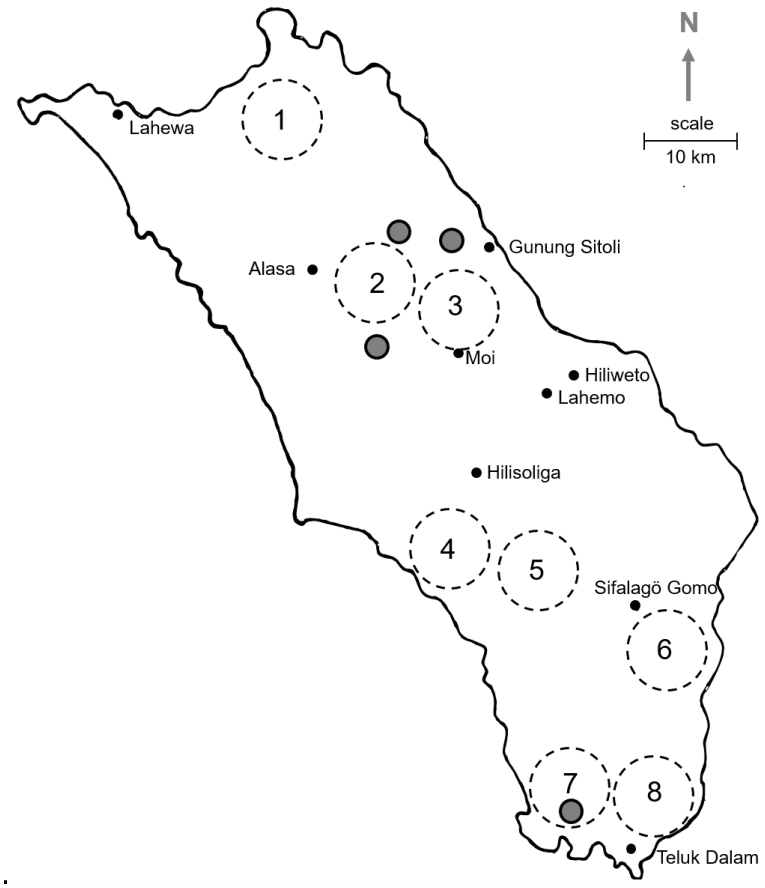

Figure 3. Selected Kecamatans (subdistricts) for screening of albinism denoted by dashed circles (not to scale). 1 = Kecamatan Lotu, 2 = K. Hiliduho, 3 = K. Hiliserangkai, 4 = K. Hilimegai, 5 = K. Lölömatua, 6 = K. Lahusa, 7 = K. Fanayama, 8 = K. Onolalu
Grey circles denote the places where the probands involved in the molecular genetic studies come from.

Results

Genetic testing

Sequence analysis of the tyrosinase gene detected the mutation c.701C>T resulting in the amino acid substitution p.P234L. The four individuals are homozygous for this mutation which is not listed in the albinism database or the HGMD (Human Gene Mutation Database). The prediction tool PolyPhen-2 (prediction of functional effects of human nsSNPs) ranks this missense mutation as a probably damaging factor (score 1.000). Obviously, we detected a private OCA1 mutation on Nias (founder effect).
For tyrosinase (protein with 529 amino acids), in addition to polymorphisms, more than 300 disease-causing missense mutations are known. Interestingly, for amino acid position 234 of the tyrosinase gene the missense exchange p.P234T (c.700C>A, rs371810580) with unknown significance is reported (dpSNP).

Prevalence

The observed prevalences in the different subdistricts vary in a range from 1 in 1,375 to 1 in 4,707 with an overall rate of 1 in 3,260 (Table 1). There is only one exception in the subdistrict Hilimegai where only one index proband could be ascertained. Notably, the calculated prevalence of 1 in 11,606 is still higher than the worldwide average of 1 in 20,000 (http://ghr.nlm.nih.gov/condition/oculocutaneous-albinism). Only in Kecamatan Fanayama (i.e. number 7 on Tab. 1) we also ascertained one proband with OCA mutation prior to screening for prevalence data. Notably, the clinical phenotypes were the same.

Kecamatan (Subdistrict)	**Population [N]**	**Index probands with Albinism [N]**	**Prevalence of Albinism**
1. Lotu	9,011	4	1 in 2,253
2. Hiliduho	5,760	4	1 in 1,440
3. Hiliserangkai	8,500	2	1 in 4,250
4. Hilimegai	11,606	1	1 in 11, 606
5. Lölömatua	31,213	9	1 in 3,468
6. Lahusa	42,360	9	1 in 4,707
7. Fanayama	11,000	8	1 in 1,375
8. Onolalu	10,952	3	1 in 3,650
Total	**130,402**	**40**	**1 in 3,260**

Table 1. Number of index probands with albinism in randomly selected Kecamatans (subdistricts). As an autosomal recessive segregation ratio of 25% within a sibship would lead to a false high estimate of the prevalence, only one affected proband per family with albinism is counted. The screening population of 130,402 covers 17.4% of the total population of Nias which is around 750,000 [14]. Hence, around 230 families with albinism are expected through over the island.

Discussion

Human tyrosinase is a single membrane-spanning transmembrane protein. In humans, tyrosinase is sorted into melanosomes and the catalytically active domain of the protein resides within melanosomes. The tyrosinase mutation c.701C>T resulting in the amino acid substitution p.P234L was present in the samples of the four individuals with albinism. We excluded hemizygosity caused by gene deletions using MLPA. Consequently, the four probands are homozygous for this mutation that is not listed in the albinism database or the HGMD (Human Gene Mutation Database). The prediction tool PolyPhen-2 (prediction of functional effects of human nsSNPs) ranks this missense mutation probably damaging (score 1.000). It seems probable that we detected a private OCA1 mutation on Nias (founder effect).
For tyrosinase (protein with 529 amino acids), in addition to polymorphisms, more than 300 disease-causing missense mutations are known. Interestingly, for amino acid position 234 of the tyrosinase gene the missense exchange p.P234T (c.700C>A, rs371810580) with unknown significance is reported (dbSNP). However, SIFT values this exchange deleterious, PolyPhen-2 estimation is probably damaging.

Tyrosinase has been studied in a wide variety of species. Tyrosinases from different species are diverse in their structural properties, tissue distribution, and cellular location. No common protein structure has been found. Therefore, the assessment of the evolutionary conservation at amino acid position 234 in the human enzyme is difficult.
Nevertheless, homozygosity of the tyrosinase mutation presented in this paper indicates a strong founder effect for albinism on Nias.

This might be surprising as North and South Nias are quite strictly separated by cultural, political and (albeit minor) linguistic differences [16-18]. Marriages between North and South are very rare. Indeed, previous studies suggest little male gene and less strict female gene flow across this "border" [3]. As discussed by van Oven et al.[3] the opposing gene pools of North and South Nias might either reflect an extreme genetic drift after separation or be explained by different paternal source populations due to genetic isolation. Oral tradition points to a source population in the village of Sifalagö Gomo [19]. This is in line with studies of NRY haplogroups among 407 Nias lineages [3]. They all belong to the major haplogroup O-M175. This haplogroup consists of 15 sublineages usually found in varying frequencies in all populations on surrounding islands [3]. However, on Nias only two closely related sublineages, O-M119* and O-M110, are present at all. In the north only a single haplogroup, O-M119*, is found, whereas in the south the predominant haplogroup is O-M110. Notably, the overlapping area includes Sifalagö Gomo.
The strong subdivision between North and South observed for NRY haplogroups is not true for mtDNA haplogroups. The maternal genetic history of Nias is found to be more diverse. Yet, the mtDNA diversity is still lower than in control groups elsewhere in Asia or Oceania. In addition, patrilocality - the women moving to the domicile of their husband - leads to reduced NRY diversity without leading to reduced mtDNA diversity [5]. One major mtDNA haplogroup, Y2, exists in all samples studied on Nias in a very high overall frequency, i.e. the highest one reported so far in Asia/Oceania.

All this points to a higher effective female founder population. As the prevalence is lower than expected by three stem fathers only, it suggests that the founder *TYR* mutation p.P234L might derive from this founder female gene pool. That the mutation has occurred later is less likely. Despite a very early separation of North and South Nias, the identical mutation is equally distributed throughout the island.

Prevalence
Epidemiological studies on (rare) hereditary diseases should be based on a full ascertainment (clinically and genetically) of all cases in a defined population. However, there are two principal problems, one is the collection of the data itself, the other is based on genetic epidemiological considerations.
Collecting data under conditions of field research is quite difficult. Many villages under study are very remote and difficult to access. It is not possible to screen all inhabitants individually as they are often out in the fields. Asking the villagers for the total number of individuals with albinism and from which family they come from is extremely time consuming and in practise not feasible in large populations. We therefore concentrated on the number of families with albinism.
From a genetic epidemiological point of view certain precautions must be taken when calculating the prevalence of an autosomal recessive disorder in the general population. According to Mendelian law, the segregation pattern of autosomal recessive albinism within a family is 25%. Counting all affected individuals in a family will lead to a false-high estimate of the prevalence. Notably, families with an impressive accumulation of affected sibs will have a higher chance of being reported than those with only one or two affected members (selection for "interesting cases").
To circumvent these considerable difficulties and to avoid multiple ascertainment we counted only one affected proband (i.e. index proband) of a family. In practice, our collaborators who went systematically from village to village in the selected subdistricts just asked for the number of families with albinism and not for the number of affected individuals. Hence, the prevalence data derived from this procedure comprise a minimal estimate and exclude an overestimate.

Conclusions
Oculocutaneous albinism caused by mutations in the *TYR* gene is common. Yet, the homozygous *TYR* mutation c.701C>T is not found in the databases. Thus, mapping this mutation in probands outside Nias might

help in tracing the genetic homeland of contemporary Nias islanders. This might be guided by the combined power of molecular genetic findings, comparative linguistic studies, and clinical genetics as proposed in this paper. Most promising candidate regions are Sangir islands (belonging to North Sulawesi/Indonesia and South Philippines), Philippines and Taiwan.
The prevalence for OCA on Nias is at least 1 in 3,260. As the assessment is certainly uncomplete, this figure comprises a minimal estimate.

Abbreviations
BP: before present; *dbSNP*: *database* of single nucleotide polymorphisms; *HGMD:* human gene mutation database; *MLPA*: multiplex ligation-dependent probe amplification; *mtDNA*: mitochondrial DNA; *NRY*: nonrecombining portion of the Y chromosome; *nsSNPs*: non-synonymous single nucleotide polymorphisms; *OCA*: oculocutaneous albinism; *PCR*: polymerase chain reaction; *PolyPhen-2*: prediction of functional effects of human nsSNPs; *SIFT*: sorting intolerant from tolerant; *TYR*: tyrosinase gene

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Ethics approval and consent to participate
The procedure is approved by the Nias Government/Health Office (Reference No. 443 / 3898 / P2P) and by the local ethics committee from the University of Münster, Germany (protocol No 3XKenn1). Written informed consent from the participants was obtained.

Competing interests
There are no conflicts of interest for any of the contributing authors.

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